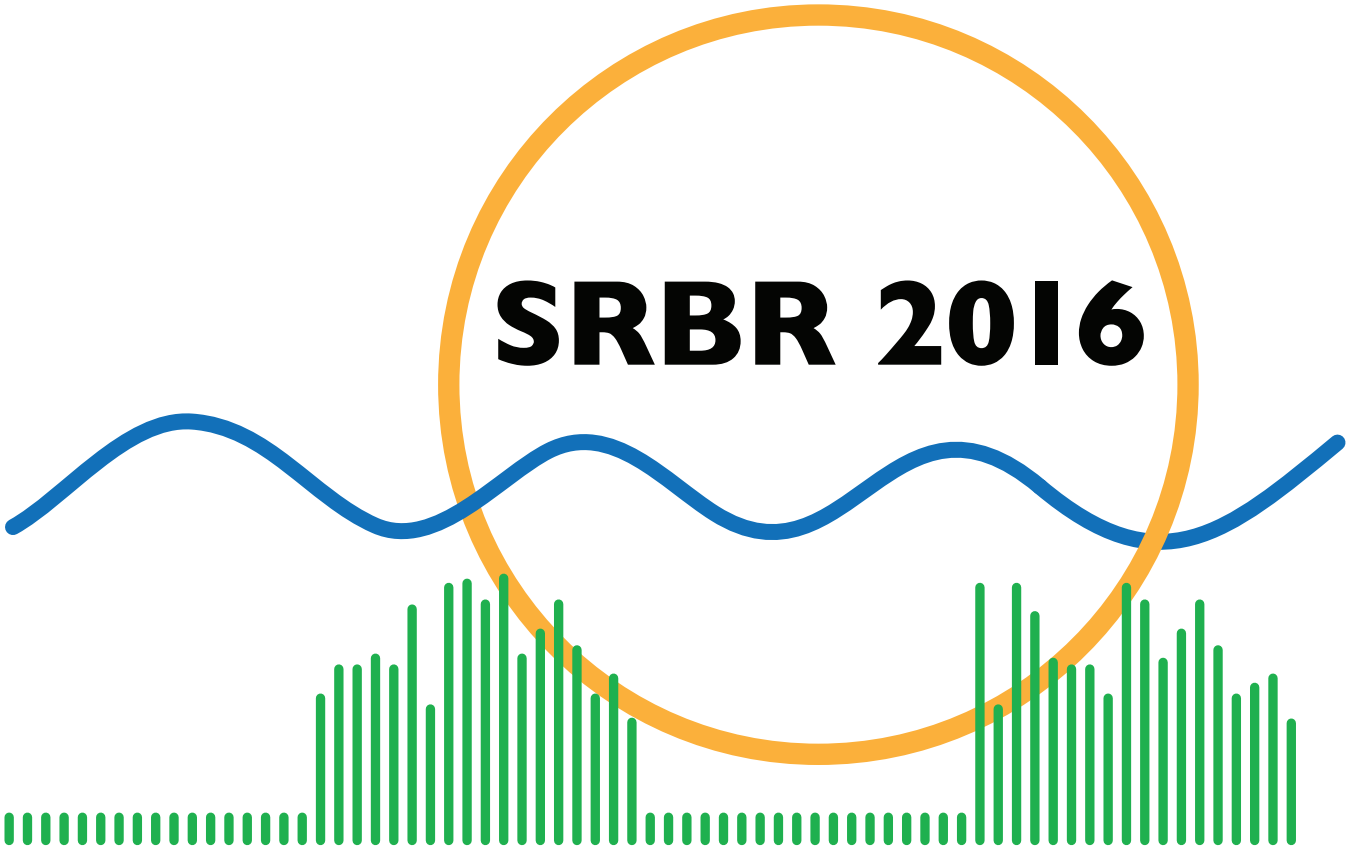
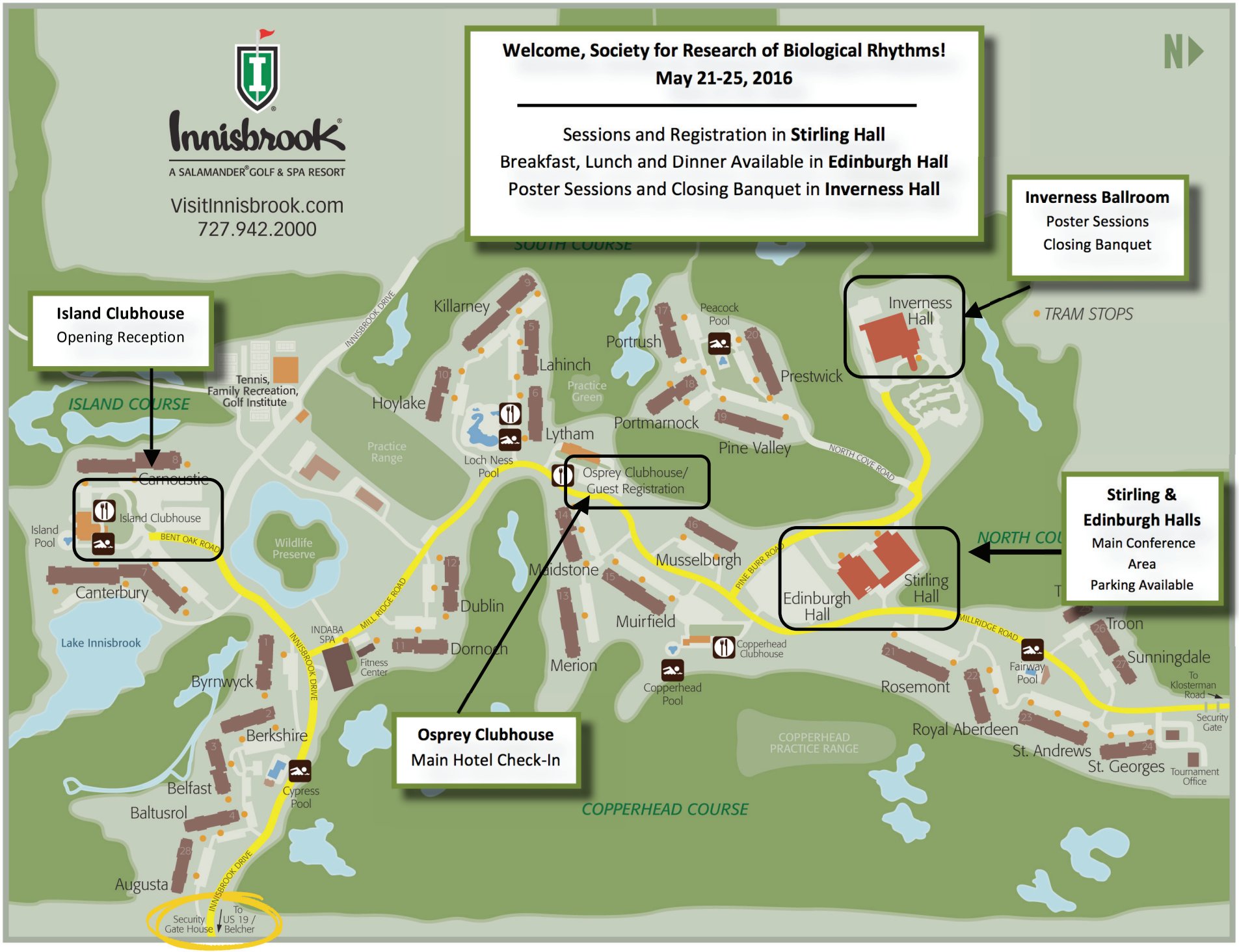


SOCIETY FOR RESEARCH ON BIOLOGICAL RHYTHMS

May 21–25, Palm Harbor, FL



Conference center floor plans can be found in the inside of the back cover.

SOCIETY FOR RESEARCH ON BIOLOGICAL RHYTHMS

May 21–25, Palm Harbor, FL

15th Biennial Meeting

Conference Program

SRBR Thanks the Sponsors of this Meeting!

National Institute of Neurological Disorders and Stroke

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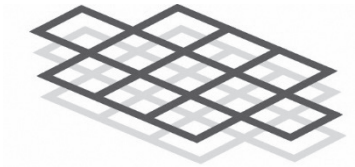

ACTIMETRICS

Private Donation from the Estate of Ronald J. Konopka

SRBR would like to acknowledge the generosity of the following companies whose unrestricted educational grants have contributed to the overall quality of this meeting:

Contents

President's Welcome to SRBR 2016

It is my pleasure to welcome you back to warm, sunny Florida for the 2016 Biennial SRBR Conference! The Society for Research on Biological Rhythms was established 30 years ago, assembled by a group of visionaries whose commitment to research, education, and scientific exchange laid the foundation for SRBR to become a leading voice in propelling the biological rhythms field into the forefront of life science and medicine. To further this remarkable progress, SRBR 2016 promises to be an exceptional forum for hearing the latest cutting-edge research, reengaging with colleagues from years past, and exchanging ideas that will shape the future of the field with a talented and diverse group of chronobiologists from around the globe. Between scientific sessions be sure to take advantage of the hiking trails, golf courses, swimming pools, tennis courts, gym equipment and other amenities at Innisbrook Resort, as well as nearby beaches and beautiful Tampa Bay.

All the scientific discourse, personal interactions and leisure activities that you will soon experience would not be possible without many people working behind the scenes who helped organize this meeting. I wish to sincerely thank the SRBR 2016 Program Chair, Nicolas Cermakian, and our Professional Development Committee Chair, Karen Gamble, our Junior Faculty Workshop Chair, Ilia Karatsoreos, and the Professional Development Committees for kicking off the meeting with terrific educational and career development events, Kelli Tometich and the Parthenon Management Group team for their meticulous planning to keep this meeting running smoothly, and our Fundraising Chair, Erik Herzog, who raised a record level of support from many generous government, corporate and individual sponsors. In addition to planning SRBR 2016, your SRBR Board of Directors made quiet progress on multiple fronts including hiring a new management firm to handle our Society and meeting activities, initiating a Governmental Affairs Committee to advocate for circadian biology and sleep, appointing committees to choose and confer the first Directors' Awards and Junior Faculty Research Awards, and enhancing diversity at biennial meetings by establishing the SRBR Diversity Travel Awards and SRBR International Travel Fellowships. I am forever grateful for the time and hard work that all SRBR committees devoted to strengthening our Society and advancing the biological rhythms field.

Finally, I want to thank all of you for being here and sharing your insights, energy and passion for biological rhythms – which is really what makes this meeting such a success. These are exciting times in the biological rhythms field, and I hope you will take full advantage of the opportunities that await you at SRBR 2016.

Best wishes for a great meeting!

Paul E. Hardin
SRBR President, 2014-2016

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Exhibitors

Exhibitor tables will be set up in the Stirling Hall Foyer throughout the entire meeting. Please take some time to visit our exhibitors, as they have provided generous support for the meeting.

CamNtech, Inc.
630 Boerne Stage Airfield
Boerne, Texas 78006, USA
Contact: Rob Davidson
830-755-8036
sales@camntech.com

Condor Instruments LTDA
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São Paulo, 05468-160 Brazil
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119 14th St NW, Suite 100
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651-481-7400

Research Diets, Inc.
20 Jules Lane
New Brunswick, NJ 08901 USA
Contact: Mark Antonio
Mark@researchdiets.com
732-247-2390

General Information

Headquarters are at the Stirling Hall in the Ballroom Foyer.

SRBR Information Desk will be in the Osprey Club House main hotel lobby on Friday, May 20 and in the Stirling Hall Foyer all other days. The Message Center will be in the Stirling Hall Foyer.

The desk hours are as follows:

Friday, May 20	3:00 pm – 7:00 pm	
Saturday, May 21	8:00 am – 12:00 pm	2:00 pm – 8:00 pm
Sunday, May 22	7:00 am – 11:30 am	3:15 pm – 6:30 pm
Monday, May 23	7:00 am – 11:30 am	3:15 pm – 6:30 pm
Tuesday, May 24	7:00 am – 11:30 am	3:15 pm – 6:30 pm
Wednesday, May 25	7:00 am – 11:30 am	

Messages can be left on the SRBR message board next to the registration desk. Meeting participants are asked to check the message board routinely for mail, notes, and messages.

Hotel check-in will be at the Osprey Club House.

Shuttle service will be available on Sunday-Tuesday from 12:30 pm to 4:30 pm and again from 6:30 pm to 8:00 pm. On Wednesday, the shuttle will be available from 12:30 pm – 4:30 pm. The shuttles will pick up and drop off from the parking lot at Edinburgh Hall and run on a continuous loop to Tarpon Springs during operation hours. Tarpon Springs has shopping, restaurants, and a grocery store.

Meals: Cash concessions will be available for breakfast, lunch and dinner in the Edinburgh Ballroom East. Weather permitting there will be seating each day on Stirling Lawn and Garden Lawn starting Sunday, May 22 thru Wednesday, May 25. Additionally, a variety of restaurants are located on the Innisbrook Resort premises and available to SRBR attendees. See below for a list of outlets scheduled to be open during meal periods. Each outlet will offer special menus and pricing for SRBR attendees.

Breakfast: Market Salamander Grille, Turnberry Pub, Packard's Steakhouse, In Suite DIning

Lunch: Market Salamander Grille, Osprey Sports Bar, The Grille at Loch Ness, Turnberry Pub, Packard's Steakhouse, In Suite Dining

Dinner: Market Salamander Grille, Osprey Sports Bar, Packard's Steakhouse, In Suite Dining

The SRBR Mobile App is now available in the App Store for iPhones and in the Google Play Store for Androids. Search SRBR and download today. View the latest schedule, attendee list and abstracts!

Follow SRBR on social media! Find us on Facebook and follow us on Twitter @SRBR2016.

Meeting Components

Professional Development

Trainee Professional Development Day

Saturday, May 21, 9:00 am – 5:00 pm

The Trainee Professional Development Day is an entire day devoted to scientific and career development activities for trainees. The day consists of a keynote address, an activity consisting of one-on-one blitz discussions, and a series of workshops on various topics. The goal of the Trainee Professional Development Day is to allow the next generation of biological rhythm researchers to learn from and interact with faculty members in a more informal and intimate setting than that allowed by the main conference.

Junior Faculty Workshops

Saturday, May 21, 1:00 pm – 4:40 pm

The goal of the Junior Faculty Workshops, which are open to investigators within 8 years of obtaining a faculty position, is to foster the growth and success rate of the next generation of biological rhythm researchers by learning from and interacting with established faculty members in a more informal and intimate setting than that allowed by the main conference. A panel of experienced members of the field will participate in each meeting, to provide tips and advice to junior faculty members and answer questions.

Meet the Professors

Sunday, May 22 – Wednesday, May 25, 10:30 am – 11:00 am

Meet the Professor Sessions are meant to provide trainees (students and postdocs) the opportunity to interact with experienced faculty members in the field and to foster scholarly conversation. Each day a number of faculty researchers will be available to talk with trainees. Any trainee interested in meeting these investigators can go to the Salon IJK and take part in this informal gathering.

Actigraphy Workshop

Wednesday, May 25, 1:15 pm - 2:15 pm

Condor Instruments will be offering a workshop on the use of actimeters and analysis of actimetry data.

Scientific Sessions

Symposia

Sunday, May 22 – Wednesday, May 25, 8:15 am - 10:30 am

Sunday, May 22 and Tuesday, May 24, 4:15 pm - 6:30 pm

Sessions of talks from guest speakers, designed by the Program Committee.

Slide Sessions

Sunday, May 22 – Wednesday, May 25, 11:00 am - 12:30 pm

Sessions of short talks selected by the Program Committee among the abstracts submitted for the meeting.

Workshops

Monday, May 23 and Tuesday May 24, 3:15 pm – 4:15 pm,
Wednesday, May 25, 3:30 pm – 4:30 pm

The aim of the workshops is to provide a forum to discuss emerging topics, big picture issues and controversies. The chair will introduce the topic and the questions to address and panelists will do a very brief presentation addressing the questions. This will be followed by an open discussion, with participation of the panelists and the audience.

Presidential Special Symposium

Monday, May 23, 4:30 pm – 6:30 pm

The Presidential Symposium is a session of two talks from special guests of the SRBR President.

Pittendrigh/Aschoff Lecture

Wednesday, May 25, 4:30 pm – 6:00 pm

The Pittendrigh/Aschoff Lecture is a keynote lecture presented by a prominent researcher in the field of chronobiology. This year's lecturer is Dr. Susan Golden. In addition to presenting the latest research from her team, she will give a summary of her history in the field, go over some of the major highlights from the meeting from her own perspective, and provide some outlook on future directions of the field of chronobiology.

Datablitz

Sunday, May 22 – Tuesday, May 24, 8:00 pm – 8:30 pm

Datablitz will showcase the research of some of the trainees presenting posters, including many of the Award recipients. Each speaker will have one minute and one slide to introduce data that they will later present in the poster session taking place immediately after.

Poster Sessions

Sunday, May 22 – Tuesday, May 24

Posters will be available for viewing in the Inverness Ballroom and Foyer starting at 10:00 am each day until 10:30 pm. All posters will remain up from May 22 – May 24. Poster setup will be from 3:00 pm to 7:00 pm on Saturday, May 21 and from 10:00 am to 4:00 pm on Sunday, May 22. Poster takedown will be on Tuesday, May 24, before 11:00 pm. Each poster will be scheduled to be presented on a certain day:

Sunday, May 22, 8:30 pm – 10:30 pm	Poster numbers S1-S113
Monday, May 23, 8:30 pm – 10:30 pm	Poster numbers M1-M112
Tuesday, May 24, 8:30 pm – 10:30 pm	Poster numbers T1-T113

Lunchtime Table Discussions

Sunday, May 22 – Wednesday, May 25, starting at 12:45 pm

Lunchtime Table Discussions will be informal discussions of selected chronobiology topics nominated from the membership. These tables are meant to bring together researchers with common interests for informal introductions and discussions. To prepare for a lunchtime table, think about questions that you would like to ask or resources you would like to share with your colleagues. Pick up your lunch in the Edinburgh Ballroom East and go to Edinburgh Ballroom West where tables have been reserved for lunchtime chat participants. Seats are limited at each table.

Special Meetings

JBR Editors Meeting, SAGE Publishers

Monday, May 23, 2:00 pm – 3:00 pm

SRBR Board of Directors Meeting

Tuesday, May 24, 12:45 pm – 2:45 pm

SRBR Governmental Affairs Meeting

Tuesday, May 24, 6:45 pm – 7:45 pm

General Meeting of SRBR Members

Wednesday, May 25, 2:30 pm – 3:30 pm

This is the biennial meeting gathering the members of the Society. **All SRBR members are welcome to attend.** Members of the outgoing Board of Directors and representatives of the meeting organization team will do a brief report, and the new Board of Directors will be presented. Members will also be invited to comment and give ideas on the future of the Society.

Social Events and Ceremonies

Welcome Reception

Saturday, May 21, 7:00 pm – 9:00 pm

Come and meet other meeting participants and old friends in this official opening event of the meeting! Drinks and small bites will be served.

Cocktail Reception

Wednesday, May 25, 6:15 pm – 7:30 pm

Closing Banquet and Awards Ceremony

Wednesday, May 25, 7:30 pm

Regular meeting registration includes participation in the banquet. For accompanying guests, banquet tickets need to be purchased in advance at the SRBR registration desk.

Meeting at a Glance

All sessions of talks will take place in the Stirling Hall. Meals will be available in the Edinburgh Hall. Poster sessions will be in the Inverness Hall.

Saturday, May 21

9:00 am – 5:00 pm **Trainee Professional Development Day** | *Stirling Hall*

1:00 pm – 4:40 pm **Junior Faculty Workshops** | *Stirling Ballroom West*

3:00 pm – 7:00 pm **Poster Session Setup** | *Inverness Ballroom and Foyer*

7:00 pm – 9:00 pm **Opening Reception** | *Island Clubhouse*

Sunday, May 22

8:00 am – 4:00 pm **Poster Session Setup** | *Inverness Ballroom and Foyer*

8:15 am – 10:30 am **Symposium 1: *Konopka Symposium: Frontiers of Molecular Chronobiology*** | *Stirling Ballroom East*

Symposium 2: *Clock Flexibility and Plasticity: Genes, Neurons and Behavior* | *Stirling Ballroom West*

Symposium 3: *Chronopharmacology in Cancer, Shift Work Sleep Disorder and Beyond* | *Stirling Salon OPQ*

10:30 am – 11:00 am **Refreshment Break** | *Stirling Hall Foyer*

Exhibits | *Stirling Hall Foyer*

Meet the Professors | *Stirling Salon IJK*

11:00 am – 12:30 pm **Slide Sessions**

A: Clocks, Feeding and Metabolism | *Stirling Ballroom East*

B: Circadian Rhythms Across the Cell | *Stirling Ballroom West*

C: Light, Brain Function and Mental Health | *Stirling Salon OPQ*

D: Temperature and Cellular Stress | *Stirling Salon LMN*

12:45 pm – 1:45 pm **Lunchtime Table Discussions** | *Edinburgh Ballroom West*

4:15 pm – 6:30 pm **Symposium 4: *SRS-SRBR Symposium: Sleep and Circadian Rhythms*** | *Stirling Ballroom East*

Symposium 5: *Circadian Rhythms in Natural Environments* | *Stirling Ballroom West*

Symposium 6: *Time Perception and Non-Circadian Timers* | *Stirling Salon OPQ*

8:00 pm – 8:30 pm **Datablitz I** | *Stirling Ballroom East*

8:30 pm – 10:30 pm **Poster Session I (S1 – S113)** | *Inverness Ballroom and Foyer*

Monday, May 23

8:15 am – 10:30 am	**Symposium 7:** ***Epigenetics and Transcription Networks in Circadian Clocks*** | *Stirling Ballroom East*
	Symposium 8: ***New Facets of Microbiology in Chronobiology: From Microbiota-Host Interactions to Natural Populations*** | *Stirling Ballroom West*
	Symposium 9: ***Role of the Circadian System in Cardiovascular Health and Disease*** | *Stirling Salon OPQ*
10:30 am – 11:00 am	**Refreshment Break** | *Stirling Hall Foyer*
	Exhibits | *Stirling Hall Foyer*
	Meet the Professors | *Stirling Salon IJK*
11:00 am – 12:30 pm	**Slide Sessions**
	E: Clocks and Immunity | *Stirling Salon LMN*
	F: Post-Transcriptional Regulation in the Clock | *Stirling Ballroom East*
	G: Photoreception and Physiology | *Stirling Salon OPQ*
	H: Neurotransmitters, Channels and Neuronal Networks | *Stirling Ballroom West*
12:45 pm – 1:45 pm	**Lunchtime Table Discussions** | *Edinburgh Ballroom West*
2:00 pm – 3:00 pm	**JBR Editors Meeting, SAGE Publishers** | *Stirling Salon DEF*
3:15 pm – 4:15 pm	**Workshop I** | ***Is it Possible to Translate Chronobiology Findings to Real Life, Health and Society?*** | *Stirling Ballroom*
4:30 pm – 6:30 pm	**Presidential Symposium: Circuits, Genes and Behavior** | *Stirling Ballroom*
8:00 pm – 8:30 pm	**Datablitz II** | *Stirling Ballroom*
8:30 pm – 10:30 pm	**Poster Session II (M1 – M112)** | *Inverness Ballroom and Foyer*

Tuesday, May 24

8:15 am – 10:30 am	**Symposium 10: *Biological Rhythms in Immune Responses and Infectious Diseases*** | *Stirling Ballroom East* **Symposium 11: *Systems Chronobiology*** | *Stirling Ballroom West* **Symposium 12: *Rhythms Over the Lifespan*** | *Stirling Salon OPQ*
10:30 am – 11:00 am	**Refreshment Break** | *Stirling Hall Foyer* **Exhibits** | *Stirling Hall Foyer* **Meet the Professors** | *Stirling Salon IJK*
11:00 am – 12:30 pm	**Slide Sessions** **I: Consequences of Circadian Disturbance** | *Stirling Ballroom East* **J: Evolution, Synthetic Biology, Environment and Circadian Clocks** | *Stirling Salon LMN* **K: Clocks and Neuropeptides** | *Stirling Salon OPQ* **L: Sleep** | *Stirling Ballroom West*
12:45 pm – 1:45 pm	**Lunchtime Table Discussions** | *Edinburgh Ballroom West*
12:45 pm – 2:45 pm	**SRBR Board of Directors Meeting** | *Stirling Salon DEF*
3:15 pm – 4:15 pm	**Workshop II** | ***Big Data Sets: How Useful Are They and How to Mine for Gold?*** | *Stirling Ballroom East*
4:15 pm – 6:30 pm	**Symposium 13: *Neuronal Networks and Central Clock Function*** | *Stirling Ballroom East* **Symposium 14: *Circadian Rhythms in Metabolism, Diabetes and Obesity*** | *Stirling Ballroom West* **Symposium 15: *Non-traditional Models: What Do They Teach Us About Biological Rhythms?*** | *Stirling Salon OPQ*
8:00 pm – 8:30 pm	**Datablitz III** | *Stirling Ballroom East*
8:30 pm – 10:30 pm	**Poster Session III (T1 – T113)** | *Inverness Ballroom and Foyer*

Wednesday, May 25

8:15 am – 10:30 am	**Symposium 16: *Post-Transcriptional/Translational Circadian Mechanisms*** | *Stirling Ballroom East*
	Symposium 17: *Non-visual Effects of Light and Other Zeitgebers* | *Stirling Ballroom West*
	Symposium 18: *Circadian Rhythms in the Context of Addiction, Mood and Neurodegenerative Disorders* | *Stirling Salon OPQ*
10:30 am – 11:00 am	**Refreshment Break** | *Stirling Hall Foyer*
	Exhibits | *Stirling Hall Foyer*
	Meet the Professors | *Stirling Salon IJK*
11:00 am – 12:30 pm	**Slide Sessions**
	M: Micro-organisms, Cancer and Cell Cycle | *Stirling Ballroom West*
	N: Clock Outputs | *Stirling Ballroom East*
	O: Light and Neuronal Networks | *Stirling Salon OPQ*
	P: Human Health, Behavior and Society | *Stirling Salon LMN*
12:45 pm – 1:45 pm	**Lunchtime Table Discussions** | *Edinburgh Ballroom West*
1:15 pm – 2:15 pm	**Actigraphy Workshop** | *Stirling Salon DEF*
2:30 pm – 3:30 pm	**General Meeting of SRBR Members** | *Stirling Ballroom*
3:30 pm – 4:30 pm	**Workshop III** | ***Are Circadian Clocks Therapeutic Targets?*** | *Stirling Ballroom*
4:30 pm – 6:00 pm	**Pittendrigh/Aschoff Lecture** | *Stirling Ballroom*
6:15 pm – 7:30 pm	**Cocktail Reception** | *Inverness Ballroom Foyer*
7:30 pm	**Closing Banquet and Awards Ceremony** | *Inverness Ballroom*

Trainee Professional Development Day

Saturday, May 21

The Trainee Professional Development Day is an entire day devoted to scientific and career development activities for trainees. The day consists of a keynote address, an activity consisting of one-on-one blitz discussions, and a series of workshops on various topics. The goal of the Trainee Professional Development Day is to allow the next generation of biological rhythm researchers to learn from and interact with faculty members in a more informal and intimate setting than that allowed by the main conference.

Only those who have pre-registered will be allowed to participate. Registered trainees should attend the workshops they selected when registering. This information will be posted on the message board in the conference center prior to the first session.

9:00 am – 9:20 am **Welcome and Orientation** | *Stirling Ballroom East*

Karen Gamble, The University of Alabama at Birmingham

Paul Hardin, Texas A&M University

9:20 am – 10:00 am **Keynote Address** | *Stirling Ballroom East*

Joseph Takahashi, University of Texas Southwestern

10:10 am – 11:00 am **Session 1**

Asking the Right Questions & Designing the Right Experiments in a Biological Rhythms Project | *Stirling Salon IJK*

Eric Bittman, University of Massachusetts Amherst

Michael Menaker, University of Virginia

Part of the scientific pursuit is having the wisdom to ask the right questions. This workshop will focus on the process of identifying and refining a research question, and optimizing experimental design to fit a hypothesis pertinent to rhythms research. Discussion of selecting appropriate controls, lighting conditions (light-dark cycle vs. skeleton photoperiod vs constant conditions), the number of time points, and the means of measurement (behavioral vs physiological vs molecular) will also take place.

Circadian Physiological and Behavioral Methods in Rodents | *Stirling Salon DEF*

Johanna Meijer, Leiden University

This workshop will describe experimental setups for the monitoring of circadian physiology in rodent models (mouse, rat, hamster, and diurnal rodents). Basic physiological and behavioral parameters and underlying protocols will be presented and discussed. At least 10 minutes will be saved for discussion and questions.

Smart-technology and Circadian Rhythms? | *Stirling Salon LMN*

Satchidananda Panda, Salk Institute for Biological Sciences

This workshop delves into emerging mobile technology, and presents smart mobile devices, applications, and sensors which allow collection of big data on various behaviors and physiological variables. Besides highlighting opportunities associated with those novel approaches, it will also discuss limitations, especially with regards to circadian rhythm research.

Basic Molecular Clocks (Definition and Current Theory) | *Stirling Salon GH*

Hanzpeter Herzel, Humboldt University of Berlin

For those that are new to the field, this workshop will give an overview of the up-to-date model of "transcriptional/translational feedback loops" in cellular clocks and review major discoveries that lead to the formation of this model. Focus will be placed on the mammalian system but a brief comparison with the *Drosophila* system will also be included. The presentation will be ~30-40 minutes, followed by a discussion of ~10-20 minutes.

Advanced Molecular Clocks (Current Open Questions and New Technical Strategies) | *Stirling Salon OPQ*

Carrie Partch, University of California, Santa Cruz

This workshop will review our current understanding of the biochemical principles underlying molecular clocks by making a comparative analysis of new advances in different systems. We will discuss commonalities and highlight new technical approaches that might be taken to answer some of the most pressing questions. It will be a mix of lecture, ~30-40 minutes and discussion, ~10-20 minutes, about how to attack these new areas of research.

What Makes up the SCN? | *Stirling Salon BC*

Martha Gillette, University of Illinois at Urbana–Champaign

What are the components that make the master clock tick? This introduction is designed as a brief background before the meeting so that new trainees will better understand new findings in SCN anatomy, inputs/outputs and interconnections. The presentation will be ~30-40 minutes, followed by a discussion of ~10-20 minutes.

11:15 am – 12:05 pm **Session 2**

Developing and Maintaining Records of Research | *Stirling Salon GH*

Horacio de la Iglesia, University of Washington

Multiple funding agencies require a plan for proper documentation of research that is not limited to the laboratory notebook. This session will stress the importance of data organization, storage, and sharing of research products; focusing on new electronic formats for record keeping. At least 10 minutes will be saved for discussion and questions.

Teaching Chronobiology | *Stirling Salon BC*

Martha Merrow, Ludwig Maximilian University of Munich

A common challenge for chronobiologists at the beginning of their careers is organising teaching. If you expect to teach chronobiology as a part of your future career, consider joining us to discuss some of the methods and models that have been developed and applied. Topics will include curricula, format and resources. If you sign up for this workshop, your 'homework' is to come with an example of a Chronobiology course curriculum that has been taught at the University or post-graduate level.

Mathematical Modeling | *Stirling Salon DEF*

Daniel Forger, University of Michigan

Decades of experimental research have revealed the immense complexity of the molecular and circuit-level construction of the circadian clock system. It is now difficult to appreciate the system in full without mathematical modeling. In this 50-minute workshop, we will discuss the basics of mathematical concepts and techniques relevant to various levels of physiology and molecular biology that make up the circadian clock system.

Zeitgebers: Entrainment of the Circadian Clock | *Stirling Salon IJK*

Jennifer Evans, Marquette University

The internal circadian clock synchronizes with the daily environmental cycles. This 50-minute workshop will introduce the basic concepts and theories of entrainment of the circadian clock as well as the common methodology that are used to study entrainment in bacteria, fungi, plants, flies, and rodents. The general principles will be the main focus, but we will also cover other aspects such as photoperiodic entrainment and non-photic entrainment.

Circadian Rhythms and Disease | *Stirling Salon LMN*

Florian Storch, Douglas Mental Health University Institute

Kenneth Wright, University of Colorado

The interplay between circadian rhythms and disease states is becoming more evident thanks to both animal and human research. This workshop is geared towards beginners in the field of chronobiology and will provide a brief background of recent findings from both the animal and human literature to help prepare the trainee for the meeting. At least 10 minutes will be saved for discussion and questions.

Questions and Controversies in Chronobiology | *Stirling Salon OPQ*

Carl Johnson, Vanderbilt University

Till Roenneberg, Ludwig Maximilian University of Munich

Despite the apparent simplicity of the circadian phenomena, their interpretations at different levels of analysis are not yet congruous. At a molecular level, does the post-translational oscillator (PTO) make a fundamental circadian oscillator even in eukaryotes? Is the entire expression of circadian transcripts driven by the transcription-translation feedback loop (TTFL) of the core clock genes? In oscillatory transcription, is the source of ultrasensitivity cooperative binding or protein sequestration? How does circadian organization in individuals emerge into circadian organization of groups? And do models add predictive power and explanatory value to our understanding of rhythmicity? These are a small sample of questions we will discuss in this 50-minute workshop. Attending this workshop will make you rethink your "givens" and hopefully take your thinking outside the box - if successful, this workshop will make you leave with more questions than you had before.

12:15 pm – 1:15 pm **Lunch** | *Stirling Ballroom East*

1:15 pm – 2:15 pm ***"Positive Feedback Looping"*** | *Stirling Ballroom East*

This activity will consist of random one-on-one blitz discussions. Participants are asked to pair randomly and discuss for seven minutes, after which they are asked to pair with another participant, and so on, for ~50 minutes. The aim of this activity is to stimulate interaction and exchanges, to allow participants to meet new people, and to "break the ice" before the SRBR conference starts.

2:25 pm – 3:55 pm **Session 3**

Statistical Methods for Time Series Analysis of Rhythms | *Stirling Salon OPQ*

John Hogenesch, University of Cincinnati

Tanya Leise, Amherst College

Analyses of time-series data sets, as frequently required in chronobiological research, can be challenging. This 90 minute workshop will cover various statistical methods that can be used to analyze periodic patterns in biological time-series data (e.g. rhythmicity, period, amplitude, phase, phase shifts). The respective strengths and limitations of each approach will also be discussed, including an overview of statistical software used for such analyses.

Publish or Perish: A Guide to When, Where, and How to Publish Your Work | *Stirling Salon LMN*

William Schwartz, University of Massachusetts Medical School

This 90-minute workshop will be run by the Editor-in-Chief of the *Journal of Biological Rhythms*, Bill Schwartz, to discuss a range of topics with workshop participants about to publish their work, whether senior graduate students or junior post-docs. Topics include authorship; deciding when and what to write; writing review articles; how to organize your writing; choosing a journal; engaging the attention of the editor; review, revision, and rejection; and serving as a journal referee. Come prepared with questions and problems!

Grantsmanship: General Principles | *Stirling Salon IJK*

Douglas McMahon, Vanderbilt University

Eva Schernhammer, Harvard University

Learn the ropes of how to write a competitive grant. This 90-minute session will cover general do's and don'ts applicable to all grant writing, independent of the funding mechanism and country of application. Special emphasis is paid to the description of biological rhythms research for a wide audience of potential reviewers.

Interview Skills & Preparing for the Transition From Postdoc to Independent Research | *Stirling Salon DEF*

Lance Kriegsfeld, University of California, Berkeley

Rae Silver, Columbia University

This 90-minute workshop will discuss how to prepare for independent research positions. We will outline a) how to keep a strong CV, track academic performance and outreach activities, and use professional social media to maximize your marketability, b) how to prepare for a successful job interview, and c) how to initiate and prepare for an independent project. It will also address how the change in roles may affect mentoring relationships and how to handle them. This session will also comprise a mock interview situation and will allow for ample discussion time.

4:10 pm – 5:00 pm **Session 4**

Best Practices for Mentors and Mentees | *Stirling Salon GH*

Eric Mintz, Kent State University

This session will address how creating a mentoring strategy can help you effectively choose the right mentor and approach mentoring others. As a discussion based session, trainees will learn how to identify multiple mentors that they can include in their mentoring network and learn how each mentor/mentee relationship is different.

Transitioning to Non-academic Careers | *Stirling Salon DEF*

Tony Gotter, Merck Research Laboratories

Eric Mabery, Reset Therapeutics Inc.

This workshop will provide an overview of working in the industry following completion of your graduate/postdoc work, and a comparison of research in an industry situation vs. an academic setting. It will also cover where and how to find jobs outside of academia. In addition, insights into the work in a non-profit research institute will be provided in contrast to the industry and academia background. At least 10 minutes will be saved for a discussion.

STAR-PROM-and RT-Biolumicording: New Technologies to Find Transcriptional Regulators and to Study Circadian Gene Expression in Vivo | *Stirling Salon IJK*

Ueli Schibler, University of Geneva

In this workshop, Dr. Schibler will discuss two novel technologies developed during the past few years in his laboratory: STAR-PROM and RT-Biolumicording. These techniques identify transcription factors with unknown DNA-binding specificities and record circadian gene expression in peripheral organs of freely moving mice, respectively. This will be an interactive, 50-minute workshop that encourages open discussion among trainees and Dr. Schibler.

History of Chronobiology | *Stirling Salon BC*

Jay Dunlap, Dartmouth Medical School

This workshop will provide a brief sketch that describes the first observations and studies that pioneered the field of chronobiology. This session is tailored to introduce trainees to the people and key experiments that paved the way for research in circadian rhythms. The presentation will be ~30-40 minutes, followed by a discussion of ~10-20 minutes.

Translational Chronobiology in Humans | *Stirling Salon LMN*

Steven Brown, University of Zurich

Phyllis Zee, Northwestern University

Translational research has been an area of emphasis, particularly given the funding climate. However, the nature and process of conducting translational research is often amorphous. This workshop will be led by both a clinical and basic science researchers in order to provide a collaborative discourse around the models and practices of translational chronobiology research. The workshop will provide a real world behind-the-scenes perspective of translational chronobiology research, and help trainees explore ways of engaging in translational research.

Clocks and Mental Health (Rhythms & Blues) | *Stirling Salon OPQ*

Samer Hattar, Johns Hopkins University

Colleen McClung, University of Pittsburgh

We tend to get moody at night. We associate spring with excitement and autumn with contemplations. But it is still unclear how the rhythms of days and seasons modulate our mood states. In this 50-minute workshop, we walk through evidence for "rhythms and blues" at brain-circuit, molecular, and genetic levels. Emphasis will be placed on molecular approaches and behavioral assay methods in the rodent system.

5:00 pm **Conclusion of Trainee Professional Development Day**

(Trainee Committee members will wrap-up in each workshop.)

2016 Junior Faculty Workshops

Saturday, May 21

The goal of the Junior Faculty Workshops is to foster the growth and success rate of the next generation of biological rhythm researchers by learning from and interacting with established faculty members in a more informal and intimate setting than that allowed by the main conference. A panel of experienced members of the field will participate in each meeting, to provide tips and advice to junior faculty members and answer questions.

Attendance is open to investigators within 8 years of obtaining a faculty position. Only those who have pre-registered will be allowed to participate. A list of registered faculty will be posted on the message board in the conference center prior to the first session.

1:00 pm – 2:00 pm **Panel Discussion 1** | *Stirling Ballroom West*

Managing a Successful Lab: Mentorship, Conflict Resolution, and Diversity

Moderator: Karyn Esser, University of Florida

Jake Chen, University of Texas Medical School

Stacey Harmer, University of California, Davis

Antonio (Tony) Nunez, Michigan State University

The panel will discuss the management skills needed to grow and run a successful lab, skills we are not usually formally trained in as scientists, but which are essential nonetheless.

2:20 pm – 3:20 pm **Panel Discussion 2** | *Stirling Ballroom West*

Navigating the Funding Environment: How to Optimize Your Efforts

Moderators: Ilia Karatsoreos, Washington State University and **Ryan Logan,** University of Pittsburgh

Charles Allen, Oregon Health and Sciences University

Hugh Piggins, University of Manchester

Mimi Shirasu-Hiza, Columbia University

Corinne Silva, National Institutes of Health, NIDDK

Funding is hard to come by in the best of times, and the current climate is very tough. This panel will help you learn strategies to optimize your efforts by specifically tailoring and targeting your proposals, and perhaps discover new sources of funding.

3:40 pm – 4:40 pm **Panel Discussion 3** | *Stirling Ballroom West*

Juggling Research, Teaching, and Service Responsibilities in Academia: Can You Really Do It All?

Moderator: Carla Finkielstein, Virginia Tech

Carla Finkielstein, Virginia Tech

Mary Harrington, Smith College

Horacio de la Iglesia, University of Washington

Even in heavily research oriented institutions, a faculty member is expected to balance teaching, training, and research. This panel will discuss strategies to help in this balancing act. In addition, involvement of undergraduates in the research effort will be a specific focus, as they can be fantastic "junior trainees", and when properly managed contribute greatly to the lab's output.

SRBR 2016 Program Details

Saturday, May 21

9:00 am – 5:00 pm **Trainee Professional Development Day** | *Stirling Hall*
(see details on pages 21-27)

1:00 pm – 4:40 pm **Junior Faculty Workshops** | *Stirling Ballroom West*
(see details on pages 28-29)

3:00 pm – 7:00 pm **Poster Session Setup** | *Inverness Ballroom and Foyer*

7:00 pm – 9:00 pm **Opening Reception** | *Island Clubhouse*

Sunday, May 22

8:00 am – 4:00 pm **Poster Session Setup** | *Inverness Ballroom and Foyer*

8:15 am – 10:30 am **Symposium 1: *Konopka Symposium: Frontiers of Molecular Chronobiology*** | *Stirling Ballroom East*
Chair: Michael Rosbash, Brandeis University

8:15 Introduction

8:30 ***From Konopka's Flies to Human Sleep Behavior***
Ying-Hui Fu, University of California San Francisco

9:00 ***Cell Size Oscillations in the Liver***
Ueli Schibler, University of Geneva

9:30 ***Circadian Clock Regulation of Translation and the Ribosome Code***
Deborah Bell-Pedersen, Texas A&M University

10:00 ***Networks of Noisy Oscillators Make up the Drosophila Circadian Circuit***
Emi Nagoshi, University of Geneva

Symposium 2: *Clock Flexibility and Plasticity: Genes, Neurons and Behavior* | *Stirling Ballroom West*
Chair: Valerie Mongrain, Université de Montréal

8:15 Introduction

8:30 ***Circadian Behavior Relies on Glycinergic Transmission Onto Switching Partners***
Maria Fernanda Ceriani, Fundacion Instituto Leloir

9:00 ***Lessons From Microbial Circadian Systems: Regulation of Virulence, Synthetic Oscillators and Clock-Based Eidetic Memory***
Luis Larrondo, Pontifica Universidad Catolica De Chile

9:30 ***Plasticity in Daily Timing: About Mice and Men***
Roelof Hut, University of Groningen

10:00 ***Circadian Plasticity in Mammals: From Epigenetics to Synapses***
Steven Brown, University of Zurich

Symposium 3: ***Chronopharmacology in Cancer, Shift Work Sleep Disorder and Beyond*** | *Stirling Salon OPQ*
Chair: Francis Lévi, University of Warwick

8:15 Introduction

8:30 ***Circadian-based Anticancer Treatments***
Pasquale Innominato, University of Warwick, The Medical Centre

9:00 ***Chronopharmacology of Antitumor Drugs Focused on Biological Clock***
Shigehiro Ohdo, Kyushu University

9:30 ***Night Shift Work and Resetting of Human Circadian Clocks***
Diane B. Boivin, Douglas Mental Health University Institute, McGill University

10:00 ***Dynamical Coupling Between the Circadian Clock and the Cell Cycle Oscillators***
David Rand, University of Warwick

10:30 am – 11:00 am **Refreshment Break** | *Stirling Hall Foyer*

Exhibits | *Stirling Hall Foyer*

Meet the Professors | *Stirling Salon IJK*

William Schwartz (mammals, SCN, circuits, social entrainment)

Ueli Schibler (mammals, tissue clocks, metabolism, molecular mechanisms)

Martha Merrow (entrainment and rhythms in humans and Neurospora, research transitions to Europe)

Johanna Meijer (mammals, SCN, circuits, light)

Samer Hattar (rodents, retina, ipRGC subtypes, photic changes in mood, sleep, learning)

Daniel Forger (modeling, SCN circuitry, molecular to electrical mechanisms)

Alec Davidson (mammals, SCN, immune clocks, pathology)

Steven Brown (mammals, molecular mechanisms, neural mechanisms, chronotype, sleep)

* = Merit Award Winner ** = Excellence Award Winner # = Diversity Travel Award Winner

11:00 am – 12:30 pm **Slide Session A:** ***Clocks, Feeding and Metabolism*** | *Stirling Ballroom East*
Chair: Richa Saxena, Massachusetts General Hospital

11:00 **SS1 • *Morning Circadian Misalignment During Insufficient Sleep is Associated With Changes in Plasma Metabolites Linked to Metabolic Dysregulation***
*Christopher Depner, University of Colorado Boulder

11:15 **SS2 • *A 5 Hour Delay in Meal Schedule Affects the Timing of the Human Circadian System***
*Skevoulla Christou, University of Surrey, UK

11:30 **SS3 • *Circadian Timing and Alignment in Healthy Adults: Associations With BMI, Body Fat, Caloric Intake and Physical Activity***
Kelly Baron, Feinberg School of Medicine, Northwestern University

11:45 **SS4 • *Natural Patterns of Food Intake Are a Weak Zeitgeber for the Liver***
Matthew Butler, Oregon Health & Science University

12:00 **SS5 • *Measuring the Physiological Cost of Circadian Desynchrony in Mammals***
David Bechtold, University of Manchester

12:15 **SS6 • *The Impact of Broad Spectrum Bright Light and Exogenous Melatonin on Plasma Glucose and Insulin in Healthy Male Participants***
Mohammed Albreiki, University of Surrey

11:00 am – 12:30 pm **Slide Session B:** ***Circadian Rhythms Across the Cell*** | *Stirling Ballroom West*
Chairs: Steven Brown, University of Zurich and Gad Asher, Weizmann Institute of Science, Israel

11:00 **SS7 • *Around the Clock Lipidomics: Insight Into Daily Oscillations in Subcellular Compartments***
***Philips Group Excellence Award* • Rona Aviram, Weizmann Institute of Science

11:15 **SS8 • *A Non-Classical Nuclear Import Pathway for Clock Proteins***
Achim Kramer, Charité - Universitätsmedizin Berlin

11:30 **SS9 • *Regulation of Second Messenger Pathways by Cryptochrome***
*Pagkapol Yhew Pongsawakul, University of California, San Diego

11:45 **SS10 • *Keeping Mitochondrial Network on Time***
Karen Schmitt, Neurobiology Lab for Brain Aging and Mental Health Transfaculty Research Platform, Molecular & Cognitive Neuroscience University of Basel

12:00 **SS11 • *BMAL1 Translation and Circadian Phenotypes in Mouse Models of Tuberous Sclerosis Complex***
Jonathan Lipton, Boston Children's Hospital, Harvard Medical School

12:15 **SS12 • *Some Rhythm, No Cry***
Marrit Putker, MRC Laboratory of Molecular Biology

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11:00 am – 12:30 pm **Slide Session C:** ***Light, Brain Function and Mental Health*** | *Stirling Salon OPQ*

Chair: Norman F. Ruby, Stanford University

11:00 **SS13 • *Independent Brain Circuits Mediate the Effects of Light on Mood and Learning***
**Vanda Pharma Excellence Award* • Diego Fernandez, Johns Hopkins University

11:15 **SS14 • *Melanopsin Regulates Both Sleep-Promoting and Arousal-Promoting Responses to Light***
Stuart Peirson, University of Oxford

11:30 **SS15 • *Light Modulates Spatial Learning and Memory in a Diurnal Rodent, the Nile Grass Rat (Arvicanthis Niloticus)***
#Joel Soler, Michigan State University

11:45 **SS16 • *Attention Deficits in Night Owls in the Morning***
Andrea Smit, Simon Fraser University

12:00 **SS17 • *Effects of Exercise Training on BMAL1 Knockout Mice***
*Sarah McLoughlin, University of Pennsylvania

12:15 **SS18 • *The Role of BMAL1 in Behavioral Responses to Pheromonal Stimuli***
*Erica Schoeller, University of California, San Diego

11:00 am – 12:30 pm **Slide Session D:** ***Temperature and Cellular Stress*** | *Stirling Salon LMN*

Chair: Leslie Griffith, Brandeis University

11:00 **SS19 • *A Calcitonin Receptor DH31R Regulates Temperature Preference Rhythm in Drosophila***
Fumika Hamada, Cincinnati Children's Hospital Medical Center

11:15 **SS20 • *Store-Operated Calcium Channels Stim and Orai Mediate Temperature Resetting of Circadian Clocks***
**Ron Konopka Excellence Award* • Ozgur Tataroglu, UMass Medical School

11:30 **SS21 • *Search for the Thermosensors Involved in Temperature Dependent Negative Masking Behavior in Mice***
Wataru Ota, Nagoya University

11:45 **SS22 • *Endogenous Temperature Cycles Impact the Formation of Pathological Aggregates***
*Bala Koritala, Institute of Medical Psychology, Ludwig Maximilians University, Munich

12:00 **SS23 • *Neurodegenerative Disease and Circadian Clock Dysfunction: Untangling the Role of Tauopathy***
Joshua Gamsby, USF Byrd Alzheimer's Institute

12:15 **SS24 • *The Chondrocyte Clock Gene BMAL1 Controls Cartilage Homeostasis and Integrity***
Qing-Jun Meng, University of Manchester

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12:45 pm – 1:45 pm **Lunchtime Table Discussions** | *Edinburgh Ballroom West*

Teaching of Chronobiology
Hosts: Luis Larrondo, Mary Harrington

Can We Distinguish Circadian Regulation From Sleep Regulation?
Hosts: Stuart Peirson, Kenneth Wright

Sex Differences in Biological Rhythms: How Extensive Are They? What Are the Challenges?
Hosts: Rae Silver, Francis Lévi

The Future for Chronobiology Research Funding
Host: Corinne Silva (NIDDK/NIH)

4:15 pm – 6:30 pm **Symposium 4: *SRS-SRBR Symposium: Sleep and Circadian Rhythms*** | *Stirling Ballroom East*
Chairs: Fred W. Turek, Northwestern University and Frank Scheer, Harvard Medical School

4:15 Introduction

4:30 ***Circadian Regulation of the Human Sleep-Wake Cycle: Some Recent Insights***
Derk Jan Dijk, University of Surrey

5:00 ***Sleep and Circadian Modulation of the Human Proteome***
Kenneth Wright, The University of Colorado Boulder

5:30 ***Clock Genes Regulate Cell Adhesion Molecules Shaping Sleep Amount and EEG***
Valérie Mongrain, Université de Montréal

6:00 ***Circadian Rhythms and Sleep in Drosophila***
Ravi Allada, Northwestern University

Symposium 5: *Circadian Rhythms in Natural Environments* | *Stirling Ballroom West*
Chair: Stacey Harmer, University of California, Davis

4:15 *Introduction*

4:30 ***Quantitative Variation in the Circadian Clock Confers Adaptation to Natural and Agricultural Settings***
Cynthia Weinig, University of Wyoming

5:00 ***Econeurogenetic Features of the Fly Circadian Clock***
Rodolfo Costa, University of Padova

5:30 ***In Search of Ancestral Sleep***
Horacio de la Iglesia, University of Washington

6:00 ***Foraging Activity Pattern is Shaped by Ecological Interactions and Water Loss Rates in a Diurnal Desert Rodent***
Noga Kronfeld-Schor, Tel Aviv University

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Symposium 6: ***Time Perception and Non-Circadian Timers*** | *Stirling Salon OPQ*
Chair: Diego Golombek, Universidad Nacional de Quilmes

4:15 Introduction

4:30 ***Regulation of Arousal Rhythms***
Kai-Florian Storch, McGill University

5:00 ***Functional and Neural Mechanisms of Interval Timing***
Warren Meck, Duke University

5:30 ***The Impact of the Moon on Bristle Worms and the Sun on Fish – The EMBO Young Investigator Lecture***
Kristin Tessmar-Raible, University of Vienna/ MFPL

6:00 ***Neuroendocrine Mechanisms Underpinning Long-Term Timing of Reproduction***
Valérie Simonneaux, CNRS INCI

8:00 pm – 8:30 pm **Datablitz I** | *Stirling Ballroom East*
Chair: #Adam Contreras, University of California, Davis

Temporal Restricted Feeding Induces Time-Dependent Behavioral Changes in Mice **(S15)**
*Victoria Acosta-Rodríguez

Individual Differences in the Rate of Re-Entrainment to a Phase Advance Predict Anxiety and Depression-Like Behavior **(S41)**
Jeff Anyan

Gravitational Loading at the Beginning of the Active Phase Attenuates Muscle Loss in Unloaded Mouse Hind Limb **(S58)**
*Shinya Aoyama

Beyond Body Weight: How Impaired Leptin Signaling Can Affect Sleep Disordered Breathing **(S94)**
Deanna Arble

Cryptochrome is a Direct Neuronal Ultraviolet Light Sensor **(S34)**
*Lisa Soyeon Baik

Circadian Misalignment and Risk-Taking in Night Shift Workers **(S21)**
*Philip Cheng

Tissue Specific Response of Clock Genes Expression in Peripheral Oscillators in a Rat Model of Shift-Work **(S71)**
*Cinthya Córdoba-Manilla

Aging Decreases Circadian Regulation of Alcohol Sensitivity and Increases Alcohol-Induced Tissue Injury and Mortality **(S38)**
*#Aliza De Nobrega

The Transcriptional Landscape Associated With Photoperiodism **(S93)**
*Laura Flavell

A Role for the Cationic Leak Channel NALCN in Daily Rhythms of Suprachiasmatic Nuclei Activity and Locomotor Behavior **(S102)**
Matt Flourakis

Abnormal PDF Expression Leads to Arrhythmicity in Vrille Mutants **(S11)**
Kushan Gunawardhana

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***Back to the Basics: A Simplified Model of Mammalian Circadian Rhythms* (S84)**
*Kevin Hannay

***ZeitZeiger: Supervised Learning for Oscillatory Data* (S98)**
*Jacob Hughey

***Endothelin-1 Regulates the Diurnal Variation of Sodium Excretion in Male and Female Rats* (S83)**
*#Jermaine Johnston

***Evaluation of Circadian Rhythms and Sleep in the APP/PS1 Mouse Model of Alzheimer's Disease* (S40)**
*Brianne Kent

***CRTC Potentiates Light-Independent timeless Transcription to Sustain Circadian Rhythms in Drosophila* (S6)**
*Mink Yung Kim

***Neuropeptide-F and Acetylcholine Mediate Photic Phase Resetting of Drosophila Circadian Behavior* (S7)**
*Pallavi Lamba

***Chronic Sleep Restriction Increases the Change in Systolic Blood Pressure Between Circadian Night and Day* (S113)**
Andrew McHill

***Photoperiod Interacts with Running Wheel Availability to Modulate Circadian Food Anticipatory Activity in Mice* (S14)**
**Mateusz Michalik

***Global and Hepatocyte-Specific Ablation of BMAL1 Induces Hyperlipidemia and Enhances Atherosclerosis* (S42)**
Xiaoyue Pan

***Nucleotide Variation in Drosophila cryptochrome Linked to Circadian Clock Function: An Association Analysis* (S12)**
Mirko Pegoraro

***Neural Correlates of Food Anticipatory Activity in Mice Subjected to Once or Twice-Daily Feeding Periods* (S16)**
**Ashutosh Rastogi

***Crosstalk Signaling Between Circadian Clock Components and Iron Metabolism* (S95)**
*Samuel Schiffhauer

***The Effect of Bmal1 Deletion in Gonadotropin-Releasing Hormone or Kisspeptin Neurons* (S91)**
*Karen Tonsfeldt

***Circadian Regulation in and by SCN Astrocytes* (S106)**
*Chak Foon Tso

***Time of Feeding Regulates Circadian Gene Expression in Mouse Peripheral Tissues* (S17)**
**Laura van Rosmalen

***The Relationship Between Light Exposure and Subsequent Sleep: What Happens Outside of the Lab?* (S30)**
*Emma Wams

8:30 pm – 10:30 pm **Poster Session I (S1 – S113)** | *Inverness Ballroom and Foyer*

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Monday, May 23

8:15 am – 10:30 am

Symposium 7: ***Epigenetics and Transcription Networks in Circadian Clocks*** | *Stirling Ballroom East*
Chair: Eva Wolf, IMB and University Mainz

8:15 Introduction

8:30 ***Competitive Mechanisms Control the Architecture of Circadian Regulatory Complexes***
Carrie Partch, University of California, Santa Cruz

9:00 ***Circadian Transcriptional Architecture in the Mouse***
Joseph Takahashi, University of Texas Southwestern

9:30 ***Structure/Function Analysis of WC-1 Reveals Mechanisms of Differential Activation of Light Versus Dark Regulation of Frequency and Clock-Controlled Genes***
Jennifer Loros, Geisel School of Medicine

10:00 ***Activation and Repression in Circadian Clock Networks***
Carl Troein, Lund University

Symposium 8: ***New Facets of Microbiology in Chronobiology: From Microbiota-Host Interactions to Natural Populations***|*Stirling Ballroom West*
Chair: Susan Golden, University of California

8:15 Introduction

8:30 ***Rhythmic Host-Microbe Signaling in Symbiosis***
Edward Ruby, Thomas Jefferson University Hospital

9:00 ***Microbiota and Circadian Rhythms***
Ali Keshavarzian, Rush University Medical Center

9:30 ***Insights From Bacterial Clocks to All Circadian Rhythms***
Carl Johnson, Vanderbilt University

10:00 ***New Perspectives on Frq-Less Rhythms in Neurospora***
Patricia Lakin-Thomas, York University

Symposium 9: ***Role of the Circadian System in Cardiovascular Health and Disease*** |*Stirling Salon OPQ*
Chair: Karen Gamble, The University of Alabama at Birmingham

8:15 Introduction

8:30 ***Role of the Circadian System in Cardiovascular Health and Disease in Humans***
Frank Scheer, Brigham and Women's Hospital, Harvard Medical School

9:00 ***Molecular Time. Consequences of Circadian Disturbances for Cardiovascular Health and Disease.***
Tami Martino, University of Guelph

9:30 ***Essential Roles of the Cardiomyocyte Circadian Clock***
Martin Young, The University of Alabama at Birmingham

10:00 ***Per1 and the Kidney Clock in the Regulation of Renal Sodium Transport***
Michelle Gumz, University of Florida

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10:30 am – 11:00 am **Refreshment Break** | *Stirling Hall Foyer*

Exhibits | *Stirling Hall Foyer*

Meet the Professors | *Stirling Salon IJK*

Kenneth Wright (humans, shift-work, metabolism, sleep)

Fred Turek (mammals, sleep, genetic mechanisms, seasonal rhythms, aging, metabolism)

Rae Silver (mammals, SCN, circuits, light)

Tanya Leise (modeling, circuits, mathematical approaches)

Achim Kramer (mammals, post-translational mechanisms, high throughput cell culture screens, immune clocks, CRIPSR/Cas9)

Carl Johnson (cyanobacteria, mammals, clock genes)

Vincent Cassone (avian clocks, photoentrainment, melatonin, bird song, GI clocks)

Ravi Allada (*Drosophila*, clock genes, genetics)

11:00 am – 12:30 pm **Slide Session E: *Clocks and Immunity*** | *Stirling Salon LMN*
Chair: Shigenobu Shibata, Waseda University

11:00 **SS25 • *Timing of Parasitic Helminth Infection is Critical in Determining Long-Term Adaptive Immune Responses***
**Thomas Hopwood, University of Manchester

11:15 **SS26 • *Achilles is a Circadian Clock Controlled Gene That Regulates Innate Immune Function in Drosophila***
Michael Hughes, UMSL

11:30 **SS27 • *Simulated Night Shift Disrupts Circadian Rhythms of Immune Functions in Humans***
Marc Cuesta, Douglas Mental Health University Institute, McGill University

11:45 **SS28 • *Characterization of the Circadian Control of Human Circulating Neutrophils***
Krisztina Ella, Semmelweis University

12:00 **SS29 • *Role of Inflammatory Signaling in the Mechanism by Which the Saturated Fatty Acid, Palmitate, Modulates Circadian Clock Properties***
***Reset Therapeutics Excellence Award* • Sam-Moon Kim, Texas A&M University

12:15 **SS30 • *A Novel Mechanism Links Inflammation to the Clock Through REV-ERBα Protein Stability***
**Marie Pariollaud, University of Manchester

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11:00 am – 12:30 pm **Slide Session F:** ***Post-Transcriptional Regulation in the Clock*** | *Stirling Ballroom East*
Chair: Seung-Hee Yoo, UT Health Science Center at Houston

11:00 **SS31 •** ***A Period2 Phosphoswitch Keeps the Beat in the Rising Heat***
Jae Kyoung Kim, Korea Advanced Institute of Science and Technology

11:15 **SS32 •** ***CNOT1 Promotes Phosphorylation of Mammalian Clock Proteins via PKA***
Guocun Huang, UT Southwestern Medical Center

11:30 **SS33 •** ***The E3-Ubiquitin Ligase Mdm2 Targets Period 2 for Degradation and Influences the Circadian Period Length***
Jingjing Liu, Virginia Tech

11:45 **SS34 •** ***Clock Transcription Factor CCA1 is Regulated Through Sumoylation***
Louise Hansen, University of Edinburgh

12:00 **SS35 •** ***Determining How CLK Promotes CYC Expression and Clock Function in Drosophila***
*Tianxin Liu, Texas A&M University

12:15 **SS36 •** ***Exploring the Connection Between Circadian Clock, Long Non-Coding RNA and Heterochromatin With Age***
Jinhee Park, Rutgers University

11:00 am – 12:30 pm **Slide Session G:** ***Photoreception and Physiology*** | *Stirling Salon OPQ*
Chair: Samer Hattar, Johns Hopkins University

11:00 **SS37 •** ***Rhodopsin 7 Reduces Light Sensitivity of the Eyes and Affects Circadian Photoreception in Fruit Flies***
Charlotte Helfrich-Förster, University Wuerzburg

11:15 **SS38 •** ***A Photoreceptor Clock is Required for Dorsal Suppression of S Opsin in the Mouse Retina***
Sujata Rao, Cleveland Clinic

11:30 **SS39 •** ***Opn5-Mediated Photoentrainment of Retinal Circadian Clocks***
Ethan Buhr, University of Washington

11:45 **SS40 •** ***Dichotomous Impact of Light Flashes on Circadian Phase Shifting and Melatonin Suppression in Humans***
Jamie Zeitzer, Stanford University

12:00 **SS41 •** ***Homeostatic Slow-Wave Sleep Response to Sleep Loss Depends on Age and Prior Light History***
Virginie Gabel, Centre for Chronobiology, Basel

12:15 **SS42 •** ***Probing Entrainment of Ostreococcus Tauri Circadian Clock by Green and Blue Light Through a Mathematical Modeling Approach***
Marc Lefranc, University of Lille

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11:00 am – 12:30 pm **Slide Session H: *Neurotransmitters, Channels and Neuronal Networks*** | *Stirling Ballroom West*
Chair: *Hugh Piggins, University of Manchester*

11:00 **SS43 • *Calcium Circadian Rhythmicity in the Suprachiasmatic Nucleus: Cell Autonomy and Network Reinforcement***
Takako Noguchi, University of California, San Diego

11:15 **SS44 • *Inferring the Functional Resynchronization Network in the Suprachiasmatic Nucleus***
**John Abel, Harvard University

11:30 **SS45 • *Inhibiting Matrix Metalloproteinases 2 and 9 Alters Circadian Neuronal Firing Patterns in the Suprachiasmatic Nucleus***
*Kathryn Abrahamsson, University of Tennessee

11:45 **SS46 • *Glial-Neuronal Signalling Controls Circuit-Level Coupling in the Suprachiasmatic Nucleus***
Marco Brancaccio, MRC Laboratory of Molecular Biology- Division of Neurobiology

12:00 **SS47 • *SCN Neurons of Cryptochrome-Deficient Mice Lack Circadian Timing in Intrinsic Excitability States and Do Not Gate Responses to Excitatory Input***
Mino Belle, University of Manchester

12:15 **SS48 • *BK Channel Inactivation Regulates Daytime SCN Excitability, Circuit and Behavioral Rhythmicity***
Andrea Meredith, University of Maryland School of Medicine

12:45 pm – 1:45 pm **Lunchtime Table Discussions** | *Edinburgh Ballroom West*

Is There Anything Left to Learn About the Circadian Timekeeping Mechanism?
Hosts: Seung-hee Yoo, Mary Cheng

Evolution of Circadian Clocks: When, How and Why Did Clock Arise?
Hosts: Eran Tauber, Cynthia Weinig

The Future for Chronobiology Research Funding – Table 1
Host: Janet He (NINDS/NIH)

The Future for Chronobiology Research Funding – Table 2
Host: Michael Sesma (NIGMS/NIH)

2:00 pm – 3:00 pm **JBR Editors Meeting, SAGE Publishers** | *Stirling Salon DEF*

3:15 pm – 4:15 pm **Workshop I** | ***Is it Possible to Translate Chronobiology Findings to Real Life, Health and Society?*** | *Stirling Ballroom*
Chair: Ellen Frank, University of Pittsburgh School of Medicine

Panelists: Francis Lévi, University of Warwick
Till Roenneberg, Institute for Medical Psychology
Eva Schernhammer, Harvard Medical School
Christopher Winrow, Merck Research Laboratories
Phyllis Zee, Northwestern University

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4:30 pm – 6:30 pm **Presidential Symposium:** ***Circuits, Genes and Behavior*** | *Stirling Ballroom*

Circuits, Genes and Behaviour: A View from the SCN
Michael Hastings, MRC Laboratory of Molecular Biology

The Circadian Brain Network and Behavior in Drosophila
Michael Rosbash, Brandeis University

8:00 pm – 8:30 pm **Datablitz II** | *Stirling Ballroom East*
Chair: Roelof Hut, University of Groningen

The MYC Oncogene Disrupts Circadian Rhythm and Metabolism in Cancer Through Modulation of REV-ERB and BMAL1 **(M2)**
*Brian Altman

Cold-Induced Period Transcription Links Environmental Temperature to the Drosophila Molecular Clock **(M12)**
**Akanksha Bafna

Genome-Wide Characterization of the Molecular Response of the Circadian Clockwork to Temperature in Drosophila **(M8)**
*Naveh Evantal

Diel Flight Activity Behavior of Wild Caught Anopheles farauti s.s and An. hinesorum Malaria Mosquitoes From Northern Queensland, Australia: Temporal Differences that Might Contribute to Speciation **(M98)**
*Gary George

Vasopressin Mediates Clock-Driven Anticipatory Thirst **(M105)**
*Claire Gizowski

Exploring Physiological Changes Underlying Protection from Severe Sleep Restriction in Migrating Birds **(M89)**
*William Horton

Circadian Rhythms in Actin Dynamics and Wound Healing **(M91)**
Ned Hoyle

24 H Metabolic Profiling in Obesity and Type 2 Diabetes (T2DM) **(M47)**
*Cheryl Isherwood

Circadian Regulation of Xenobiotic Metabolism **(M46)**
*Anna Kriebs

Diapause in Drosophila melanogaster **(M11)**
*Ane Martin Anduaga

Clock Genes Regulate Circadian Gating of Parturition and Gestation Length **(M4)**
*Carmel Martin-Fairey

4C-Seq in Mouse Liver Reveals Clock-Dependent Rhythmic Chromatin Contacts **(M50)**
*Jérôme Mermet

Molecular Description of the Poised CRY:CLOCK:BMAL1 Repressive Complex **(M51)**
*Alicia Michael

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Chloride Cotransporter KCC2 Essential for GABAergic Hyperpolarization in the SCN **(M101)**
Anneke Olde Engberink

Regulation of Mitochondrial Dynamics by the Circadian Deadenylase Nocturnin **(M72)**
*Yasemin Onder

CRY Acts as a Cofactor for the SCF-FBXL3 Mediated Degradation of Novel Substrates **(M53)**
*Stephanie Papp

Integration of Light Intensity Information Into the Clock Neuron Network of Drosophila melanogaster **(M6)**
*Matthias Schlichting

Ultradian Feeding in Mice Not Only Affects the Peripheral Clock in the Liver, But Also the Master Clock in the Brain **(M90)**
Satish Sen

Circadian Profiles of Light, Activity, and Body Temperature for Non-Invasive Physiology Prediction in Humans **(M22)**
*Benjamin Smarr

Regulation of the Mammalian Circadian Clock Transcriptional Output by CLOCK:BMAL1 **(M52)**
Alexandra Trott

The Effects of Circadian Misalignment During Adolescence on Mood and Alcohol Sensitivity **(M37)**
*Chelsea Vadnie

Trends in Self-Reported Hourly Lighting and Sleep in a Global Dataset of Travelers **(M21)**
Olivia Walch

Per1: Venus Arcuate Neurons Exhibit Robust Rhythms in Excitability **(M61)**
*Adam Watson

Circadian Translational Profiling of the Drosophila Head Fat Body Reveals Potential Novel Roles for a Peripheral Oscillator **(M10)**
*Amy Yu

Circadian Clock Control by Polyamine Levels Through a Mechanism That Declines with Age **(M96)**
*Ziv Zwighaft

8:30 pm – 10:30 pm **Poster Session II (M1 – M112)** | *Inverness Ballroom and Foyer*

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Tuesday, May 24

8:15 am – 10:30 am

Symposium 10: ***Biological Rhythms in Immune Responses and Infectious Diseases*** | *Stirling Ballroom East*
Chair: Andrew Loudon, University of Manchester

8:15 Introduction

8:30 ***How Immune Cell Clocks Regulate Inflammatory Responses***
Julie Gibbs, University of Manchester

9:00 ***Circadian Rhythms in Leukocyte Migration***
Christoph Scheiermann, Ludwig-Maximilians-University Munich

9:30 ***Circadian Modulation of the Innate and Adaptive Immune Response***
Ruud Buijs, Institute for Biomedical Research

10:00 ***Circadian Regulation of Allergic Reaction***
Atsuhito Nakao, University of Yamanashi

Symposium 11: ***Systems Chronobiology*** | *Stirling Ballroom West*
Chair: Felix Naef, EPFL

8:15 Introduction

8:30 ***Transcriptional Response of Neurospora to Light Cues***
Michael Brunner, Heidelberg University Biochemisty Center

9:00 ***The Rhythmic Transcriptome in Tissues of Aging Mice***
Pål Westermark, Charite-Universitatsmedizin Berlin

9:30 ***Time for Precision Medicine: From Big Data to Improved Therapeutics***
John Hogenesch, University of Cincinnati College of Medicine

10:00 ***Orchestration of Liver Proteome by Circadian and Feeding Rhythms***
Frédéric Gachon, Nestlé Institute of Health Sciences

Symposium 12: ***Rhythms Over the Lifespan*** | *Stirling Salon OPQ*
Chair: Elizabeth Klerman, Brigham and Women's Hospital

8:15 Introduction

8:30 ***Circadian Rhythms in Older Adults***
Jeanne Duffy, Brigham & Women's Hospital, Harvard Medical School

9:00 ***The Adolescent Central Circadian Clock and Its Response to Bright Light***
Stephanie Crowley, Rush University Medical Center

9:30 ***Entrainment of the Circadian Clocks During Early Developmental Stages***
Alena Sumova, Institute of Physiology, Czech Academy of Sciences

10:00 ***Circadian Rhythms in Long-Living Naked Mole Rat***
Roman Kondratov, Cleveland State University

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10:30 am – 11:00 am **Refreshment Break** | *Stirling Hall Foyer*

Exhibits | *Stirling Hall Foyer*

Meet the Professors | *Stirling Salon IJK*

Phyllis Zee (humans, translational-clinical rhythms, sleep)

Debra Skene (humans, aging, treatment of circadian disruption, light, melatonin)

Frank Scheer (humans, clinical rhythms, and sleep)

Till Roenneberg (sleep, chronotypes, entrainment, *Neurospora*)

Hugh Piggins (mammals, SCN, circuits, neuropeptide signaling, electrophysiology)

Satchin Panda (mammals, SCN, light, clock genes, tissue clocks, feeding rhythms)

Michael Hastings (rodents, SCN, molecular mechanisms, circuits)

Patrick Emery (*Drosophila*, behavioral genetics, light and temperature entrainment, circuitry)

11:00 am – 12:30 pm **Slide Session I: *Consequences of Circadian Disturbance*** | *Stirling Ballroom East*

Chair: Carolina Escobar, Universidad Nacional Autónoma de México

11:00 **SS49 • *"Of Islands and Pancakes": A Novel Method to Quantify and Visualize Mistimed Rhythms***
Dorothee Fischer, Harvard T.H and Chan School of Public Health

11:15 **SS50 • *Developmental Origin of Health and Disease (DOHaD) and the Circadian Clock: Later Life Health Effects of Gestational Circadian Rhythm Disturbance in Mice***
Gijsbertus van der Horst, University Medical Center

11:30 **SS51 • *Metabolic Consequences of Internal Desynchrony***
*Vincent van der Vinne, Umass Med School

11:45 **SS52 • *Circadian Rhythm De-Synchronization Exacerbates Pathological Outcomes in an Animal Model of Ischemic Stroke***
David Earnest, Texas A&M University Health Science Center

12:00 **SS53 • *Night Shift Work Disrupts Fractal Regulation of Human Motor Activity***
Kun Hu, Brigham & Women's Hospital/Harvard Medical School

12:15 **SS54 • *Unravelling the Mechanisms of Chronic Circadian Rhythm Disturbance Using Transcriptomics and Metabolomics Approaches***
Linda van Kerkhof, National Institute for Public Health and the Environment

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11:00 am – 12:30 pm **Slide Session J:** ***Evolution, Synthetic Biology, Environment and Circadian Clocks*** | *Stirling Salon LMN*
Chair: Charalambos Kyriacou, University of Leicester

11:00 **SS55 • *A Tunable Artificial Circadian Clock in Clock-Defective Mice***
Choogon Lee, Florida State University

11:15 **SS56 • *Circadian and Infradian Clocks in the Urochordate Botryllus schlosseri***
Rachel Ben-Shlomo, University of Haifa - Oranim

11:30 **SS57 • *Pollutant Affects on the Circadian Rhythm of Daphnia pulicaria***
Jennifer Hurley, Renssealer Polytechnic Institute

11:45 **SS58 • *New Insights Into the Genetics of Diurnal/Nocturnal Preference***
Eran Tauber, University of Leicester

12:00 **SS59 • *Codon Usage Affects Drosophila Period Protein Structure and Function***
Jingjing Fu, UT Southwestern Medical Center

12:15 **SS60 • *Circadian Genes, Photoperiodic Clock and Diapause in Insect, Pyrrhocoris apterus***
David Dolezel, Institute of Entomology

11:00 am – 12:30 pm **Slide Session K:** ***Clocks and Neuropeptides*** | *Stirling Salon OPQ*
Chair: Christopher Colwell, UCLA

11:00 **SS61 • *Synchronous Drosophila Circadian Pacemakers Display Non-Synchronous Ca2+ Rhythms in Vivo***
***Ron Konopka Excellence Award* • Xitong Liang, Washington University in St. Louis

11:15 **SS62 • *The Small GTPase RHO1 is Required in a Dosage-Dependent Manner to Align Peptidergic Control of Behavioural Rhythms With Clock-Controlled Gene Expression***
Miguel Ramírez Moreno, University of Southampton

11:30 **SS63 • *Reciprocal Communications of Clock Neurons via PDF and CCHa1 Neuropeptides in Drosophila***
Taishi Yoshii, Okayama University

11:45 **SS64 • *The PTTH Neuropeptide Couples Central and Peripheral Clocks in Drosophila***
John Ewer, CINV, Universidad de Valparaiso

12:00 **SS65 • *Decoding the Firing Patterns of SCN Vip Neurons***
*Cristina Mazuski, Washington University in St. Louis

12:15 **SS66 • *Doublecortin-Like Regulates Circadian Rhythms of Locomotor Activity by Controlling Vasopressin Signaling in the Suprachiasmatic Nucleus***
Erno Vreugdenhil, Leiden University Medical Center

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11:00 am – 12:30 pm **Slide Session L: *Sleep*** | *Stirling Ballroom West*
Chair: Martha Vitaterna, Northwestern University

11:00 **SS67 • *Cerebral Underpinnings of Human Circadian Performance Modulations During Sleep Loss***
Christian Cajochen, Centre for Chronobiology, Psychiatric Hospital of the University of Basel

11:15 **SS68 • *Social Regulation of Naturally Occurring Plasticity in Sleep and Circadian Rhythms in Bees***
Guy Bloch, Hebrew University of Jerusalem

11:30 **SS69 • *Light-Dependent Regulation of Sleep/Wake States by Prokineticin 2 in Zebrafish***
David Prober, California Institute of Technology

11:45 **SS70 • *The Lateral Line Confers Evolutionarily Derived Sleep Loss in the Mexican Cavefish***
Alex Keene, Florida Atlantic University

12:00 **SS71 • *Sexually Dimorphic Regulation of Sleep in Drosophila***
Kyunghee Koh, Thomas Jefferson University

12:15 **SS72 • *Dissection of the Downstream Circadian Circuitry Involved in Sleep Regulation***
Fang Guo, HHMI/Brandeis University

12:45 pm – 1:45 pm **Lunchtime Table Discussions** | *Edinburgh Ballroom West*

Circadian Outreach Strategies: How to Disseminate Chronobiology Knowledge to the Public and Medical Doctors?
Hosts: David Welsh, Susan Golden, Martha Merrow

Have We Forgotten Nonphotic Entrainment?
Hosts: Eric Mintz, Debra Skene

The Future for Chronobiology Research Funding – Table 1
Host: Janet He (NINDS/NIH)

The Future for Chronobiology Research Funding – Table 2
Host: Michael Sesma (NIGMS/NIH)

12:45 pm – 2:45 pm **SRBR Board of Directors Meeting** | *Stirling Salon DEF*

3:15 pm – 4:15 pm **Workshop II** | ***Big Data Sets: How Useful Are They and How to Mine for Gold?*** | *Stirling Ballroom East*
Chair: John Hogenesch, University of Cincinnati College of Medicine

Panelists: Michael Hughes, UMSL
Tami Martino, University of Guelph
David Rand, University of Warwick
Debra J. Skene, University of Surrey
Joseph Takahashi, University of Texas Southwestern/Howard Hughes Medical Institute

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4:15 pm – 6:30 pm

Symposium 13: ***Neuronal Networks and Central Clock Function*** | *Stirling Ballroom East*

Chair: Patrick Emery, University of Massachusetts Medical School

4:15 Introduction

4:30 ***Reciprocal Interactions Between Behaviour and SCN Electrical Activity***
Johanna Meijer, Leiden University Medical Center

5:00 ***Assembling a Clock System: Ontogeny of Circadian Synchrony in the SCN***
Erik Herzog, Washington University

5:30 ***Beyond Simple Timekeeping in the SCN***
Daniel Forger, University of Michigan

6:00 ***Physiological Effects of Temperature on a Circadian Clock Neuron Network***
Orie Shafer, University of Michigan

Symposium 14: ***Circadian Rhythms in Metabolism, Diabetes and Obesity*** |*Stirling Ballroom West*

Chair: Frank Scheer, Brigham and Women's Hospital, Harvard Medical School

4:15 Introduction

4:30 ***Behavioral and Dietary Chronotype: Predictors and Metabolic Consequences***
Kristen Knutson, University of Chicago

5:00 ***Obesity and the Clocks: Are We Predestinated?***
Marta Garaulet, Universidad de Murcia

5:30 ***Time-Restricted Feeding Imparts Pleiotropic Effects on Multiple Organs***
Satchidananda Panda, Salk Institute for Biological Studies

6:00 ***A Clock Mediated Trade-Off Between Growth and Starvation Tolerance in Cyanobacteria***
Michael Rust, University of Chicago

Symposium 15: ***Non-Traditional Models: What Do They Teach Us About Biological Rhythms?*** | *Stirling Salon OPQ*

Chair: Nicholas Foulkes, Karlsruhe Institute of Technology

4:15 Introduction

4:30 ***Monarch Butterfly CRYPTOCHROME 2 Represses Circadian Transcription Through BMAL1 C-Terminal Domain***
Christine Merlin, Texas A&M University

5:00 ***From Genome to Function: Timing Adaptations in the Intertidal Insect Clunio Marinus***
Tobias Kaiser, Center for Integrative Bioinformatics / Max F Perutz Laboratories

5:30 ***Diel and Circadian Timing in the Anopheles gambiae Malaria Mosquito***
Giles Duffield, University of Notre Dame

6:00 ***Life at Extremes: Circadian Clocks in the Dark and Cold***
Cristiano Bertolucci, University of Ferrara

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8:00 pm – 8:30 pm **Datablitz III** | *Stirling Ballroom East*

Chairs: Lisa Soyeon Baik, University of California Irvine and Yong Zhang, University of Nevada Reno

Lhx1-Regulated Transcriptional Networks Control Sleep/Wake Coupling and Thermal Resistance of the SCN Clockworks **(T103)**
*Joseph Bedont

Comparison of the Circadian Clock of Social and Solitary Bees **(T78)**
*Katharina Beer

Translation Across Time and Space **(T88)**
Violeta Castelo-Szekely

Circadian Clock Regulation of mRNA Translation Through the Eukaryotic Elongation Factor eEF-2 **(T55)**
Stephen Caster

Hepatic miRNA Loss Resulted in Altered Adaptation to Food Restriction in Mice **(T93)**
**Ngoc-Hien DU

A Functional Synthetic Hybrid Circadian Oscillator Generated Through Transcriptional Rewiring **(T53)**
*#Alejandra Goity

A Systems-Driven Experimental Approach Reveals the Complex Regulatory Distribution of p53 by Circadian Factors **(T96)**
Tetsuya Gotoh

Using Signal Processing to Explore Diversity: Analyses of Locomotor Activity and Core Body Temperature Reveal Sex Differences in Mice **(T82)**
*Azure Grant

A Piece of Chocolate in the Dark Phase Prevents Circadian Desyncrhony and Overweight in Male Shift-Worker Rats **(T15)**
#Mara Guzman-Ruiz

Novel Transcriptional Mechanisms of Muscle-Specific Clock Output **(T56)**
*Brian Hodge

Integrative Analysis of Multiple Genomics Datasets Reveals Key Networks and Pathways Underlying the Circadian and Homeostatic Regulation of Sleep **(T110)**
*Peng Jiang

TNF Signaling Regulates the Circadian Rhythm of Myogenic Responsiveness and Systemic Blood Pressure **(T83)**
*Jeff Kroetsch

Selective Inhibition of Casein Kinase I Delta Enhances Hippocampal Dependent Learning and Alters Expression of Circadian Clock Proteins in the Hippocampus **(T36)**
*Heather Mahoney

Ion Channels that Regulate Neuronal Physiology and Circadian Behavior in Drosophila melanogaster **(T7)**
Nara Ines Muraro

Circadian Control of CD8+ T Cell Response **(T28)**
*Chloé Nobis

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***Phase-Angle Differences Between Dim-Light Melatonin Onset and Sleep Onset in Patients Diagnosed With Delayed Sleep Phase Syndrome* (T22)**
*Catia Reis

***An Evolutionary Hotspot in CRYPTOCHROME's Structure Tunes the Period of the Mammalian Circadian Rhythm* (T52)**
*Clark Rosensweig

***Mice Are Able to Acquire Multiple Independent Time Memories* (T75)**
*Choden Shrestha

***Sleep and Circadian Regulation of Metabolic Rate in Drosophila* (T98)**
*Melissa Slocumb

***Transcriptional Regulatory Logic of the Diurnal Cycle in the Mouse Liver* (T77)**
*Jonathan Sobel

***Entrainment Ability of the Peripheral Circadian Clocks by Light, Food, Stress, and Exercise in Aged Mice* (T97)**
*Yu Tahara

***Differential Roles for Mammalian Cryptochromes in the Retinal Circadian Clock* (T32)**
*Jovi Wong

***Circadian Clocks Modulate Huntington's Disease via Stress Response Pathways* (T35)**
*Fangke Xu

***BMAL1 Deletion in Adulthood Facilitates Adaptation to Disrupted Light/Dark Schedules in Mice* (T87)**
Guangrui Yang

***Transgenerational Epigenetic Effects of Cocaine on Circadian Behavior and Cocaine Reward* (T40)**
*Alexandra Yaw

***Circadian Rhythm of Redox State in Hippocampal CA1 Regulates Neuronal Membrane Excitability* (T58)**
*Mia Yu

***A Dissociation Between Diurnal Cycles in Locomotor Activity, Feeding Behavior and Hepatic PERIOD2 Expression in Chronic Alcohol-Fed Mice* (T65)**
*Peng Zhou

8:30 pm – 10:30 pm **Poster Session III (T1 – T113)** | *Inverness Ballroom and Foyer*

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Wednesday, May 25

8:15 am – 10:30 am

Symposium 16: ***Post-Transcriptional/Translational Circadian Mechanisms*** | *Stirling Ballroom East*
Chair: Eun Young Kim, Ajou University School of Medicine

8:15 Introduction

8:30 ***Rhythmic Post-Transcriptional Control Mechanisms***
Carla Green, UT Southwestern Medical Center

8:55 ***Post-Transcriptional Modification Regulates Clock Oscillation and Extends mRNA Rhythms***
Yoshitaka Fukada, School of Science,The University of Tokyo

9:20 ***Alternative Splicing and Post-Transcriptional Regulation of Timeless mRNA is Essential for the Adaptation of the Circadian System to Temperature in Drosophila***
Sebastian Kadener, The Hebrew University of Jerusalem

9:45 ***Atomic-Resolution Mechanism of the Cyanobacterial Circadian Clock***
Andy LiWang, University of California, Merced

10:10 ***Novel Elements Effecting the Circadian Oscillator and Output in Neurospora***
Jay Dunlap, Geisel School of Medicine at Dartmouth

Symposium 17: ***Non-Visual Effects of Light and Other Zeitgebers*** | *Stirling Ballroom West*
Chair: Claude Gronfier, Inserm, Université Claude Bernard

8:15 Introduction

8:30 ***Visual Information Reaching the Mouse SCN***
Robert Lucas, University of Manchester

8:55 ***Non-Canonical Light Signalling Contributes to Drosophila Circadian Clock Entrainment***
Ralf Stanewsky, University of London, University College London

9:20 ***Circadian Rhythms and Light Response in Humans***
Charles Czeisler, Brigham & Women's Hospital

9:45 ***Chemical Integration of Circadian and Photoperiodic Clocks in Plants***
Brian Zoltowski, Southern Methodist Univerity

10:10 ***The Role of Pseudo-Response Regulators in Maintaining Cyclic Gene Expression in Arabidopsis***
Eva Farre, Michigan State University

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Symposium 18: ***Circadian Rhythms in the Context of Addiction, Mood and Neurodegenerative Disorders*** | *Stirling Salon OPQ*
Chair: Colleen McClung, University of Pittsburgh

8:15 Introduction

8:30 ***Ethanol-Induced Plasticity in the SCN***
Rebecca Prosser, University of Tennessee Knoxville

8:55 ***Late Life Cyclers: The Old Clock That Could***
Jadwiga Giebultowicz, Oregon State University

9:20 ***Evidence for Circadian Modulation of Reward in Humans and Its Relevance to Adolescent Substance Abuse***
Brant Hasler, University of Pittsburgh School of Medicine

9:45 ***Circadian Clocks in Fibroblast and Mouse Models of Mood Disorders***
David Welsh, University of California, San Diego

10:10 ***Sleep and Circadian Rhythm Characteristics Across the Psychosis Spectrum***
Katharina Wulff, University of Oxford

10:30 am – 11:00 am **Refreshment Break** | *Stirling Hall Foyer*

Exhibits | *Stirling Hall Foyer*

Meet the Professors | *Stirling Salon IJK*

Erik Herzog (rodents, SCN, astrocytes, neuronal circuits)

David Welsh (single cells, SCN, bioluminescence imaging)

Joseph Takahashi (mouse genetics, clock genes, SCN circuits)

Colleen McClung (rodents, role of clock mechanisms in neuropsychiatric disease)

Robert Lucas (retina, entrainment, responses to light)

Green Carla (rodents, clock output, metabolism, post-transcriptional mechanisms)

Charles Czeisler (humans, sleep and circadian rhythms)

Susan Golden (cyanobacteria, clock genes, functional genomics, biofuels)

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11:00 am – 12:30 pm **Slide Session M:** ***Micro-Organisms, Cancer and Cell Cycle*** | *Stirling Ballroom West*
Chair: Carla Finkielstein, Virginia Polytechnic Institute and State University

11:00 **SS73 • *A Human Gut Bacterium Express Circadian Rhythms and Swarming Response to Melatonin***
***Condor Instrument Excellence Award* • Jiffin Paulose, University of Kentucky

11:15 **SS74 • *Trypanosoma Brucei Infection Accelerates the Mouse Circadian Clock***
*Filipa Rijo-Ferreira, UT Southwestern / IMM

11:30 **SS75 • *Activating Circadian Clock Function in Cancer Cells Inhibits Tumor Growth***
Silke Kiessling, McGill / Douglas Mental Health University Institute

11:45 **SS76 • *Real-Time Bioluminescence Reporters of Circadian Rhythms and Signaling Pathways in Solid Tumours in Vitro and in Vivo***
Robert Dallmann, University of Warwick

12:00 **SS77 • *Intercellular Coupling of Cell Cycle and Circadian Clock in Adult Stem Cell Cultures***
Toru Matsu-ura, University of Cincinnati

12:15 **SS78 • *Cry2 and Fbxl3 Promote Circadian Destruction of c-Myc***
Katja Lamia, The Scripps Research Institute

11:00 am – 12:30 pm **Slide Session N:** ***Clock Outputs*** | *Stirling Ballroom East*
Chair: Han Wang, Soochow University

11:00 **SS79 • *Loss of ZBTB20 Causes Unimodal Behavioral Rhythms and Impairs Circadian Output***
Ying Xu, Nanjing University to Soochow University

11:15 **SS80 • *How Does the Mammalian Circadian Clock Generate Tissue-Specific Rhythmic Outputs?***
Joshua Beytebiere, Texas A&M University

11:30 **SS81 • *Integrating Functional Genomics Data Reveals Tissue-Dependent Mechanisms Underlying Circadian Gene Expression***
*Jake Yeung, EPFL

11:45 **SS82 • *Mining for Novel Candidate Clock Genes in the Circadian Regulatory Network***
Bharath Ananthasubramaniam, Charite Universitaetsmedizin Berlin

12:00 **SS83 • *Transcriptional Variation Across SCN Subregions***
Eric Mintz, Kent State University

12:15 **SS84 • *Altered Bile Acid Dynamics in Mice Lacking Nocturnin***
***DSI Excellence Award* • Jeremy Stubblefield, UT Southwestern Medical Center

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11:00 am – 12:30 pm **Slide Session O:** ***Light and Neuronal Networks*** | *Stirling Salon OPQ*
Chair: François Rouyer, CNRS

11:00 **SS85 •** ***Drosophila Clockwork Dynamics: Functional Contributions of Strong and Weak Neuronal Oscillators to Circadian Synchrony and Light Response***
Todd Holmes, University of California at Irvine School of Medicine

11:15 **SS86 •** ***Dual-Mode Control of Network Flexibility in the Drosophila Clock Circuit***
Abhishek Chatterjee, NeuroPSI, CNRS UMR-9197

11:30 **SS87 •** ***Optogenetic Investigation of SCN Communication and Photoperiodicity***
*Michael Tackenberg, Vanderbilt University

11:45 **SS88 •** ***Polarity of GABAA Signaling Influences the Dynamics of SCN Coupling***
Jennifer Evans, Marquette University

12:00 **SS89 •** ***Geniculohypothalamic GABAergic Signalling Modulates Suprachiasmatic Nuclei Responses to Retinal Input***
Lydia Hanna, University of Manchester

12:15 **SS90 •** ***Atypical Opsins in Photoentrainment and Development***
Richard Lang, Cincinnati Children's Hospital Medical Center

11:00 am – 12:30 pm **Slide Session P:** ***Human Health, Behavior and Society*** | *Stirling Salon LMN*
Chair: Phyllis Zee, Northwestern University

11:00 **SS91 •** ***Genome-Wide Association Analysis and Functional Follow-Up Identifies Novel Loci for Chronotype in 100,420 Individuals From the UK Biobank***
*Jacqueline Lane, Massachusetts General Hospital

11:15 **SS92 •** ***Differential DNA Methylation at Circadian Clock (Related) Gene Loci in Pre-Eclampsia***
Inês Chaves, Erasmus MC Rotterdam

11:30 **SS93 •** ***Ultradian Rhythms of Locomotor (In)Activity in a Real-World Sample of 120,000 Hours of Human Sleep***
Eva Winnebeck, Ludwig Maximilian University, Munich

11:45 **SS94 •** ***The Effect of Chronotype and Time of Year on School Attendance and Performance***
Giulia Zerbini, University of Groningen

12:00 **SS95 •** ***Sleep Hygiene and Academic Performance in College Undergraduates***
Gideon Dunster, University of Washington

12:15 **SS96 •** ***Long Weekly Work Hours Increase the Risk of Adverse Health and Safety Outcomes in First-Year and More Experienced Resident Physicians***
*Celine Vetter, Brigham and Women's Hospital and Harvard Medical School

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12:45 pm – 1:45 pm **Lunchtime Table Discussions** | *Edinburgh Ballroom West*

Melatonin: An Important Player too Often Overlooked in our B6 Mouse-Centric Research World?
Hosts: Vincent Cassone, Takashi Yoshimura

Human Chronotyping: How to Do It? Is It Relevant in Diseased Individuals as Well?
Hosts: Till Roenneberg, Martin Ralph

The Future for Chronobiology Research Funding
Host: Corinne Silva (NIDDK/NIH)

1:15 pm – 2:15 pm **Actigraphy Workshop**|*Stirling Salon DEF*
Organized by Condor Instruments
Chair: Till Roenneberg, Ludwig MaxMillian University

2:30 pm – 3:30 pm **General Meeting of SRBR Members** | *Stirling Ballroom*

3:30 pm – 4:30 pm **Workshop III** | ***Are Circadian Clocks Therapeutic Targets?*** | *Stirling Ballroom*
Chair: Thomas Burris, Saint Louis University School of Medicine

Panelists: Diane B. Boivin, Douglas Mental Health University Institute, McGill University
Zheng (Jake) Chen, UT Health Science Center at Houston
Steve Kay, The Scripps Research Institute
Colleen McClung, University of Pittsburgh
Travis Wager, Pfizer

4:30 pm – 6:00 pm **Pittendrigh/Aschoff Lecture** | ***The Time of Our (Cyanobacterial) Lives: elucidating the Kai oscillator*** | *Stirling Ballroom*
Susan Golden, University of California, San Diego

6:15 pm – 7:30 pm **Cocktail Reception** (Cash Bar) | *Inverness Ballroom Foyer*

7:30 pm **Closing Banquet and Awards Ceremony** | *Inverness Ballroom*

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Poster Titles

Sunday, May 22

S1 **Constant Light Promotes Tumor Development via Insulin Resistance and Altered Inflammatory Response** • Natalí N Guerrero-Vargas, Facultad de Medicina, Universidad Nacional Autónoma de México

S2 **Circadian Rhythm of Proteins in Breast Cancer Tissue Cultured Cells** • Sean-Patrick Scott, Tecnologico de Monterrey

S3 **Disruption of the Cardiomyocyte Circadian Clock Influences Myocardial Insulin Signaling** • Graham McGinnis, The University of Alabama at Birmingham

S4 #**Environmental Circadian Disruption Increases Ischemic Brain Damage** • Anne Ramsey, Morehouse School of Medicine

S5 **Developmental Regulation of the Narrow Abdomen Ion Channel in the *Drosophila* Circadian Pacemaker.** • Bridget Lear, University of Iowa

S6 ***CRTC Potentiates Light-Independent Timeless Transcription to Sustain Circadian Rhythms in *Drosophila*** • MinkYung Kim, KAIST

S7 ***Neuropeptide-F and Acetylcholine Mediate Photic Phase Resetting of *Drosophila* Circadian Behavior** • Pallavi Lamba, University of Massachusetts Medical School

S8 **Grooming Behavior of *Drosophila* is Under Circadian Regulation** • Bing Qiao, University of Miami

S9 **Igf-li mRNA-Binding Protein Regulates Night Sleep in *Drosophila*** • Xueyan Pang, University of Nevada

S10 **Light-Induced Plasticity of *Drosophila* Clock Function** • Charles Hurdle, University of Southampton

S11 **Abnormal PDF Expression Leads to Arrhythmicity in Vrille Mutants** • Kushan Gunawardhana, Texas A&M University

S12 **Nucleotide Variation in *Drosophila* Cryptochrome Linked to Circadian Clock Function: An Association Analysis.** • Mirko Pegoraro, University of Leicester

S13 **Identification and characterization of Genes Controlling Development of PDF-Positive Clock Neurons in the Fruit Fly *Drosophila melanogaster*** • Outa Uryu, Faculty of Life and Environmental Sciences, University of Tsukuba

S14 ****Photoperiod Interacts With Running Wheel Availability to Modulate Circadian Food Anticipatory Activity in Mice** • Mateusz Michalik, Simon Fraser University

S15 ***Temporal Restricted Feeding Induces Time-Dependent Behavioral Changes in Mice** • Victoria Acosta-Rodríguez, UTSW Medical Center Dallas

S16 ****Neural Correlates of Food Anticipatory Activity in Mice Subjected to Once or Twice-Daily Feeding Periods** • Ashutosh Rastogi, Kent State University

S17 ****Time of Feeding Regulates Circadian Gene Expression in Mouse Peripheral Tissues** • Laura van Rosmalen, UT Southwestern Medical Center

S18 **Phase Shifts in Circadian Peripheral Clocks Caused by Exercise Are Dependent on the Feeding Schedule in PER2::LUC Mice** • Shigenobu Shibata, Waseda University

S19 **Levofloxacin-Induced QT Prolongation Depends on the Time of Drug Administration** • Laura Kervezee, Leiden University Medical Center

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S20 **Automatic Scoring of Heart Rate and Wrist Movements to Assess Sleep Architecture** • Antoine Viola, PPRS-Research

S21 *__Circadian Misalignment and Risk-Taking in Night Shift Workers__ • Philip Cheng, Henry Ford Health System

S22 **Evaluation of Biomathematical Models in Predicting Cognitive Impairment Among Short-Haul Airline Pilots** • Siera Martinez, San Jose State University Research Foundation

S23 **Resetting of Human Peripheral Clocks by Phototherapy During Simulated Night Shift Work** • Marc Cuesta, Douglas Mental Health University Institute, McGill University

S24 **Chrono-Typing and Political Orientation: Evidence of Left-Leaning Owls and Right- Leaning Larks** • Christian Cajochen, Centre for Chronobiology, Psychiatric Hospital of the University of Basel

S25 **Gestational Day Length and Risk of Depression in Adulthood in Women** • Elizabeth Devore, Brigham & Women's Hospital

S26 #__Association of Allostatic Load and Shift Work Among Us Adults__ • Nicole Bowles, The Rockefeller University

S27 **EPd, a Clock Controlled Gene, Mediates Rhythmic Immune Response in *Drosophila*** • Jiajia Li, University of Missouri-St. Louis

S28 **Chronotoxicity of Everolimus on the Immune System** • Dilek Ozturk, Bezmialem Vakif University

S29 **Time of Day-Dependent Sensitivity to LPS: A Sensory Role for the Autonomic Nervous System** • Eva Soto-Tinoco, Universidad Nacional Autónoma de México

S30 *__The Relationship Between Light Exposure and Subsequent Sleep: What Happens Outside of the Lab?__ • Emma Wams, University of Groningen, NL

S31 **Constant Light During Lactation Programs Circadian and Metabolic Functions in Rat Pups** • Madahi Palma Gomez, UNAM

S32 **A New Standardized Method to Assess the Endogenous and Light–Response of the Retinal Clock in Mammals** • Hugo Calligaro, INSERM U1208

S33 **Pineal Serotonin Modulates Entrainment of Central Circadian Clock by Light** • Keisuke Ikegami, Kindai University Faculty of Medicine

S34 *__Cryptochrome is a Direct Neuronal Ultraviolet Light Sensor__ • Lisa Soyeon Baik, University of California- Irvine

S35 **Can a Poor Sleep/Wake Cycle Contribute to Hippocampal Malfunction in a Mouse Model of Neurodevelopmental Disabilities?** • Cristina Ghiani, David Geffen School of Medicine at UCLA

S36 **COMT Allelic Variation and Sleep Organization in Human Neonatal Opioid Withdrawal** • Marie Hayes, University of Maine

S37 **Diurnal Regulation of Cocaine Self-Administration** • Ian Webb, University of Mississippi Medical Center

S38 *#__Aging Decreases Circadian Regulation of Alcohol Sensitivity and Increases Alcohol-Induced Tissue Injury and Mortality__ • Aliza De Nobrega, Florida State University

S39 **Rev-Erbα Deficiency is Associated With Mixed Affective Behaviors in Mice.** • Tsuyoshi Otsuka, Wakayama Medical University

S40 *__Evaluation of Circadian Rhythms and Sleep in the APP/PS1 Mouse Model of Alzheimer's Disease__ • Brianne Kent, University of British Columbia

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S41 **Individual Differences in the Rate of Re-Entrainment to a Phase Advance Predict Anxiety and Depression-Like Behavior** • Jeff Anyan, Concordia University

S42 **Global and Hepatocyte-Specific Ablation of BMAL1 Induces Hyperlipidemia and Enhances Atherosclerosis** • Xiaoyue Pan, SUNY Downstate Medical Center

S43 **Interdisciplinary Approaches for Identification of Circadian-Controlled Glycogen Metabolism in Neurospora Crassa** • Lily(Mokryun) Baek, College of Medicine, University of Cincinnati

S44 **Effects of Wheel Running Exercise on Feeding Patterns and Glucose Tolerance in C57BL/6J Mice** • Eric McGann, Rider University

S45 **Mouse Strain Differences in Response to Glucose Tolerance Test.** • Bretton Nabit, Rider University

S46 **Dosing Time-Dependent Changes in Beneficial Effects of Sesamin on High Fat-Induced Hyperlipidemia in Rats** • Norifumi Tateishi, Suntory Wellness Limited

S47 **Circadian Control of Oscillations in Mitochondrial Rate-Limiting Enzymes and Nutrient Utilization by PERIOD Proteins** • Gad Asher, Weizmann Institute of Science, Israel

S48 **Characterizing DNA Binding Activities of Mammalian Circadian Clock Protein Complexes** • Alfred Tamayo, Harvard Medical School

S49 **Prolyl Isomerases-Flipping the Circadian Switch** • Hande Asimgil, UC Santa Cruz

S50 **HITS-CLIP Reveals a Role for the RNA-Binding Protein FBP3 in the Circadian Clock** • Peng Gao, UT Southwestern Medical Center

S51 #**Regulation of Reverbα by the Spsb1-4 E3 Ligase Family** • Tsedey Mekbib, Morehouse School of Medicine

S52 **Circadian Clock Regulation of Translation Initiation Through eIF2α Phosphorylation** • Shanta Karki, Texas A&M University

S53 **Applications of Machine Learning in the Processing and Analysis of Large Circadian Proteomics Time-Series Datasets** • Alexander Crowell, Dartmouth College

S54 **Roles for Period Binding Domain of dCLOCK in *Drosophila* Circadian Clock** • Euna Lee, Ajou University

S55 **Important Roles of the RNA Editing Enzyme in the Mammalian Circadian Clockwork** • Hikari Yoshitane, The University of Tokyo

S56 **A Slow Conformational Switch in the BMAL1 Transactivation Domain Modulates Circadian Cycling** • Chelsea Gustafson, University of California, Santa Cruz

S57 **Changes in Titin Isoform Composition Following Inducible Knockout of BMAL1 in Skeletal Muscle** • Lance Riley, University of Florida

S58 ***Gravitational Loading at the Beginning of the Active Phase Attenuates Muscle Loss in Unloaded Mouse Hind Limb** • Shinya Aoyama, Waseda University

S59 **Diurnal Variation in G-Protein-Coupled Inwardly Rectifying Potassium (GIRK) Channels in Hippocampus** • Venkata Tekumalla, UAB

S60 **Circadian Transcription Factor NPAS2 and Metabolic Redox Sensor SIRT1 Interact in the Mouse Striatum to Regulate Reward-Related Behavior** • Darius Becker-Krail, University of Pittsburgh

S61 **Alterations of the Circadian System with Chronic Administration of the Serotonin (1A) Mixed Agonist/Antagonist BMY7378** • Jhenkruthi Vijaya Shankara, University of Calgary

S62 **Chronic Sleep Deprivation Inhibits Short and Long Term Memory in Aplysia** • Harini Krishnan, Florida State University

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S63 **Daily and Annual Rhythms of Activity in the Alpine Chamois under Natural Conditions** • Cristiano Bertolucci, University of Ferrara

S64 **Clock-Modulation of Virulence in the Phytopathogenic Fungus *Botrytis cinerea* and the Evolution of Clock Negative Elements in Fungi** • Luis Larrondo, Pontifica Universidad Catolica De Chile

S65 **A Fear-Entrained Oscillator in the Mouse** • Horacio de la Iglesia, University of Washington

S66 #**Histone Demethylase JARID1a Regulates Hepatic Glucose Metabolism and Enables Rapid Transcriptional Response to Food Intake** • Kacee DiTacchio, University of Kansas Medical Center

S67 **Circadian Rhythms in the Sea Anemone *Nematostella vectensis*** • Rebecca Helm, Woods Hole Oceanographic Institution

S68 **The New Main Factor Influence on a Circannual Rhythm** • Dmitrii Borisov, Nizhniy Novgorod State Agricultural Academy, Russia

S69 **Red and Green Luciferases Reveal Phase-Dependent Protein Productivity During Metabolic Rhythms of Yeast** • James Robertson, Middle Tennessee State University

S70 **Is the Zugunruhe Oscillator Related to MASCO?** • Paul Bartell, Pennsylvania State University

S71 ***Tissue Specific Response of Clock Genes Expression in Peripheral Oscillators in a Rat Model of Shift-Work** • Cinthya Córdoba-Manilla, Universidad Nacional Autónoma de México

S72 **Cryptochromes Suppress Ppard and Limit Exercise Endurance** • Megan Vaughan, The Scripps Research Institute

S73 **The Arcuate Nucleus: Site for Time-Of-Day-Dependent Negative Feedback on Corticosterone Secretion** • Luis Abel León-Mercado, Universidad Nacional Autónoma de México

S74 **Circadian Clock Regulation of the Melatonin MTNR1B Receptor in Human Myometrial Cells** • James Olcese, Florida State University College of Medicine

S75 **Lack of Exercise Leads to Altered Activity Patterns in Wild-Type and Vip-Deficient Mice During Light-Dark Cycles** • Kun Hu, Brigham & Women's Hospital/Harvard Medical School

S76 **Differences in Circadian Light Response of Nasonia Wasps from Different Latitudes** • Theresa Floessner, University of Groningen

S77 **Same-Phase Circadian Rhythms of Trimethylated Lysine 4 on Histone 3 at Promoters of Diversely-Expressed Genes in the Green Alga *Chlamydomonas*** • Sigrid Jacobshagen, Western Kentucky University

S78 **Insulin Resets the Circadian Clock via Induction of Clock Gene PER2** • Priya Crosby, MRC Laboratory of Molecular Biology

S79 **Coupled Oscillators, Synchronization and (Photoperiodic) Entrainment of Circadian Clocks** • Christoph Schmal, ITB, Charité Berlin

S80 **How Can You Tell Your Signal Is Rhythmic?** • Andrey Lazopulo, University of Miami

S81 **Circadian Rhythms in Wound Healing in Female Siberian Hamsters** • Erin Cable, University of Chicago

S82 **Dosing-Time Dependent Reproductive Toxicity of Everolimus in Male Mice** • Narin Ozturk, Istanbul University

S83 *#**Endothelin-1 Regulates the Diurnal Variation of Sodium Excretion in Male and Female Rats** • Jermaine Johnston, The University of Alabama at Birmingham

S84 ***Back to the Basics: A Simplified Model of Mammalian Circadian Rhythms** • Kevin Hannay, University of Michigan

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S85 **Don't Luc Now: How Firefly Luciferase Behaves in Mammalian Cells** • John O'Neill, MRC Laboratory of Molecular Biology

S86 **The Adrenal Clock Limits Disruption of Circadian Glucocorticoid Rhythms by Aberrant Light Exposure.** • William Engeland, University of Minnesota

S87 **Chronopharmacology of Everolimus by Ubiquitin Pathway in Mouse Renal Cell Carcinoma** • Shigehiro Ohdo, Kyushu University

S88 **Effects of the Duper Mutation on Phase Shifts and Estrous Cycles.** • Eric Bittman, University of Massachusetts at Amherst

S89 **Aryl Hydrocarbon Receptor Deficiency Alters Circadian and Metabolic Rhythmicity** • Shelley Tischkau, Southern Illinois University

S90 **PRD-1, a Component of the Circadian System of Neurospora Crassa, is a Member of the Dead-Box RNA Helicase Family** • Di Wu, York University

S91 ***The effect of BMAL1 Deletion in Gonadotropin-Releasing Hormone or Kisspeptin Neurons** • Karen Tonsfeldt, University of California San Diego

S92 **A Mathematical Model of the Liver Circadian Clock Linking Feeding/Fasting Cycles to Clock Function** • Marc Lefranc, University of Lille

S93 ***The Transcriptional Landscape Associated With Photoperiodism** • Laura Flavell, University of Leicester

S94 **Beyond Body Weight: How Impaired Leptin Signaling Can Affect Sleep Disordered Breathing** • Deanna Arble, University of Michigan

S95 ***Crosstalk Signaling Between Circadian Clock Components and Iron Metabolism** • Samuel Schiffhauer, Virginia Tech University

S96 **Extensive Regulation of Diurnal Transcription and Metabolism by Glucocorticoids** • Meltem Weger, University of Birmingham

S97 **Daily Magnesium Fluxes Regulate Cellular Timekeeping and Energy Expenditure** • Gerben van Ooijen, University of Edinburgh

S98 ***ZeitZeiger: Supervised Learning for Oscillatory Data** • Jacob Hughey, University of California, San Francisco

S99 **Characterizing Core Clock Gene Dynamics in Mouse and Human Peripheral Blood Using Simulated Shift Work Protocols** • Shobhan Gaddameedhi, Washington State University

S100 **Examining the Contributions of the BrLKP2 Gene Family to the Circadian Clock in *Brassica rapa*** • Jin A. Kim, National Academy of Agricultural Science(NAAS)

S101 **Exposure to Long Photoperiods Induces Changes in Coupling Between Single Neurons of the Mouse Suprachiasmatic Nucleus** • Renate Buijink, Leiden University Medical Centre

S102 **A Role for the Cationic Leak Channel NALCN in Daily Rhythms of Suprachiasmatic Nuclei Activity and Locomotor Behavior** • Matt Flourakis, Northwestern University

S103 **Circadian Rhythms in the Expression and Function of Synaptic and Extrasynaptic GABAA Receptors in the Suprachiasmatic Nucleus.** • James Walton, Georgia State University

S104 **Clock Gene Expression in the SCN of Arctic Ground Squirrels** • Lily Yan, Michigan State University

S105 **AVP Signaling Reprograms SCN Organization** • Kayla Rohr, Marquette University

S106 ***Circadian Regulation in and by SCN Astrocytes** • Chak Foon Tso, Washington University in St. Louis

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S107 **Spatial Segregation of PER1 and PER2 Expression in the Mouse SCN** • Malini Riddle, Barnard College

S108 **Role of grk2 in Circadian Behavior and Molecular Rhythms** • Lucia Mendoza-Viveros, University of Toronto Mississauga

S109 **Examination of the Suprachiasmatic Nucleus Expression in Forebrain BMAL1 Knockout Mice** • Mariko Izumo, UT Southwestern Medical Center

S110 **Dim Light at Night Disturbs the Daily Sleep-Wake Cycle and Sleep Architecture in Rats** • Andries Kalsbeek, Netherlands Institute for Neuroscience

S111 **Deficits in Temporal Processing in a Mouse Model of Autism** • Diego Golombek, Universidad Nacional de Quilmes

S112 **Nitrergic Neural Communication for the Synchronization of the Mammalian Circadian Clock: A Putative Redox-Regulation** • Diego Golombek, Universidad Nacional de Quilmes

S113 **Chronic Sleep Restriction Increases the Change in Systolic Blood Pressure Between Circadian Night and Day** • Andrew McHill, Harvard Medical School/Brigham and Women's Hospital

Monday, May 23

M1 **Correlation of Between Circadian Rest Activity Rhythm and Nucleic Acid Turnover in Patients With Metastatic Colorectal Cancer** • Sandrine Dulong, INSERM

M2 ***The MYC Oncogene Disrupts Circadian Rhythm and Metabolism in Cancer Through Modulation of REV-ERB and BMAL1** • Brian Altman, University of Pennsylvania Perelman School of Medicine

M3 **Active-Phase Restricted Feeding Restores the Blood Pressure Circadian Rhythm in Type 2 Diabetic db/db Mice** • Tianfei Hou, University of Kentucky

M4 ***Clock Genes Regulate Circadian Gating of Parturition and Gestation Length** • Carmel Martin-Fairey, Washington University

M5 **Magnetic Field Effects in *Drosophila melanogaster*** • Giorgio Fedele, University of Leicester

M6 ***Integration of Light Intensity Information Into the Clock Neuron Network of *Drosophila melanogaster*** • Matthias Schlichting, Brandeis University

M7 **An RNAi Screen for RNA Binding Proteins Controlling *Drosophila* Circadian Behavior** • Lauren Foley, UMass Medical School

M8 ***Genome-Wide Characterization of the Molecular Response of the Circadian Clockwork to Temperature in *Drosophila*** • Naveh Evantal, Hebrew University of Jerusalem

M9 **Light Induced Bursts in *Drosophila* Locomotion** • Stanislav Lazopulo, University of Miami

M10 ***Circadian Translational Profiling of the *Drosophila* Head Fat Body Reveals Potential Novel Roles for a Peripheral Oscillator** • Amy Yu, Tufts Medical School

M11 ***Diapause in *Drosophila melanogaster*** • Ane Martin Anduaga, University of Leicester

M12 ****Cold-Induced Period Transcription Links Environmental Temperature to the *Drosophila* Molecular Clock** • Akanksha Bafna, University of Southampton

M13 **#The Dopamine Transporter is Not Required for Entraining Circadian Rhythms to Scheduled Feeding** • Jennifer Enriquez, California State Polytechnic University, Pomona

M14 **Mapping Dopaminergic-D1R Circuitry That Mediate Circadian Entrainment to Feeding** • Andrew Steele, California State Polytechnic University, Pomona

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M15 **Time-Restricted Feeding of a High-Fat Diet Attenuates Its Deleterious Effects on Middle-Aged Mice** • Marilyn Duncan, University of Kentucky Medical School

M16 **Role of Gonadal Hormones in Food Anticipatory Activity in Response to Timed Restricted Feeding** • Jessica Krizo, Kent State University

M17 #**Decreased Food Anticipatory Activity of Obese (Neotomodon Alstoni) Mice Relates to Changes in Hypothalamic Fos Expression** • César Luna Illades, UNAM

M18 **Time Perception Relates to Cognitive Performance, Anxiety and Subjective Reports of Well-Being** • Natalia Bobko, Institute for Occupational Health, Kyiv, Ukraine

M19 **Objectively Measured Late-Morning Physical Activity Predicts Mortality in the NHANES 2003-2006 Cohorts** • Vadim Zipunnikov, Johns Hopkins Bloomberg School of Public Health

M20 **MEQ Predicts Optimal Performance Time in Addition to Morning-Evening Preference** • Martin Ralph, University of Toronto

M21 **Trends in Self-Reported Hourly Lighting and Sleep in a Global Dataset of Travelers** • Olivia Walch, University of Michigan

M22 ***Circadian Profiles of Light, Activity, and Body Temperature for Non-Invasive Physiology Prediction in Humans** • Benjamin Smarr, University of California, Berkeley

M23 **Molecular Basis for Chronotype and Time-Of-Day Effects on Decision-Making** • Krista Ingram, Colgate University

M24 **A Prospective Study of Rotating Night Shift Work and Incident Depression in the Nurses' Health Study 2** • Celine Vetter, Brigham and Women's Hospital and Harvard Medical School

M25 **Clock Regulation of Circadian Rhythms in the Human Neocortex** • Miles Fontenot, University of Texas Southwestern

M26 **TNF-Alpha and Ccl2 Mediate the Immune-Circadian Interaction in Inflammation and Cancer Animal Models** • Diego Golombek, Universidad Nacional de Quilmes

M27 **Circadian Rhythmicity in Bone Marrow-Derived Macrophages** • Shan Chen, Dartmouth College

M28 **Identifying CK1e/d Activity as a Potential Link Between Circadian Rhythm Disruption and CXCL-1 Mediated Neuroinflammation** • Jonathan Shelton, Janssen Pharmaceutical R&D

M29 **How Outer Retinal Photoreception and Melanopsin Phototransdcution Control Non-Image Forming Visual Functions** • Samer Hattar, Johns Hopkins University

M30 **Characterization of Non-Visual Responses to Light Using Spectral, Temporal and Spatial Properties of Rods/Cones and ipRGCs in Humans** • Abhishek Prayag, INSERM U1208

M31 **Red Light at Night Does Not Suppress Melatonin in the Horse** • Barbara Murphy, University College Dublin

M32 **Photoreceptor Weighted Light Intensities and Their Dose-Response Relationships for Non-Visual Effects of Light** • Luc Schlangen, Philips Lighting Research

M33 **Chronotype Differences in the Distribution of Excitatory and Inhibitory Cell Populations in ipRGC Target Areas** • Jennifer Langel, Johns Hopkins University

M34 **The Multifunctional Nature of Cryptochromes in the Mammalian Retina** • Nicola Smyllie, MRC Laboratory of Molecular Biology

M35 **Blue Light Therapy Improves Circadian Dysfunction in Two Mouse Models of Huntington's Disease** • Huei-Bin Wang, UCLA

M36 **Time-of-Day Disruption of GSK3β Phosphorylation and Cognitive Impairment in the Tg-SWDI Mouse Model of Alzheimer's Disease** • Jennifer Davis, The University of Alabama at Birmingham

* = Merit Award Winner ** = Excellence Award Winner # = Diversity Travel Award Winner

M37 ***The Effects of Circadian Misalignment During Adolescence on Mood and Alcohol Sensitivity** • Chelsea Vadnie, University of Pittsburgh

M38 **Treating Circadian Dysfunction Delays Disease Progression in Mouse Models of Huntington's Disease** • Dawn Loh, University of California Los Angeles

M39 **Constant Darkness and Constant Light Suppress Voluntary Alcohol Intake in Mice** • Alan Rosenwasser, University of Maine

M40 **Clock-HIF Interaction Establishes Rhythmic Skeletal Muscle Exercise Tolerance and the Hypoxic Response** • Clara Peek, Northwestern University

M41 **Disruption of Daily Rhythms by High-Fat Diet is Reversible** • Julie Pendergast, University of Kentucky

M42 **Short-Term Effect of Nocturnal Transportation Noise on Glucose Metabolism** • Laurie Thiesse, Centre for Chronobiology of Basel

M43 **The Liver Circadian Clock Modulates Blood Glucose Lowering Efficacy of Metformin** • Katja Lamia, The Scripps Research Institute

M44 **Effects of Photoperiod on Locomotor Activity and Glucose Regulation in C57BL/6J Male Mice** • Kevin Munoz, Rider University

M45 **Glucose Tolerance in Nocturnal Animals Experiencing Light-Dark Stimulus Patterns Mirroring Patterns Measured from Dayshift and Rotating Shift Workers** • Mariana Figueiro, Lighting Research Center, Rensselaer Polytechnic Institute

M46 ***Circadian Regulation of Xenobiotic Metabolism** • Anna Kriebs, The Scripps Research Institute

M47 ***24 H Metabolic Profiling in Obesity and Type 2 Diabetes (T2DM)** • Cheryl Isherwood, University of Surrey

M48 **Composition and Structure of Cytoplasmic PERIOD Complexes in Relation to the Nuclear PERIOD Complex** • Pieter Bas Kwak, Harvard Medical School

M49 **Determination of poly(A)-Tail Lengths and 3'-End Modifications of mRNAS by Tail-Seq in Circadian Systems** • Hua Jin, HHMI at Brandeis University

M50 ***4C-Seq in Mouse Liver Reveals Clock-Dependent Rhythmic Chromatin Contacts** • Jérôme Mermet, EPFL SV IBI UPNAE

M51 ***Molecular Description of the Poised CRY:CLOCK:BMAL1 Repressive Complex** • Alicia Michael, University of California, Santa Cruz

M52 **Regulation of the Mammalian Circadian Clock Transcriptional Output by CLOCK:BMAL1** • Alexandra Trott, Texas A&M University

M53 ***CRY Acts as a Cofactor for the SCF-FBXL3 Mediated Degradation of Novel Substrates** • Stephanie Papp, The Scripps Research Institute

M54 **Epigenetic Regulation of the *Drosophila* Circadian Clock Involves the Interaction of a SWI/SNF Chromatin-Remodeler with Histone Deacetylases to Repress Transcription** • Rosanna Kwok, University of California, Davis

M55 **CLOCKWORK ORANGE Enhances PER Mediated Circadian Transcriptional Repression by Competing with CLK-CYC for E-Box Binding** • Jian Zhou, Texas A&M University

M56 **CRY Drives Cyclic CK2-Mediated BMAL1 Phosphorylation to Control the Mammalian Circadian Clock** • Teruya Tamaru, Toho University School of Medicine

M57 **Selective Knockout of BMAL1 in Skeletal Muscle and Not the Brain Regulates Circadian Rhythms of Wheel Running and Sleep** • Allison Brager, Morehouse School of Medicine

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M58 **Simulated Light Therapy Enhances Recognition Memory and Alters Daily Rhythms in Hippocampal Gene Expression** • Jennifer Evans, Marquette University

M59 **Integrated Multimodal Analysis of Cell- And Circuit-Specific Processes in Circadian Hippocampal Functions** • James Chu, University of Illinois Urbana Champaign

M60 **The Circadian Transcription Factor CLOCK Represses the Expression of the Dopamine Rate-Limiting Enzyme Tyrosine Hydroxylase via Recruitment of the Metabolic Sensor SIRT1** • Gabrielle Pittman, University of Pittsburgh

M61 ***'Per1::Venus Arcuate Neurons Exhibit Robust Rhythms in Excitability'** • Adam Watson, University of Manchester

M62 **Local Adaptation by Losing Circadian Control of Asexual Development in *Neurospora discreta*** • Kwangwon Lee Lee, Rutgers University - Camden

M63 **Regressive Evolution in the Somalian Cavefish *Phreatichthys andruzzii:* Loss of Selective Constraint on Circadian Opsin Genes** • Cristiano Bertolucci, University of Ferrara

M64 **Investigating Neural Correlates of Rhythm Deterioration in Seasonal Adaptive Behavior in Aging Mice** • Anneke Olde Engberink, Leiden University Medical Center

M65 **Does the Id2 Null Mouse Have a Disturbed Circadian Profile in Core Body Temperature?** • Peng Zhou, Harvard Medical School

M66 **Functional Segmentation of the Clock by Lim-Type** • Liu Zhihua, Harvard Medical School

M67 **Evaluation of Novel Methods to Non-Invasively Monitor Core Body Temperature Rhythms in the Horse** • Margaret Nolan, Equilume Ltd

M68 **Meta-Analysis of Transcriptomic Datasets Identifies Genes Enriched in the Circadian Pacemaker** • Laurence Brown, University of Oxford

M69 **Real-Time Ticking of a Biological Clock Assembled in a Test Tube** • Joel Heisler, UC Merced

M70 **Hyper-Flexible and Light-Driven Rest/Activity Rhythms Under Non-24h Conditions** • Thijs Johannes Walbeek, University of California San Diego

M71 **The Evolution of Neural Circuitry Regulating Sleep and Arousal in the Blind Mexican Cavefish** • Bethany Stahl, Florida Atlantic University

M72 ***Regulation of Mitochondrial Dynamics by the Circadian Deadenylase Nocturnin** • Yasemin Onder, UT Southwestern Medical Center

M73 **Aging and the Gastrointestinal Clock: Influence of Melatonin on the Gut Microbiome** • Jiffin Paulose, University of Kentucky

M74 **Isolated Retina Müller Cells Exhibit Sustained Circadian Rhythms in Culture** • Nadia Mazzaro, Institute for Cellular and Integrative Neurosciences

M75 **Effects of Chronic Alcohol + Binge on Liver Rhythms and Bile Acid Metabolism in Mice** • Jessica Ferrell, Northeast Ohio Medical University

M76 **The Role of Melatonin in the Photoperiodic Control of Bird Song Distribution and Repertoire in the House Sparrow, Passer Domesticus** • Clifford Harpole, University of Kentucky

M77 **Urokinase Plasminogen Activator (uPA) Regulates Phase Resetting of the Mammalian Circadian Clock** • Joanna Cooper, University of Tennessee

M78 **Polymorphisms in the Human Clock Gene Period3 Are Associated With Diurnal Preference, Subjective Sleepiness and the Response to Morning Light** • Gabriella Mazzotta, Università di Padova

M79 **Binge Eating Behavior for Sucrose is Time-Of-Day Dependent: Effects on Reward Brian Areas** • Rodrigo Osnaya Ramirez, National Autonomous University of Mexico

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M80 **Removing the Brakes on Photic Entrainment in the Circadian System** • Ryan Chan, University of Calgary

M81 **JmjC Domain Protein JMJD5: A Repressor of the Mammalian Circadian Clock and a Potential Mediator of Circadian Control of Energy Metabolism.** • Anand Saran, University of Kansas Medical Center

M82 **Beyond *Drosophila*: Analysis of Cycling Genes in the Jewel Wasp *Nasonia*, An Emerging Model Organism** • Nathaniel Davies, University of Leicester

M83 **Zebrafish Liver Diurnal Gene Expression and Comparative Transcriptomics** • Ghislain Breton, University of Texas Health Science Center

M84 **An Assay to Characterize the Dampening Tendency of the Photo-Periodic Oscillator** • Koustubh Vaze, University of Wuerzburg

M85 **Quantitative Analysis of mRNA-Protein Flux in Circadian Rhythms by Ribosomal Profiling and Mass Spectrometry** • Arthur Millius, RIKEN Quantitative Biology Center

M86 **Long Term High-Fat Diet Consumption and Wheel-Running Access Produces Alterations in Circadian Locomotor Activity** • Joseph Seggio, Bridgewater State University

M87 **A Model-Based Analysis of Light-Induced Circadian Arrhythmia in the Siberian Hamster** • Andrew Phillips, Brigham and Women's Hospital / Harvard Medical School

M88 **CalfluxVTN, a New, Bright Bioluminescent Ca2+ Sensor That Can Be Coupled With Excitatory, Optogenetic Stimulation** • Derrick Cumberbatch, Vanderbilt University

M89 ***Exploring Physiological Changes Underlying Protection From Severe Sleep Restriction in Migrating Birds** • William Horton, Pennsylvania State University

M90 **Ultradian Feeding in Mice Not Only Affects the Peripheral Clock in the Liver, But Also the Master Clock in the Brain** • Satish Sen, University of Amsterdam and University of Strasbourg

M91 **Circadian Rhythms in Actin Dynamics and Wound Healing** • Ned Hoyle, MRC-Laboratory of Molecular Biology

M92 **Crystal Clear: Solving the Structures of Cyanobacterial KaiABC Subcomplexes** • Nicolette Goularte, UC Santa Cruz

M93 **Feedback Loops of the Mammalian Circadian Clock Constitute Repressilator** • Hanspeter Herzel, Institute for Theoretical Biology

M94 **Investigating the Role of Zinc in the Circadian System** • Mahtab Moshirpour, University of Calgary

M95 **Variability of Behavioral Chronotypes of 16 Mammalian Species Under Controlled Conditions** • Roberto Refinetti, Boise State University

M96 ***Circadian Clock Control by Polyamine Levels Through a Mechanism That Declines With Age** • Ziv Zwighaft, Weizmann Institute of Science

M97 **TTDP, a Primate-Specific Gene Exclusively Expressed in Testis Which Regulates the Circadian Rhythms** • Jinhu Guo, Sun Yat-sen University

M98 ***Diel Flight Activity Behavior of Wild Caught *Anopheles farauti* s.s. and *An. Hinesorum* Malaria Mosquitoes from Northern Queensland, Australia: Temporal Differences That Might Contribute to Speciation** • Gary George, University of Notre Dame

M99 **Open Board**

M100 **Natural Variation and Co-Expression Network Approaches to Assess the Contribution of the Circadian Clock to Plant Fitness** • Kathleen Greenham, Dartmouth College

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M101 **Chloride Cotransporter KCC2 Essential for GABAergic Hyperpolarization in the SCN** • Anneke Olde Engberink, Leiden University Medical Center

M102 **Does the Polarity of SCN GABAaR Signaling Regulate Phase Advances of the Behavioral Clock?** • John McNeill, Georgia State University

M103 **Phase it! Strength Isn't Everything, At Least in the Mammalian SCN** • Bharath Ananthasubramaniam, Charite Universitaetsmedizin Berlin

M104 **Stem-Like Cell Cultures of the Adult Mouse Suprachiasmatic Nucleus** • Michael Geusz, Bowling Green State University

M105 ***Vasopressin Mediates Clock-Driven Anticipatory Thirst** • Claire Gizowski, Research Institute of the McGill University Health Centre

M106 **Manipulating the Cellular Circadian Period of AVP Neurons Alters the Behavioral Circadian Period** • Michihiro Mieda, Kanazawa University

M107 **Rhythms in VIP Cell Output Within the Mouse Suprachiasmatic Nuclei** • Timothy Brown, University of Manchester

M108 **BK Channels Are Activated by Distinct Calcium Sources During Day and Night in SCN Neurons** • Andrea Meredith, University of Maryland School of Medicine

M109 **Sex Differences in Age-Related Sleep Changes in Mice** • Martha Vitaterna, Northwestern University

M110 **Identifying Neurons that Regulate Plasticity in Sleep Duration** • Seana Lymer, New York University

M111 **Open Board**

M112 **How Biological Clocks Yield Negative Entropy – A Novel Concept of Entrainment** • Manfred Goedel, Institute of Medical Psychology, University of Munich

Tuesday, May 24

T1 **The Circadian Clock Proteins BMAL1 and CLOCK Control G2/M Cell Cycle Transition** • Elham Farshadi, Erasmus University Medical Center, Rotterdam

T2 **Cardiovascular Dysfunction in a Mouse Model of Huntington's Disease** • Christopher Colwell, UCLA

T3 **Endogenous Circadian Rhythm in Vascular Function and Cardiovascular Risk** • Saurabh Thosar, Oregon Health and Science University

T4 **Determining the Ontogeny of Synchronization in the *Xenopus laevis* Embryo Using Gene Expression and Behavior** • Kristen Curran, University of Wisconsin Whitewater

T5 **MicroRNA-92a Acts as a Circadian Regulator of Neuronal Excitability in *Drosophila*** • Xiao Chen, Brandeis University

T6 ***Drosophila* DH31 Neuropeptide and PDF Receptor Control Night-Onset Temperature Preference** • Tadahiro Goda, Cincinnati Children's Hospital Medical Center

T7 **Ion Channels that Regulate Neuronal Physiology and Circadian Behavior in *Drosophila melanogaster*** • Nara Ines Muraro, Instituto de Investigación en Biomedicina de Buenos Aires (IBioBA)-CONICET-MPSP

T8 **Regulation of Chromatin Accessibility on CLOCK/CYCLE Direct Targets in *Drosophila*** • Katharine Abruzzi, Howard Hughes Medical Institute; Brandeis University

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T9 **Integration of Clock and Temperature Circuits Drives Pre-Dawn Temperature Preference in *Drosophila*** • Yujiro Umezaki, Cincinnati Children's Hospital Medical Center

T10 **The Expression of *period* and *timeless* in the Early Development of *Drosophila melanogaster*** • Jia Zhao, The University of Auckland

T11 **A *Drosophila apterous mutation* Uncouples Locomotor Activity From Circadian Clock Control** • Bernard Possidente, Skidmore College

T12 **How Does Electrical Activity Regulate Circadian Gene Expression in *Drosophila* Pacemaker Neurons?** • Zhonghua Zhu, New York University

T13 **Ovarian Hormones Prevent Disruption of Daily Rhythms From High-Fat Feeding in Female Mice** • Julie Pendergast, University of Kentucky

T14 **Oscillations in Circadian Gene Expression in Liver and Brain in Response to Scheduled, Calorie Restricted Feeding** • Charles Zhang, California State Polytechnic University, Pomona

T15 #**A Piece of Chocolate in the Dark Phase Prevents Circadian Desyncrhony and Overweight in Male Shift-Worker Rats** • Mara Guzman-Ruiz, Universidad Nacional Autónoma de México

T16 **Relationship Between Timing of Food Intake and Insulin Sensitivity** • Vittobai Rashika Rangaraj, University of Chicago

T17 **Circadian and Feeding Rhythms Differentially Affect Rhythmic mRNA Transcription and Translation in Mouse Liver** • Cédric Gobet, NIHS/EPFL

T18 **Circadian Fluctuations in Hemodynamic of Surgeons Under 24-Hour Duties** • Natalia Bobko, Institute for Occupational Health, Kyiv, Ukraine

T19 **The Effect of Daytime Napping Under Bright Light Condition After Simulated Night Work on Biological Rhythm in Healthy Human** • Shunsuke Nagashima, Human Health Sciences, Graduate School of Medicine, Kyoto University

T20 **Insights Into the Human Chronobiome** • Carsten Skarke, University of Pennsylvania

T21 **Human Circadian Timing After Weekend Exposure to the Modern Versus Natural Light-Dark Cycle** • Ellen Stothard, University of Colorado Boulder

T22 ***Phase-Angle Differences Between Dim-Light Melatonin Onset and Sleep Onset in Patients Diagnosed With Delayed Sleep Phase Syndrome** • Catia Reis, CENC - Sleep Medicine Center, Lisbon

T23 **Glucocorticoid Signalling is Disrupted by Mistimed Sleep** • Simon Archer, University of Surrey

T24 **Identifying Circadian Transcripts in Human Subcutaneous Adipose Tissue** • Skevoulla Christou, University of Surrey

T25 **Sleep-Wake Rhythms and Safety: Using a Meta-Analytic Risk Index Model to Predict Occupational Injuries** • Dorothee Fischer, Harvard T.H. Chan School of Public Health, Boston, MA, USA

T26 **Inflamatory Markers During Night Work and Vacation** • Leana Araujo, Adventist University of Health Sciences

T27 **Photoperiod Influences Circadian Rhythms of Adaptive and Innate Immune Responses of Male Siberian Hamsters** • Kenneth Onishi, University of Chicago

T28 ***Circadian Control of CD8+ T Cell Response** • Chloé Nobis, Douglas Mental Health University Institute

T29 **Cyclically Expressed Heme Oxygenase Protects the Fruit Fly's Retina Against Light-Induced Damage** • Milena Damulewicz, Jagielllonian University

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T30 **Beta Arrestins Shape Melanopsin-Dependent Responses to Light** • Ludovic Mure, The Salk Institute

T31 **Colour Processing in the Non-Image Forming Visual System** • Lauren Walmsley, The University of Manchester

T32 ***Differential Roles for Mammalian Cryptochromes in the Retinal Circadian Clock** • Jovi Wong, University of Oxford

T33 **Are Intrinsically Photosensitive Retinal Ganglion Cells (ipRGCS) Necessary for Light Entrainment of Peripheral Clocks?** • Paulo Kofuji, University of Minnesota

T34 **Circadian Forced Desynchrony Leads to Behavioral Manifestations of Depression in Rats** • Horacio de la Iglesia, University of Washington

T35 ***Circadian Clocks Modulate Huntington's Disease via Stress Response Pathways** • Fangke Xu, Northwestern University

T36 ***Selective Inhibition of Casein Kinase I Delta Enhances Hippocampal Dependent Learning and Alters Expression of Circadian Clock Proteins in the Hippocampus** • Heather Mahoney, University of South Florida

T37 **Behavioral and SCN Neurophysiological Disruption in the Tg-SwDI Mouse Model of Alzheimer's Disease** • Jodi Paul, The University of Alabama at Birmingham

T38 **Circadian Abnormalities in the BTBR Mouse Model of Autism Spectrum Disorder** • Michael Antle, University of Calgary

T39 **Alcohol Abuse in Circadian Desynchrony: Impact of Age, Genetics, and Environment** • Danielle Gulick, University of South Florida

T40 ***Transgenerational Epigenetic Effects of Cocaine on Circadian Behavior and Cocaine Reward** • Alexandra Yaw, Kent State University

T41 **Measuring Time in Adipocytes-The Effect of Insulin on Clock Gene Expression** • Neta Tuvia, Charite University Medical Center Berlin

T42 **Circadian Rhythms Disturbances, Depression and Type 2 Diabetes: Possible Interrelationships** • Carmel Bilu, Ben-Gurion University

T43 **Effects of a Forced Desynchrony Protocol on Feeding Patterns and Glucose Tolerance in C57BL/6J Mice** • Melissa Rasimowicz, Rider University

T44 **Oscillations in Bat Thermogenesis Are Independent of the Adipocyte Circadian Clock** • Georgios Paschos, University of Pennsylvania

T45 **Circadian Rhythms of Triglyceride Accumulation in NIH3T3-L1 Cells** • Satomi Morita, Meiji University

T46 **Nocturnal Light Exposure Acutely Disrupts Glucose Metabolism** • Anne-Loes Opperhuizen, Netherlands Institute for Neuroscience

T47 **Metabolic Defects in BMAL1 Ko Mice** • Céline Jouffe, IDO, Helmholtz Zentrum München

T48 **Characterization and Behavior of Multimeric Protein Complexes of the Mammalian Circadian Clock Across the Circadian Cycle** • Rajindra Aryal, Harvard Medical School

T49 **Identification of RE-VERB-α Degradation Mechanisms** • Ting-Chung Suen, Morehouse School of Medicine

T50 **CNOT1 Promotes Phosphorylation of Mammalian Clock Proteins via PKA** • Zhang Yunfeng, Soochow University

T51 **Cry2 Suppresses Transformation by Destabilizing c-Myc** • Anne-Laure Huber, The Scripps Research Institute

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T52 *__An Evolutionary Hotspot in CRYPTOCHROME's Structure Tunes the Period of the Mammalian Circadian Rhythm__ • Clark Rosensweig, UT Southwestern Medical Center

T53 *#__A Functional Synthetic Hybrid Circadian Oscillator Generated Through Transcriptional Rewiring__ • Alejandra Goity, PUC

T54 #__Re-Evaluating the Roles of Protein Kinase a (PKA) and Camp Signaling in Circadian Core-Clock Mechanisms__ • Consuelo Olivares-Yañez, PUC

T55 __Circadian Clock Regulation of mRNA Translation Through the Eukaryotic Elongation Factor eEF-2__ • Stephen Caster, Texas A&M University

T56 *__Novel Transcriptional Mechanisms of Muscle-Specific Clock Output__ • Brian Hodge, University of Florida

T57 __Circadian Rhythm of Muscle Mitochondrial Metabolism__ • Paul de Goede, Academic Medical Center Amsterdam (AMC)

T58 *__Circadian Rhythm of Redox State in Hippocampal CA1 Regulates Neuronal Membrane Excitability__ • Mia Yu, University of Illinois at Urbana-Champaign

T59 __Altered Circadian Phenotype in Cannabinoid Receptor 1 Knockout Mice__ • Kirsten Maricic, Kent State University

T60 __Circadian Effects of Conditional Serotonin Knockdown in the Midbrain Raphe Nuclear Complex of Adult Mice__ • Ashley Shemery, Kent State University

T61 __Transcriptomic Study of Circadian Rhythm in Astrocytes__ • Shao'ang Wen, Institute of Neuroscience

T62 __Entrainment Pathways of *C. elegans* Circadian Rhythms__ • Diego Golombek, Universidad Nacional de Quilmes

T63 __Daily Changes in Opsin mRNA Levels in the Antarctic Krill__ • Cristiano Bertolucci, University of Ferrara

T64 __Stress Alters Adrenal Clock Function in a Sexually Dimorphic Manner__ • Jennifer Evans, Marquette University

T65 *__A Dissociation Between Diurnal Cycles in Locomotor Activity, Feeding Behavior and Hepatic PERIOD2 Expression in Chronic Alcohol-Fed Mice__ • Peng Zhou, Harvard Medical School

T66 __KaiA Mutant on Oxidized Quinone Binding Site Overcomes Jet Lag Faster__ • Yong-Ick Kim, New Jersey Institute of Technology

T67 __Cohabiting Grass Rats Synchronize Their Activity Bouts on a Non-Circadian Scale__ • Alexandra Castillo-Ruiz, Georgia State University

T68 __The Circadian Clock Regulates Autophagy Directly Through Nuclear Hormone Receptor Rev-Erbα and Indirectly via C/EBPβ in Zebrafish__ • Han Wang, Soochow University

T69 __Time Restricted Foraging Activity and Clock Gene Expression in Honey Bees__ • Rikesh Jain, National Centre for Biological Sciences-Tata Institute of Fundamental Research, Bangalore, India

T70 __The Bioclock Studio: Undergraduate Students Connecting Circadian Biology and Sleep Research from Laboratories to Classrooms and the Public__ • Pagkapol Yhew Pongsawakul, University of California, San Diego

T71 __Effects of Constant Bright Light and Heavy Water on Interval Timing in Rats__ • Christian Petersen, Simon Fraser University

T72 __Per2 Expression Rhythms in Mice with Early Senescence and Bimodal Locomotor Rhythms__ • Mugdha Mokashi, The University of Alabama at Birmingham School of Medicine

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T73 **Does Duper Alter Pacemaker Function or the Core Molecular Clock?** • Ajay Kumar, University of Massachusetts Amherst

T74 **Aging of the Circadian System in Short and Long Circadian Period Mutant Mice** • Malgorzata Oklejewicz, Erasmus MC

T75 ***Mice Are Able to Acquire Multiple Independent Time Memories** • Choden Shrestha, University of Toronto

T76 **RAS2 is a Regulator of the Circadian Clock in *Neurospora crassa*** • Krisztina Káldi, Semmelweis University

T77 ***Transcriptional Regulatory Logic of the Diurnal Cycle in the Mouse Liver** • Jonathan Sobel, EPFL SV IBI UPNAE

T78 ***Comparison of the Circadian Clock of Social and Solitary Bees** • Katharina Beer, University of Wuerzburg

T79 **Entrainment Maps: A New Tool for Understanding Properties of Circadian Oscillator Models** • Casey Diekman, New Jersey Institute of Technology

T80 **Genome-Wide Profiling of Diurnal Rhythmic Gene Expression in the Water Flea *Daphnia pulex*** • Giles Duffield, University of Notre Dame

T81 **Open Board**

T82 ***Using Signal Processing to Explore Diversity: Analyses of Locomotor Activity and Core Body Temperature Reveal Sex Differences in Mice** • Azure Grant, UC Berkeley

T83 ***TNF Signaling Regulates the Circadian Rhythm of Myogenic Responsiveness and Systemic Blood Pressure** • Jeff Kroetsch, University of Toronto Faculty of Medicine

T84 **Phase of Circadian Entrainment: A Simple Theory Behind Complex Data?** • Grigory Bordyugov, Charite Berlin

T85 **Using MRI to Observe Migratory Related Neurophysiological Changes** • Bruce Langford, Pennsylvania State University

T86 **Testing Novel Objective Parameters for Alertness** • Renske Lok, University of Groningen

T87 **BMAL1 Deletion in Adulthood Facilitates Adaptation to Disrupted Light/Dark Schedules in Mice** • Guangrui Yang, University of Pennsylvania

T88 **Translation Across Time and Space** • Violeta Castelo-Szekely, Center for Integrative Genomics - University of Lausanne

T89 **Mitochondrial Network Morphology Changes With a Circadian Rhythm in Cell Lines** • Sarah Lueck, Humboldt University Berlin

T90 **Circadian Rhythms in *Neurospora crassa* Are Regulated by a Component of a Conserved Nutrient-Sensing Pathway** • Lalanthi Ratnayake, York University

T91 **Differences in the Circadian Phenotype Among Substrains of Cba Mice** • Suzuka Itoh, Meiji University

T92 **Understanding Timekeeping in an Intertidal Crustacean *Eurydice pulchra*** • Lin Zhang, University of Leicester, UK

T93 ****Hepatic miRNA Loss Resulted in Altered Adaptation to Food Restriction in Mice** • Ngoc-Hien Du, Center for Integrative Genomics

T94 **Secreted Proteins Exhibit Diurnal Profiles in Human Plasma** • Benjamin Weger, Netlé Institute of Health Sciences

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T95 **Circadian Control of Global Proteomic Output in *Neurospora crassa*** • Jennifer Hurley, Renssealer Polytechnic Institute

T96 **A Systems-Driven Experimental Approach Reveals the Complex Regulatory Distribution of p53 by Circadian Factors** • Tetsuya Gotoh, Virginia Tech

T97 ***Entrainment Ability of the Peripheral Circadian Clocks by Light, Food, Stress, and Exercise in Aged Mice** • Yu Tahara, Waseda university

T98 ***Sleep and Circadian Regulation of Metabolic Rate in *Drosophila*** • Melissa Slocumb, Florida Atlantic University

T99 **Amplitude Response of Circadian Clock System to External Stimuli** • Tao Zhang, Soochow University

T100 **Neuronal Activity Induced Changes of Energy Metabolites in the Mouse Suprachiasmatic Nucleus** • Renate Buijink, Leiden University Medical Centre

T101 **SCN Phosphoproteomic Analysis Reveals GRK2 as an Important Modulator of Neuronal Structure and Cytoskeleton Organization** • Cheng-Kang Chiang, Ottawa Institute of Systems Biology, University of Ottawa

T102 **Ontogeny of Circadian Synchrony in the Suprachiasmatic Nucleus** • Vania Carmona-Alcocer, Washington University in St. Louis

T103 ***Lhx1-Regulated Transcriptional Networks Control Sleep/Wake Coupling and Thermal Resistance of the SCN Clockworks** • Joseph Bedont, University of Pennsylvania

T104 **Isoflurane Anaesthesia Phase Shifts the SCN: Recordings From PER2::LUC Mice** • Nicola Ludin, The University of Auckland

T105 **CRTC1-SIK1 Pathway is Significant to Light Adaptation Capability** • Yu Liu, Soochow University

T106 **Cannabinoid Signaling Alters Clock Phase and GABAergic Neurotransmission Within the SCN** • Lauren Hablitz, Oregon Health and Science University

T107 **GRK2 Regulates Nucleocytoplasmic Distribution of PERIOD1/2 and Major Ligand-GPCR Systems in Circadian Timekeeping** • Arthur Cheng, University of Toronto

T108 **Gpr176 is an SCN-Specific Gz-Coupled Orphan GPCR That Controls Circadian Behavior** • Masao Doi, Graduate School of Pharmaceutical Sciences, Kyoto University

T109 **Circadian Arrhythmia Disrupts Theta Oscillations in the EEG** • Adrienne Thom, Stanford University

T110 ***Integrative Analysis of Multiple Genomics Datasets Reveals Key Networks and Pathways Underlying the Circadian and Homeostatic Regulation of Sleep** • Peng Jiang, Northwestern University

T111 **Open Board**

T112 **Recovery of Circadian Time-Place Learning in Rats with Hippocampal Lesions** • Ralph Mistlberger, Simon Fraser University

T113 **Sex Differences in Circadian Food Entrainment Are Unrelated to Gonadal Sex Hormones** • Antonio Aguayo, California State Polytechnic University, Pomona

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Slide Session Abstracts

Sunday, May 22, 2016

11:00 am - 12:30 pm

Slide Session: ***Clocks, Feeding and Metabolism***

Chair: Richa Saxena

11:00 am SS1

Morning Circadian Misalignment during Insufficient Sleep is Associated with Changes in Plasma Metabolites Linked to Metabolic Dysregulation

Christopher Depner[1], Rachel Markwald[1], Charmion Cruickshank-Quinn[2], Kevin Quinn[2], Nichole Reisdorph[2], Kenneth Wright[1]

[1]*University of Colorado Boulder,* [2]*University of Colorado, Anschutz Medical Campus*

Abstract: Introduction: Impaired sleep and circadian misalignment are associated with elevated type 2 diabetes risk (T2D). We previously reported insufficient sleep results in morning circadian misalignment (i.e. morning wakefulness during the biological night), and such circadian misalignment is associated with reduced insulin sensitivity. Here we investigated the plasma metabolome to better understand mechanisms underlying metabolic dysregulation during insufficient sleep and associated circadian misalignment.

Methods: We conducted a randomized cross-over 14-15 day in-laboratory study where 16 (8M/8F) healthy participants aged 22.4±4.8y (mean±SD) completed 3 baseline days (9h sleep opportunity/night) followed by 5 days of insufficient (5h/night), and adequate (9h/night) sleep conditions. Food intake was designed to meet energy needs at baseline and was ad libitum during insufficient and adequate sleep. Circadian phase was analyzed by dim-light melatonin offset (DLMOff). Plasma was analyzed from morning fasted samples by untargeted LC/MS on the final day of baseline, insufficient, and adequate sleep.

Results: During insufficient sleep, DLMOff timing relative to waketime was ($P < 0.01$) ~3h later than observed for baseline and adequate sleep conditions. After filtering, 70 metabolites (29 putatively identified) were altered ($P < 0.05$; Bonferroni corrected) during insufficient sleep versus baseline and adequate sleep. Specifically, three short-chain triglycerides (TAG) were elevated ($P <0.05$) during insufficient sleep versus baseline and adequate sleep. Furthermore, across all conditions, a later DLMOff after waketime was associated ($P < 0.05$) with elevated levels of a specific short-chain TAG (TAG-40:0), and elevated TAG-40:0 was associated ($P < 0.01$) with reduced oral and intravenous insulin sensitivity.

Conclusion: Insufficient sleep resulted in morning circadian misalignment and increased short-chain TAGs. More severe circadian misalignment was associated with increased TAG-40:0, and elevated TAG-40:0 was associated with lower insulin sensitivity. Previous findings show accumulation of similar short-chain TAGs increase T2D risk. Thus, our findings suggest morning circadian misalignment during insufficient sleep may increase T2D risk via increases in short-chain TAGs.

Research Funding: Supported by NIH R01HL109706 and UL1 TR000154

11:15 am SS2

A 5 Hour Delay in Meal Schedule Affects the Timing of the Human Circadian System

Skevoulla Christou[1], Sophie M.T. Wehrens[1], Cheryl Isherwood[1], Benita Middleton[1], Michelle A. Gibbs[1], Simon N. Archer[1], Debra J. Skene[1], Jonathan D. Johnston[1]

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Abstract: In humans, the entraining effect of environmental cues on peripheral clocks is poorly understood. Timed feeding entrains peripheral clock rhythms of rodents maintained under fixed light-dark (LD) cycles. We hypothesised that a 5-hr delay in the timing of meals would delay the phase of peripheral clock rhythms, but not markers of the suprachiasmatic nuclei clock (e.g. plasma melatonin and cortisol), in humans kept in fixed sleep-wake and LD cycles.

Ten healthy male participants (aged 22.9 ± 4 years (mean ± SD), BMI 23.1 ± 2.5kg/m2) conducted a 13 day controlled laboratory study. Three daily isocaloric meals adjusted to individual energy needs, were given for three days at 5-hr intervals, beginning 30 minutes after wake under 16:8 LD and sleep-wake conditions. Participants then completed a 37-hr constant routine (CR) (awake, semi-recumbent posture, dim light <8 lux, isocaloric hourly meals, social isolation) where serial blood samples, adipose biopsies and subjective questionnaires were taken to assess circadian gene expression, endocrine and other rhythms at baseline. Following this, participants completed six days where only meals were delayed by 5-hr (other parameters remained unchanged). A second 37-hr CR was then completed to assess the circadian system after the delayed food intervention.

There was no difference in subjective hunger or sleepiness between each CR. Melatonin phase, estimated by 25% dim light melatonin onset (DLMO) and plasma cortisol acrophase showed no significant differences between the 2 CRs, as hypothesised. Plasma glucose rhythms showed a significant 5-hr delay in phase between the 2 CRs. Plasma insulin and triglyceride rhythms, however, were not significantly altered. Analysis of clock gene expression (PER2, PER3) in serial adipose biopsies showed a significant delay in acrophase times of both genes in the second CR whereas blood clock gene expression (PER3) showed no significant difference between CRs.

Our data demonstrate that meal timing differentially affects peripheral but not central components of the human circadian system.

Research Funding: Funded by UK BBSRC grant BB/I008470/1 and BB/J01445/1.

11:30 am SS3

Circadian Timing and Alignment in Healthy Adults: Associations with BMI, Body Fat, Caloric Intake and Physical Activity

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Abstract: Background: Late sleep timing has been associated with higher BMI but few studies have included measures of biological timing or evaluated alignment of circadian rhythms outside of laboratory settings. The goal of this study was to determine the relationship between circadian timing

and alignment of sleep-wake with the melatonin rhythm (phase angle) with measures of obesity and obesity related behaviors. We hypothesized that shorter phase angle would be associated with higher BMI, body fat and caloric intake and lower physical activity independent of circadian timing and sleep duration.

Methods: Healthy adults with sleep duration >6.5 hours completed 7 days of wrist actigraphy, food diaries and SenseWear arm band monitoring to measure sleep, dietary intake and physical activity. Participants spent one night in the clinical research unit to measure dim light melatonin onset (DLMO). Phase angle was calculated as the duration between DLMO and average sleep onset time in the prior week. Body fat was evaluated using dual axis absorptiometry (DXA). Data were analyzed using multivariable regression analyses controlling for age, gender, sleep duration and DLMO time and sample day.

Results: Participants included 97 adults (61 F, age 26.8 + 7.3 years) with average sleep duration 443.7 (SD= 50.4) minutes. Average phase angle was 2.2 hours (SD= 1.5). Phase angle and DLMO were not associated with BMI or body fat. In multivariable models, shorter phase angle was associated with higher caloric intake (p=0.03) and with higher body fat (p=0.04) and higher android/gynoid fat ratio (p=0.03) among males only. Phase angle and DLMO were not associated with average daily physical activity.

Conclusions: Circadian timing and alignment are not associated with elevated BMI or body fat, among healthy adults with >6.5 hours of sleep, but circadian alignment may confer increased risk for weight gain through higher caloric intake.

Research Funding: K23HL109110, UL1TR000150, P01 AG11412, R01 HL090873

11:45 am SS4

Natural Patterns of Food Intake are a Weak Zeitgeber for the Liver

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Abstract: Background. Temporally restricted feeding studies have shown that food is a strong zeitgeber for the liver clock, suggesting that circadian control of food intake is critical in synchronizing peripheral tissues. Objective. Here we tested whether natural patterns of food intake, without long fasting intervals, were sufficient to entrain the liver clock. Methods. Eleven Per2::LUC mice (B6.129S6-Per2tm1Jt/J, Jackson Laboratory) were housed individually in cages on a constant 12L:12D light cycle with water freely available. Each cage was equipped with a custom built automatic feeder. Feeders dropped 20 mg pellets (S0163, Bio-Serv, Flemington, NJ) either when a pellet was removed from the trough (Ad Lib) or on a specified schedule. Mice experienced four feeding conditions in the following order: Ad Lib, Scheduled, Reversed, and Daytime-Restricted. From eating behavior during the Ad Lib condition, we calculated an average feeding profile across the day for the group. In the Scheduled condition, this average profile was then imposed on all mice. Mice received a meal every 90 minutes, with the meal size varying according to the Ad Lib average profile (total food per day adjusted for baseline intake on a per mouse basis). After 4 weeks on Scheduled feeding, the pattern was shifted by 12 hours to the Reversed condition, and 4 weeks after that, all mice were shifted to Daytime-Restricted feeding. At the end of each feeding condition, PER2::LUC bioluminescence was measured in vivo every 4 hours in isoflurane-anesthetized mice to quantify phase in the liver and submandibular gland. Results. Peak PER2::LUC bioluminescence in the liver occurred at ZT20.4 and ZT20.5 in the Ad Lib and Scheduled feeding conditions, respectively. Reversing the schedule caused a significant but small advance to ZT18.1. Daytime-Restricted feeding further advanced the peak phase

to ZT14.2. In all conditions, the PER2::LUC rhythms in the submandibular gland remained entrained to the light-dark cycle. Conclusions. Natural patterns of eating are a weak zeitgeber for the liver.

Research Funding: Medical Research Foundation New Investigator Grant

12:00 pm SS5

Measuring the Physiological Cost of Circadian Desynchrony in Mammals

Alexander West[1], Laura Smith[1], Andrew Loudon[1], David Bechtold[1]

[1]*University of Manchester*

Abstract: Almost all organisms on the planet rely on intrinsic timekeeping to anticipate fluctuations within the environment and adapt their physiology accordingly. Mammals are certainly no exception, and coordinated daily rhythms are evident in most aspects of our behaviour and physiology (e.g. from sleep/wake and feeding cycles to hormone rhythms and metabolism), all driven by a circadian clock network. The circadian clockwork is responsive to environmental signals (such as light/dark and food availability), which not only reinforces synchronisation with the external environment, but also facilitates internal synchrony between brain and tissue clocks located across the body. Unfortunately, within our modern society the natural framework of perpetual and predictable environmental rhythmicity has been undermined. Both epidemiological evidence and controlled intervention studies now clearly associate circadian disruption with cancer, immune dysfunction and numerous of metabolic and cardiovascular problems. However, the underpinning biology that associates environmental disruption of the clock with such disease states is not well understood. We therefore set out to define the impact of circadian misalignment in mice by imposing stable, but non-resonant (i.e. non-24hr) cycles of light and/or food. As expected, mice exhibited robust entrainment to environments that were within the limits of the circadian clock (e.g. 22.5hr to 27hr cycles). However, despite achieving stable entrainment, chronic exposure (>16 weeks, and up to 1 year) to non-resonant light/dark cycles resulted in widespread and tissue specific disruption of clock regulated transcriptional rhythms. Moreover, mice maintained in non-resonant light/dark cycles also exhibited a number of physiological consequences, including altered metabolic rate, reduced glucose tolerance, and cardiac dysfunction. Together these studies highlight the profound impact of circadian misalignment on mammalian physiology, and provide mechanistic insight into the clock disruption-associated pathophysiology associated with circadian disruption in human populations (such as shift work and forced desynchrony).

Research Funding: This work is funded through a David Phillips Research Fellowship (DB) and Research Grant (DB) from the Biotechnology and Biological Sciences Research Council (UK).

12:15 pm SS6

The Impact of Broad Spectrum Bright Light and Exogenous Melatonin on Plasma Glucose and Insulin in Healthy Male Participants

Mohammed Albreiki[1], Benita Middleton[1], Shelagh Hampton[1]

[1]*University of Surrey*

Abstract: Light at night is the major component for disruption of SCN function, resulting in melatonin suppression via intrinsically photosensitive retinal ganglion cells. Human Evidence from sleep deprivation (Wehrens et al 2010) and circadian misalignment (Scheer et al 2009) have reported changes in insulin sensitivity. Our previous study has shown that light at night is associated with changes in glucose tolerance and insulin sensitivity (Albreiki et al 2015). This study aims to investigate the impact of light and/or exogenous melatonin on plasma hormones and metabolites prior to and after a set meal in healthy sleep deprived subjects.

Nine healthy male participants, (26 years (SD 4.03) BMI 24.8 kg/m2 (SD 2.4)) were randomised to a three way cross over design protocol; light session (LS) (>500lux), dark session + exogenous melatonin (DSM) (<5lux), and light session + exogenous melatonin (LSM) (>500lux), separated by at least seven days. Each session started at 18:00h and finished at 06:00h the next day. Participants consumed an isocaloric meal (1066 Kcal, 38g protein, 104g CHO, 54g fat, 7g fibre), meal timings were individualised based on estimated melatonin onset. Exogenous melatonin (Circadin) was administered 90 minutes prior to the evening meal. Plasma and saliva samples were collected at specific time intervals to assess glucose, insulin and melatonin levels. Three factor repeated measures ANOVA, followed by post hoc tests and paired Student's t-test were performed.

Salivary melatonin was significantly higher in DSM and LSM compared to LS ($P < 0.001$). Postprandial glucose and insulin were significantly greater in LS than in DSM and LSM ($p = 0.01$). No significant differences were shown between DSM and LSM in all 3 measures. There were significant effects of time in all 3 measures ($p < 0.001$).

Melatonin suppression in LS was expected due to the light intensity, whereas high melatonin level in LSM and DSM was due to slow release exogenous melatonin. Postprandial plasma glucose levels were greater in LS than in DSM and LSM despite the presence of higher insulin levels and lower melatonin levels in the LS. These effects can be explained by changes in insulin sensitivity. The study confirms our findings of a previous study that reported changes in glucose and insulin responses due to the presence of endogenous melatonin in the late evening.

Research Funding: This project is supported by Abu Dhabi health services company (SEHA).

11:00 am - 12:30 pm

Slide Session: *Circadian Rhythms Across the Cell*

Chairs: Steven Brown and Gad Asher

11:00 am SS7

Around the Clock Lipidomics: Insight into Daily Oscillations in Subcellular Compartments

Rona Aviram[1], Gal Manella[1], Adi Neufeld-Cohen[1], Ziv Zwighaft[1], Meytar Elimelech[1], Yaarit Adamovich[1], Marina Golik[1], Chunyan Wang[2], Xianlin Han[2], Gad Asher[1]

[1]*Weizmann Institute of Science*, [2]*Sanford Burnham Prebys Medical Discovery Institute*

Abstract: The field of circadian clocks in mammals was revolutionized upon the discovery that circadian clocks are present in virtually every cell in the body. The presence of cell autonomous oscillators raises the question whether circadian oscillations are also found in intracellular organelles, a conjecture that so far was never thoroughly examined. Mounting evidence links circadian clocks with lipid homeostasis, and lipids as essentials of structure/function of organelles that measurably define their identity. Hence, we employed lipidomics approaches on nuclei and mitochondria isolated around the clock from mouse liver, and found that they can be used as a robust readout for endogenous time. Our results show the existence of circadian oscillations that were never reported so far in intracellular compartments. About 30% of the quantified lipids accumulate in a daily manner, with distinct and opposite phases between the nucleus and mitochondria. Remarkably, these variations are highly responsive to even mild changes in feeding time and appear to be coupled to each other. Interestingly, we found that different lipid species largely differ in their response to nighttime feeding, in an organelle-dependent manner. Our temporal and spatial analyses advance our knowledge regarding lipid homeostasis and reveal new insight regarding rhythmicity in subcellular organelles.

Research Funding: G.A: ISF 138/12, ERC-2011 METACYCLES 310320. X. H: National Institute of General Medical Sciences R01 GM105724, American Diabetes Association, Intramural institution

11:15 am SS8

A Non-Classical Nuclear Import Pathway for Clock Proteins

Sandra Korge[1], Achim Kramer[1]

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Abstract: Critical for the period of circadian rhythms is the delay between the production of the negative-limb clock proteins (e.g., PERs, CRYs in mammals) and their auto-repression. Posttranslational events such as complex formation, phosphorylation, de-phosphorylation, but also nuclear import and export dynamics and regulated degradation have been implicated in the generation of this delay. However, whereas our knowledge of the identity of the involved players is growing, the timing of many of these events is little understood. To better characterize the role of regulated subcellular localization of clock proteins, we performed a systematic RNAi-based loss-of-function screen in synchronized U2 OS cells to identify clock-regulating, nucleo-cytoplasmic transport-associated genes. In addition to

members of classical nuclear import pathway involving IMPORTINα/β, we found that knockdown of the non-classical nuclear import carrier transportin1 (TNPO1) led to a period shortening of up to 1.5h (a finding confirmed in knockout cells generated by CRISPR/Cas9). In contrast to the classical nuclear localization signal (NLS) the consensus sequence of non-classical NLS of TNPO1 cargos is less defined with PY-containing motifs being overrepresented, several of which are present in PER and CRY proteins. Performing nuclear import assays using fluorescence-recovery after photobleaching (FRAP), TNPO1-dependent nuclear accumulation of PY-containing PER- and CRY-derived peptides is demonstrated. In addition to direct binding of PER1 and TNPO1, we found that the import rate of full-length PER1 but not PER2 is substantially altered upon TNPO1 knockdown. In summary, we provide evidence for an unexpected alternative nuclear import pathway of clock proteins that is essential for normal circadian dynamics.

Research Funding: Deutsche Forschungsgemeinschaft (SFB740/D2) to AK

11:30 am SS9

Regulation of Second Messenger Pathways by Cryptochrome

Pagkapol Yhew Pongsawakul[1], Michelle Juarez[2], Tsuyoshi Hirota[3], Marc Montminy[4], Steve Kay[5]

[1]*University of California, San Diego*, [2]*The City College of New York*, [3]*Nagoya University*, [4]*The Salk Institute for Biological Studies*, [5]*The Scripps Research Institute*

Abstract: The circadian clock anticipates daily changes in environmental cycles to synchronize cellular, physiological, and behavioral rhythms. Rhythms in feeding-fasting and metabolic pathways are controlled by the clock, which at the molecular level comprises circuitries of activators and repressors. These include Cryptochromes (CRYs) that function as potent transcriptional repressors of the CLOCK/BMAL1 activators. We have shown that CRYs also function as regulators of hepatic gluconeogenesis by inhibiting glucagon-mediated stimulation of cAMP production through an interaction with the Gsα subunit of the heterotrimeric G protein in the cytoplasm.

To further investigate the novel role of cytoplasmic CRYs beyond their transcriptional functions in the nucleus, we employed the cytoplasmic mutant of Cry1 (Cry1ΔCCm) and Cry2 (Cry2ΔCCm). Overexpressing wild-type Cry1/2 and Cry1/2ΔCCm results in distinct nuclear and cytoplasmic CRY localizations, respectively, in cultured cells. We found that cytoplasmic CRY1ΔCCm or CRY2ΔCCm inhibits cAMP signaling with a higher potency than that of the nuclear counterpart CRY1 or CRY2. This inhibition of Gsα-induced cAMP signaling activity by cytoplasmic CRY is independent of the CLOCK/BMAL1 repressive activity. The inhibition of cytoplasmic CRY upon a Gsα-mediated stimulation does not depend upon the repressive activity of the Giα subunit, an inhibitory G protein.

Furthermore, we found that cytoplasmic CRYs potently and dose-dependently inhibit Gqα-stimulated intracellular Ca2+ signaling via phospholipase C/inositol triphosphate, independently of the CLOCK/BMAL1 repression. A co-immunoprecipitation assay reveals that CRY1ΔCCm and CRY2ΔCCm interact with Gsα and Gqα, but not Giα. In conclusion, our study showed that cytoplasmic CRY inhibits cAMP and Ca2+ signaling pathways by interacting with the respective Gsα and Gqα subunits of the G protein, and this inhibition is independent of the CLOCK/BMAL1 repression.

As cAMP and Ca2+ are regarded as integral cytosolic components that drive transcriptional oscillator in the SCN and possibly other tissues, we provide reciprocal evidence of core clock repressors that regulate cytosolic signals. Our study places CRY as a molecular link that connects core circadian feedback loops with the cytosolic cAMP and Ca2+ signaling.

Research Funding: This study was supported by NIH grants R01GM074868 and R01MH051573 (to S.A.K), R01DK091618 (to M.M.), and T32GM008666 (to P.Y.P) and the Thai Government Fellowship (to P.Y.P).

11:45 am SS10

Keeping Mitochondrial Network on Time

Karen Schmitt[1], Amandine Grimm[1], Robert Dallmann[2], Naotada Ishihara[3], Katsuyoshi Mihara[4], Jürgen A. Ripperger[5], Urs Albrecht[5], Stephan Frank[1], Steven A. Brown[2], Anne Eckert[1]

[1]*University of Basel* [2]*University of Zurich*, [3]*Kurume University*, [4]*Kyushu University*, [5]*University of Fribourg*

Abstract: A wide range of metabolic processes follows the rhythmicity of the circadian cycle to anticipate energetic requirements of diverse cellular functions in response to cellular and environmental constraints. Yet, only a few studies have characterized the mechanisms involved, demonstrating coupling via cyclic redox metabolite levels (e.g: NAD+ and sirtuins), mitochondrial protein acetylation and chromatin modification. To preserve the integrity of a healthy mitochondrial population within the cell but also the integrity of the cell itself, mitochondrial networks come in varied shapes and ultrastructures to ensure, among other functions, the main energy supply, stored in the form of adenosine triphosphate (ATP), by oxidative reactions from nutritional sources. Within this network, the mechanistic relationship between the clock and the mitochondrial network remains mostly elusive.

Both in vivo and in vitro, our findings indicate that the circadian clock controls rhythmic mitochondrial fission-fusion dynamics, as well as all other aspects of mitochondrial bioenergetic homeostasis, including oxidative phosphorylation, generation of ATP and reactive oxygen species (ROS). Therefore, our findings establish a novel mitochondrial dynamics-based regulatory connection between the circadian clock and metabolism, suggesting a key role for global circadian regulation of mitochondrial function in controlling circadian metabolism.

Research Funding: Swiss National Science Foundation (#31000_122572 to SAB, SF, AE and #31003A_149728 to AE), Novartis Foundation for Biomedical Research Basel, Synapsis Foundation (all to AE)

12:00 pm SS11

BMAL1 Translation and Circadian Phenotypes in Mouse Models of Tuberous Sclerosis Complex

Jonathan Lipton[1], Lara Boyle[1], Elizabeth Yuan[1], Ashwin Nathan[1], Kevin Hochstrasser[1], Peter Tsai[2], Fred Davis[3], Mustafa Sahin[1]

[1]*Boston Children's Hospital, Harvard Medical School*, [2]*Univeristy of Texas, Southwestern*, [3]*Northeastern University*

Abstract: Background: We have previous demonstrated that BMAL1 associates with the protein synthesis machinery in the cytoplasm in response to signaling through the mTOR pathway (Lipton et al., Cell, 2015). Tuberous Sclerosis Complex (TSC) is a neurodevelopmental disorder characterized by epilepsy (90%), tumor formation (>90%), autism (~50%), intellectual disability (>50%) and sleep dysfunction and is a paradigmatic "mTOR-opathy" (Lipton & Sahin, Neuron, 2014). We hypothesized that mice with deficiencies in the genes responsible for TSC – Tsc1 or Tsc2 – would demonstrate circadian rhythm abnormalities.

Methods: We have used a combination of cell culture, biochemistry, circadian luminometry, gene expression, bioorthogonal non-canonical amino acid labeling, and mouse behavior to assess cell biological, biochemical and behavioral phenotypes of Tsc1 and Tsc2-deficiency mice and cells.

Results: Here we show that mouse models of Tsc-deficiency result in abnormalities in free-running circadian activity and physiology. We find that in Tsc-deficient cells – in which mTOR signaling is elevated – the translation of BMAL1 is increased (without a concurrent increase in its overall transcription). The increase in BMAL1 is coincident with marked increases in BMAL1/CLOCK-dependent transcripts, most notably Per1 and Per2. Tsc2-deficient cells demonstrate abnormally exuberant responses to synchronization and similarly, Tsc+/- mice phase shift rapidly in response to light.

Conclusion: We have found that dysregulated BMAL1 translation may underlie circadian phenotypes in models of a devastating neurodevelopmental disorder.

Research Funding: NIH K08 Award.

12:15 pm SS12

Some Rhythm, No Cry

Marrit Putker[1], Ned Hoyle[1], Estere Seinkmane[1], Johanna Chesham[1], Mathew Edwards[1], Kevin Feeney[1], Robin Fischer[2], Nikolai Peschel[2], Ko-Fan Chen[3], John O'Neill[1]

[1]*MRC Laboratory of Molecular Biology*, [2]*Biozentrum Universität Würzburg, Germany*, [3]*Institute of Neurology, University College London*

Abstract: Circadian timekeeping in mammalian cells is facilitated by a network of transcriptional, translational and post-translational feedback mechanisms that drive oscillations in most aspects of cell biology. The generally accepted clock model consists of a core gene expression feedback loop (GEFL) involving activating transcription factors, CLOCK and BMAL1, and their repressors, Period (PER) and Cryptochrome (CRY). Of these negative factors, CRY is thought the main repressor, requiring PER to perform its full function. CRY proteins are therefore believed to be absolutely required for the ticking of the mammalian cellular clock, and CRY-deficient cells and animals are often used as arrhythmic experimental models. Here, we show that CRY1/2-deficient adult fibroblasts exhibit circadian oscillations of PER2::LUC reporter activity. The clock in these CRY-deficient cells is less robust compared with wild type, but critically exhibits the canonical characteristics of a circadian rhythm. We have employed live imaging, biochemical and pharmacological approaches to characterise the nature of CRY-independent cellular rhythms. Rhythmic PER2::LUC expression is regulated in a post-transcriptional fashion, and correlates with a rhythmic regulation of PER2 stability that persists in the absence of transcriptional cycles. Our findings add another layer of complexity to the current model of molecular circadian timekeeping.

Research Funding: Dutch Cancer Society (KWF) (BUIT-2014-6637), EMBO (ALTF-654-2014, non-stipendiary), Research Council (MC_UP_1201/4)

11:00 am - 12:30 pm

Slide Session: *Light, Brain Function and Mental Health*

Chair: Norman F. Ruby

11:00 am SS13

Independent Brain Circuits Mediate the Effects of Light on Mood and Learning

Diego Fernandez[1], Michelle Fogerson[2], Lorenzo Lazzerini Ospri[1], David Berson[2], Samer Hattar[1]

[1]*Johns Hopkins University*, [2]*Brown University*

Abstract: Light exerts profound effects on several behaviors, including synchronization of internal circadian rhythms to the solar day, sleep, mood, and learning. This photic influence requires intrinsically photosensitive retinal ganglion cells (ipRGCs) that project to a broad number of brain regions. Alterations in regular lighting conditions lead to circadian and/or sleep disruptions, which secondarily result in mood and cognitive deficits. Here we reveal a neuronal circuit involving an ipRGC-brain target located in the dorsal thalamus, which relays photic information to the medial prefrontal cortex (mPFC) to mediate the direct effects of light on mood. This dorsal thalamic brain region, however, is dispensable for the direct light effects on learning, which instead are mediated by the connection from the retina to the suprachiasmatic nucleus, the central circadian pacemaker. These results provide evidence for a novel sensory pathway, emanating from ipRGCs, whereby light can affect the mPFC, an area known to be involved in mood disorders.

Research Funding: This work was supported by the generous contributions of the PEW Charitable Trusts, and the National Institutes of Health grant GM076430.

11:15 am SS14

Melanopsin Regulates both Sleep-Promoting and Arousal-Promoting Responses to Light

Violetta Pilorz[1], Eric Tam[1], Steven Hughes[1], Carina Pothecary[1], Mark Hankins[1], Stafford Lightman[2], Vladyslav Vyazovskiy[1], Patrick Nolan[3], Russell Foster[1], Stuart Peirson[1]

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Abstract: Light plays a critical role in the regulation of numerous aspects of physiology and behaviour, including the entrainment of circadian rhythms and the regulation of sleep. These responses involve melanopsin (OPN4)-expressing photosensitive retinal ganglion cells (pRGCs) in addition to rods/cones. Nocturnal light exposure in rodents has been shown to result in rapid sleep induction, in which melanopsin plays a key role. However, studies have also shown that light exposure can result in elevated corticosterone, a response that is not compatible with sleep. To investigate these contradictory findings and to dissect the relative contribution of pRGCs and rods/cones, we assessed the effects of light of different wavelengths on behaviourally defined-sleep. Here we show that blue light (470nm) causes behavioural arousal, elevating corticosterone and delaying sleep onset. By contrast, green light (530nm) produces rapid sleep induction. Compared to wildtype mice, these responses are altered in melanopsin-deficient mice (Opn4-/-), resulting in enhanced sleep in response

to blue light, but delayed sleep induction in response to green or white light. We go on to show that blue light evokes higher Fos induction in the SCN compared to the sleep-promoting ventrolateral preoptic area (VLPO), whereas green light produced greater responses in the VLPO. Collectively, our present study demonstrates that nocturnal light exposure can have either an arousal- or sleep-promoting effect, and that these responses are melanopsin-mediated via different neural pathways with different spectral sensitivities. These findings raise important questions relating to how artificial light may alter behaviour in both the work and domestic setting.

Research Funding: This work was supported by a project grant from the BBSRC to SNP, PMN and RGF (BB/I021086/1).

11:30 am SS15

Light Modulates Spatial Learning and Memory in a Diurnal Rodent, the Nile Grass Rat (Arvicanthis Niloticus)

Joel Soler[1], Tomoko Ikeno[1], Antonio Nunez[1], Lily Yan[1]

[1]*Michigan State University*

Abstract: Bright light enhances cognitive functions in school children, healthy adults and patients in early stages of dementia. However, the underlying mechanisms of these effects of ambient illumination in humans are not well understood due to the lack of an adequate animal model; i.e., one with a day-active profile like that of our species. To fill this gap, we have used hippocampal-dependent spatial learning in the diurnal grass rat (Arvicanthis Niloticus) as a model system to investigate the effects of ambient light on cognition in diurnal species. The animals were housed in either 12:12 hr bright light-dark (BLD) or dim light-dark (DLD) condition. Chronic daylight deficiency impaired hippocampal-dependent learning and memory in a Morris Water Maze (MWM) task, with DLD animals having longer latencies to locating the platform and spending less time searching in the goal quadrant when compared to animals housed in BLD. The expression of hippocampal brain-derived neurotrophic factor (bdnf) and its protein product (BDNF) were also attenuated in the DLD group as revealed by qPCR and immunohistochemistry. The reduction of BDNF immunoreactivity was significant within CA1 region. Consistently, proliferation of apical dendritic spines was also decreased in the DLD group in the CA1 region as revealed by Gogi staining. Additionally, placing the animals previously housed in the DLD into the BLD condition for 4 weeks significantly improved performance in the MWM and upregulate BDNF expression in the hippocampus. Morphological analysis of hippocampal dendritic spines using Golgi staining is being carried out in animals that were transferred from DLD to BLD. These experiments provide evidence that light modulates cognition in a diurnal species, and serve as a starting point for determining the neural underpinnings of that modulation.

Research Funding: NSF grant IOS 1051919

11:45 am SS16

Attention Deficits in Night Owls in the Morning

Andrea Smit[1], Ashley Livingstone[1], Mateusz Michalik[1], Ralph Mistlberger[1], John McDonald[1]

[1]*Simon Fraser University*

Abstract: Synchrony effects of performance between chronotype and time of day have been shown in many tasks at the level of behavioural output (Schmidt et al. 2007 for review). Multiple neural processes contribute to behavioural performance, yet it is currently unknown which stages of processing are sensitive to the interaction between chronotype and time of day. The present study sought to examine if synchrony effects were evident at early stages of visual processing. The Event Related Potential (ERP) technique was used to measure brain responses to visual stimuli. This within-subject study directly examined variability in attentional performance in a visual search task using ERP components associated with attentional selection (N2pc) and suppression (PD). Extreme chronotypes (early versus late types) were tested at 9 AM and 4 PM. Two predictions were put forward: 1) For late types, the ability to ignore distracting information will improve from 9 AM to 4 PM, and; 2) Social jetlag would be highest at the 9 AM testing session for both chronotypes. Consistent with the first hypothesis, the ability to ignore distractions (indexed by the PD) was lower for late types during 9 AM testing times, while early types showed no difference between sessions. Consistent with the second hypothesis, social jetlag was higher at 9 AM compared to 4 PM, regardless of chronotype. Additionally, late types showed reduced visual short term memory capacity and increased sleep loss at the 9 AM session. These results are the first to show clear electrophysiological evidence of the synchrony effect, highlighting that differences in performance between chronotype are occurring at early stages of visual processing.

Research Funding: NSERC

12:00 pm SS17

Effects of Exercise Training on BMAL1 Knockout Mice

Sarah McLoughlin[1], Guangrui Yang[1], Seth Rhoades[1], Aalim Weljie[1], Garret A FitzGerald[1]

[1]*University of Pennsylvania*

Abstract: Circadian rhythms orchestrate the timing of numerous physiological processes, behaviours and more than 40% of protein-coding genes. Unsurprisingly, disruption of circadian rhythms by shift work or jet lag can have detrimental effects on human health. Prenatal loss of the circadian gene BMAL1 leads to disruption of circadian rhythms in gene expression and activity concomitant with a range of defects including premature aging, shortened lifespan, corneal inflammation, memory deficits and neurodegeneration. Loss of BMAL1 in adulthood leads to similar arrhythmia in gene expression and locomotion but a milder phenotype including corneal inflammation, neurodegeneration and dysregulation of muscle transcriptome without lifespan effects. Endurance exercise training has been shown to improve mouse models of aging, muscle dysfunction and neurodegeneration. The aims of this study are to determine if 1) loss of BMAL1 affects exercise tolerance, and 2) endurance exercise can ameliorate defects of BMAL1 knockout mice. We found that, despite differential phenotypes, both prenatal and adult-inducible loss of BMAL1 led to deficits in exercise tolerance. We showed that a mild, controlled exercise program improved exercise tolerance, activity levels and markers

of neurodegeneration in BMAL1 prenatal KO mice compared to sedentary controls. However, no improvements were observed in habituation or hippocampal-dependent memory deficits of these mice. Targeted LC-MS analysis of plasma revealed a disparity in long-chain acylcarnitines between WT and KOs that was partially rescued with mild exercise. We conclude that, regardless of timing, loss of BMAL1 leads to deficiencies in exercise performance and BMAL1 prenatal knockout mice have the capacity to respond to exercise training leading to improvements in neurodegeneration and metabolism. Ongoing work investigates the mechanisms by which exercise can ameliorate the overlapping deficits arising from temporally discrete loss of BMAL1.

Research Funding: NIH

12:15 pm SS18

The Role of BMAL1 in Behavioral Responses to Pheromonal Stimuli

Erica Schoeller[1], Daniel Clark[2], Sandeepa Dey[3], Lisa Stowers[3], Pamela Mellon[1]

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Abstract: Circadian rhythms are a biological mechanism by which the body synchronizes its physiological processes with the light-dark cycle. Disruptions of circadian rhythms are associated with behavioral impairments, including depression, anxiety, and addiction. Here we show that systemic deletion of BMAL1 in male mice results in complete loss of copulatory, aggressive, and fear behaviors. Since these behaviors are all driven by detection of pheromonal stimuli, we analyzed the function of the olfactory systems responsible for processing pheromones: the main olfactory epithelium and the vomeronasal organ. We found that general olfactory function was intact, demonstrated by the ability to uncover food hidden from view. We next analyzed function of the vomeronasal organ (VNO), a specialized scent organ that detects pheromones, and found that VNO neurons from BMAL1 KO males detected pheromones derived from female estrus urine at the same rate as WT. Finally, we analyzed the neural circuits involved in mating behavior in BMAL1 KO males exposed to estrus female scent by quantifying the a marker of neuronal activation, c-FOS, in the copulatory neural circuit following pheromone exposure. While the olfactory bulb and anterior olfactory bulb were activated in response to estrus scent in BMAL KO males, neuronal regions critical for regulating male copulatory behavior, including the medial preoptic area and the bed nucleus of the stria terminalis were not activated in response to estrus scent. Thus, we conclude that the failure of the circadian mutant BMAL KO male mice to respond to pheromonal stimulus is due to abnormal neuronal responses to pheromonal cues from receptive females, and thus results in complete infertility of BMAL KO males. We anticipate the aggressive and fear behavioral deficits result from similar defects in neural circuitry, and future studies will be performed to evaluate these circuits as well as underlying molecular mechanisms.

Research Funding: Lalor Foundation, R01 HD072754, R01 HD082567, U54 HD012303

11:00 am - 12:30 pm

Slide Session: ***Temperature and Cellular Stress***

Chair: Leslie Griffith

11:00 am SS19

A Calcitonin Receptor DH31R Regulates Temperature Preference Rhythm in Drosophila

Tadahiro Goda[1], Yujiro Umezaki[1], Michelle Chu[1], Fumika Hamada[1]

[1]*Cincinnati Children's Hospital Medical Center*

Abstract: Body temperature fluctuates over the span of 24 hours. Although this body temperature rhythm (BTR) influences sleep and metabolism, its underlying mechanisms are largely unclear. We previously showed that Drosophila exhibit a temperature preference rhythm (TPR), which resembles mammalian BTR. Here, we demonstrate that a class II G-protein coupled receptor (GPCR), Diuretic Hormone 31 receptor (DH31R), a homologue of the mammalian Calcitonin receptor, regulates TPR during the daytime, but not locomotor activity rhythms. While DH31R is expressed in the clock cells, the Dh31r mutation does not affect the molecular clock. On the other hand, the mutant of Dh31, a ligand of DH31R, exhibited normal daytime TPR and locomotor activity rhythms. However, surprisingly, a double peptide mutant of Dh31 and pdf exhibited a more severe abnormality in both daytime TPR and locomotor activity rhythms than each single peptide mutant. Excess of either tethered-DH31 (t-DH31) or t-PDF in the clock cells rescued these double peptide mutant phenotypes. Our data suggest that DH31 has a synergistic role with PDF to regulate both daytime TPR and locomotor activity rhythms. These findings identify that DH31R plays an important role in regulating TPR independently from the locomotor activity rhythms.

Research Funding: JST (Japan Science and Technology)/Precursory Research for Embryonic Science and Technology (PRESTO), the March of Dimes, and NIH R01 grant GM107582.

11:15 am SS20

Store-Operated Calcium Channels Stim and Orai Mediate Temperature Resetting of Circadian Clocks

Ozgur Tataroglu[1], Patrick Emery[1]

[1]*UMass Medical School*

Abstract: Circadian clocks integrate inputs such as light and temperature to remain synchronized with the environment. Although light input to the clock is well studied, the molecular mechanisms by which circadian clocks respond to temperature are not yet well understood. Recently, we showed that temperature shifts the Drosophila circadian clocks through an increase in intracellular calcium, which leads to Calmodulin (CaM) binding to TIM (TIMELESS) and its subsequent degradation by the atypical calcium-dependent protease SMALL OPTIC LOBES (SOL). Similarly, the mammalian homolog of SOL (SOLH) promotes thermal mPER2 degradation and phase-shifts the clock. We now show that circadian temperature resetting is mediated by store-operated calcium entry channels ORAI

and its partner STIM. Down-regulation of either STIM or ORAI, but not of other calcium channels, reduces thermal degradation of TIM, blocks thermal phase shifts and compromises entrainment to temperature cycles in flies. Thermal degradation of mPER2-LUC is also blocked when STIM or ORAI are downregulated in mammalian liver cells. The sensory subunit of these channels, STIM, has been shown to have intrinsic temperature sensitivity and can also be activated by oxidative stress, hypoxia and changes in pH levels, which can all entrain circadian clocks. Furthermore, STIM and ORAI are highly conserved from worms to humans and are ubiquitously expressed in all tissues. Combined with our results, these observations suggest that STIM might act as a conserved, broadly-tuned environmental sensor for circadian clocks that enables resetting of the clock through temperature and other signals.

Research Funding: This work was supported by NIH grants (GM079182 and GM066777) (to P.E.).

11:30 am SS21

Search for the Thermosensors Involved in Temperature Dependent Negative Masking Behavior in Mice

Wataru Ota[1], Makiko Kashio[2], Makoto Tominaga[2], Takashi Yoshimura[1]

[1]*Nagoya University*, [2]*Okazaki Institute for Integrative Bioscience, National Institute for Physiological Sciences*

Abstract: Adaptations to environmental changes are crucial to the survival of animals. Masking behavior is thought to be an acute adaptive response to environmental changes. However, the regulatory mechanisms of masking behavior are not well understood. Here we report that ambient temperature cycles induce negative masking behavior in mice.

We were originally interested in the function of a novel UV-sensitive opsin (OPN5), which is expressed in the mouse brain and is known as a deep brain photoreceptor in birds. We first observed decreased locomotor activity (negative masking response) in blinded mice during UV light exposure. Since the eye is believed to be the only photoreceptive organ in mammals, we examined if this response was light-dependent by injecting India ink under the scalp. The observed results suggested that temperature changes caused by the UV light source triggered the negative masking response. Accordingly, we examined the effect of various ambient temperature cycles (24/24°C, 24/26°C, 24/28°C, 24/30°C, 24/32°C, 24/34°C) on locomotor activity under constant darkness. These results showed that the increase in the masking ratio was directly proportional to the increase in temperature difference. Thus, the observed negative masking responses in blinded mice are demonstrated as a temperature-dependent behavior.

Since transient receptor potential channels (TRP channels) are known as the main thermosensors in mammals, we are now determining which thermosensors mediate temperature-dependent masking behavior by analyzing several TRP channel knockout mice. Currently, we found that TRPM2 deficient mice show impaired response to ambient temperature cycles, suggesting that TRPM2 is involved in temperature-dependent negative masking behavior.

We have also examined thermally-induced Fos expression in mouse brain by in situ hybridization. Several parts of the brain, including the preoptic area (POA), which is known as the thermoregulatory center, were activated by an acute change in ambient temperature (24°C to 34°C). These results are expected to contribute to identify the neural circuits regulating temperature dependent negative masking behavior in future studies.

Research Funding: This work was supported by the NEXT Program (LS055), JSPS KAKENHI (26000013), HFSP (RGP0030/2015), Grant-in-Aid for JSPS Fellows (14J03915), and IGER program.

11:45 am SS22

Endogenous Temperature Cycles Impact the Formation of Pathological Aggregates

Bala Koritala[1], Maria Olmedo[2], Mirjam Geibel[3], Martha Merrow[4]

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Abstract: Misfolded proteins are thought to be causative for neurodegenerative diseases. Protein folding and aggregation are controlled by components of the proteostasis network, namely by heat shock proteins which function as chaperones. Reinke et al. (G&D, 2008) showed that endogenous temperature rhythms induce rhythmic binding of HSF-1 (Heat Shock Factor-1, a transcription factor that regulates expression of numerous heat shock genes) to heat shock (promoter) elements. Clinical studies showed that the amplitude of the endogenous temperature rhythms is reduced in patients with neurodegenerative disease (Pierangeli et al. Springer, 1997) and bright light exposure allays symptoms in dementia patients (Riemersma-van Der Lek et al. JAMA, 2008).

We attempted to develop a simple protocol with which to study how zeitgebers impact protein folding. We hypothesized endogenous temperature rhythms may regulate the abundance of heat shock proteins and thus impact protein folding and/or aggregate formation. To test this hypothesis, we used C. elegans as model and found that temperature cycles with a 24h structure induce rhythmic expression of a subset of genes encoding heat shock proteins and of the proteins themselves. In the C. elegans Huntington's disease model animal, poly-glutamine aggregate formation is decreased in 24h temperature cycles. Proteomic analysis of the aggregates reveals a subset of distinct components depending on zeitgeber condition.

Research Funding: Ludwig Maximilian University, Munich, Germany which includes mainly reagents.

12:00 pm SS23

Neurodegenerative Disease and Circadian Clock Dysfunction: Untangling the Role of Tauopathy

Joshua Gamsby[1], Danielle Gulick[2], Korey Stevanovic[2], Amara Yunus[2], Dan Pham[2], Aurelie Joly-Amado[2]

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Abstract: BACKGROUND: Alzheimer's disease (AD) is the 6th leading cause of death in the United States and one of the most costly diseases impacting the aged population. AD affects 1 out of every 8 elderly Americans and consumes an annual healthcare budget of $200 billion dollars. Dementia and memory loss are the hallmarks of this disease and are associated with the formation of Amyloid Beta (Aβ) plaques and Neurofibrillary Tangles (NFTs) in regions of the brain associated with behavior and memory. In addition to dementia and memory loss, disruption of normal circadian rhythm physiology – e.g. sleep/wake cycle and body temperature rhythms – frequently occurs. For example, up to 40% of all AD patients have reported sleep disruption with patients exhibiting excessive daytime sleepiness and unintentional sleep episodes during the day, as well as a decrease in total sleep at night. Although troubling for the patient and care giver on its own, circadian disruption (CD) may also contribute to the dementia and memory loss associated with this devastating illness. Most of the work directed

towards elucidating the link between CD and AD has focused on mouse models of Aβ pathology, thus it is presently unclear how NFTs or tauopathy, the accumulation of hyperphosphorylated tau protein that leads to NFT formation, contributes to CD. RESULTS: Preliminary data generated from our lab using a transgenic mouse model of tauopathy (Tg4510) show that these mice display increased bouts of wakefulness during the light cycle (when they should be sleeping), and display a long free-running period at an age when tauopathy is present. In these same mice, we found evidence of tauopathy in the suprachiasmatic nucleus nucleus as well as a disruption in the cyclic expression of the core clock proteins PER2 and BMAL1 in the hypothalamus. Interestingly, we also observed disruption in the cyclic expression of PER2 and BMAL1 in the Tg4510 mouse hippocampus, which is involved in normal memory function and is a key site of neurodegeneration in AD, suggesting a possible role for tauopathy-induced CD in this loss.

CONCLUSIONS: These results demonstrate that tauopathy can disrupt normal circadian clock function both in the master clock in the hypothalamus, and in peripheral clocks, such as the hippocampus.

Research Funding: This work is funded by the USF Department of Molecular Medicine, the USF Health Byrd Alzheimer's Institute, and the Alzheimer's Association.

12:15 pm SS24

The Chondrocyte Clock Gene Bmal1 Controls Cartilage Homeostasis and Integrity

Michal Dudek[1], Nicole Gossan[1], Nan Yang[1], Hee-Jeong Im[2], Jayalath Ruckshanthi[1], Hikari Yoshitane[3], Xin Li[2], Yoshitaka Fukada[3], Ray Boot-Handford[1], Qing-Jun Meng[1]

[1]*University of Manchester*, [2]*Rush University Medical Center*, [3]*The University of Tokyo*

Abstract: Osteoarthritis (OA) is the most prevalent and debilitating joint disease, with no effective disease modifying treatments. It has multiple risk factors including ageing and results in the progressive damage and loss of articular cartilage. Autonomous circadian clocks have been demonstrated in mouse cartilage and environmental disruption of circadian rhythms in mice predisposes to OA-like damage. However, the contribution of the cartilage clock mechanisms to tissue homeostasis is still unclear. Here we show that the expression of BMAL1, a core clock transcription factor, was disrupted in human OA cartilage and in aged mouse cartilage. Furthermore, targeted Bmal1 ablation in mouse chondrocytes abolished their circadian rhythm and caused progressive degeneration of articular cartilage. BMAL1 directs the circadian expression of many genes implicated in cartilage homeostasis including those involved in catabolic/anabolic and apoptosis pathways. Loss of BMAL1 reduced the levels of pSMAD2/3 and NFATc2, decreased the expression of major matrix-related genes Sox9, Acan and Col2a1, but increased the levels of pSMAD1/5. These results define a novel regulatory mechanism linking the chondrocyte BMAL1 to the maintenance and repair of cartilage, and suggest circadian rhythm disruption as a risk factor for joint diseases such as OA.

Research Funding: Medical Research Council; Arthritis Research UK; Wellcome Trust (UK); NIH/NIAMS; Veterans Affairs (VA) BLD&R Merit Review Award (USA); Arthritis Foundation; MEXT of Japan

11:00 am - 12:30 pm

Slide Session: ***Clocks and Immunity***

Chair: Shigenobu Shibata

11:00 am SS25

Timing of Parasitic Helminth Infection is Critical in Determining Long-Term Adaptive Immune Responses

Thomas Hopwood[1], Sarah Otto[1], Julie Gibbs[1], David Ray[1], Kathryn Else[1], Andrew Loudon[1]

[1]*University of Manchester*

Abstract: The circadian clock has been implicated in immune coordination across a range of diseases, but little is known of its role in parasitology. Although gastrointestinal parasite infection affects more than one billion people globally, causing significant morbidity and mortality, our knowledge of exactly how the immune system combats these parasites is incomplete. The nematode Trichuris muris provides a physiologically useful model to study gastrointestinal parasite infection in mice. Following infection, time-course of response is typically 3-4 weeks for a full immunological response and worm expulsion. Extensive studies describe a paradigm whereby generation of a polarised Th2 response yields efficient worm expulsion, and resistance to infection, whereas an immune response polarised towards Th1 renders a mouse susceptible to persistent infection. Our data reveal that mice infected with T. muris in the morning (ZT0) generate strong Th2 responses, and efficient worm expulsion by 3-4 weeks. In marked contrast, mice infected in the evening (ZT12) generate Th1-polarised responses and are significantly less able to expel parasites. To dissect mechanisms underlying temporal control, we used a reverse feeding schedule (mice only have access to food during the photo-phase). This shifted gut circadian gene expression by 12h. Despite both groups being exposed to the parasite at ZT0, day-fed mice were significantly more susceptible to infection than night-fed mice, implicating the importance of an intrinsic oscillator within the gut. Next, we used conditional genetics to target BMAL1 in T-cells (CD4+ve), but this failed to disrupt time-of-day effects. We then investigated the role of dendritic cells (CD11c+ve), gut-resident immune cells that prime the local adaptive immune response. Mice deficient for BMAL1 in dendritic cells generated a "resistant" Th2-type immune response irrespective of the time at which they were infected. Thus, our data implicate a critical role for the circadian clock within antigen-presenting dendritic cells in coordinating a local adaptive immune response to facilitate worm expulsion. Further, our data suggest that education of the dendritic cell into either a Th1- or Th2 promoting antigen-presenting cell occurs early in infection.

Research Funding: BBSRC, Welcome Trust

11:15 am SS26

Achilles is a Circadian Clock Controlled Gene that Regulates Innate Immune Function in Drosophila

Michael Hughes[1]

[1] *University of Missourin, St. Louis*

Abstract: The circadian clock is a transcriptional / translational feedback loop that drives the rhythmic expression of downstream mRNAs. Termed "clock-controlled genes", these molecular outputs of the circadian clock orchestrate cellular, metabolic, and behavior rhythms. A major challenge for the circadian field is to identify key upstream regulators of circadian mRNA expression and to identify how they control rhythmic outputs at an organismal level. We have identified a novel clock-controlled gene in Drosophila neurons, Achilles (Achl), which is rhythmic at the mRNA level and which represses expression of anti-microbial peptides in the innate immune system. Achl knock-down in neurons results in dramatically elevated levels of crucial immune response genes, including IM1, Mtk, and drosomysin. As a result, animals with knocked-down Achl expression are resistant to immune challenge with both gram-positive and gram-negative bacteria. Notably, Achl knock-down in the absence of immune challenge significantly diminishes the fly's overall lifespan, indicating an energetic or metabolic cost of constitutively activating this pathway. We speculate that Achl signals through neuropeptidergic pathways involved in immunity and metabolism. Taken together, these results demonstrate that (1) Achl is a novel clock-controlled gene, (2) Achl links circadian clocks to regulation of the innate immune system, and (3) suggest that Achl signals through neuropeptides to relay circadian signaling from neurons to the fatbody, a principal metabolic and immunological tissue in flies.

Research Funding: We acknowledge funding from the University of Missouri Research Board and the University of Missouri - St. Louis College of Arts and Science.

11:30 am SS27

Simulated Night Shift Disrupts Circadian Rhythms of Immune Functions in Humans

Marc Cuesta[1], Geneviève Dubeau-Laramée[1], Nicolas Cermakian[1], Phillipe Boudreau[1], Diane B. Boivin[1]

[1]*Douglas Mental Health University Institute, McGill University*

Abstract: Introduction: Recent research has unveiled a circadian regulation of the immune system in rodents. Yet, little is known about rhythms of immune functions in humans and how they are affected by circadian disruption. Here, we assessed rhythms of cytokine secretion by immune cells and tested their response to simulated night shifts. Methods: Nine healthy subjects (22.8±3.6 years old; 1 woman) were studied individually in time isolation for 6 days. Peripheral blood mononuclear cells were collected from each participant kept in constant posture over 24 h under a day-oriented schedule (baseline) and after 3 days under a night-oriented schedule (night shift). Monocytes and T lymphocytes were stimulated ex vivo with lipopolysaccharide and phytohemagglutinin, respectively. Cytokines secreted in response to this stimulation were measured by ELISA. In addition, the relative proportion of monocytes and T lymphocytes was measured by flow cytometry following surface

staining Results: At baseline, a bimodal rhythmic secretion was detected for different cytokines, namely IL-1β, IL-6, and TNFα: a night peak was mainly due to a higher responsiveness of monocytes and a day peak was partly due to a higher proportion of monocytes. A rhythmic release was also observed for IL-2 and IFNγ with a nighttime peak due to a higher cell count and responsiveness of T lymphocytes. Following night shifts, except for IL-2, cytokine secretion was still rhythmic but with peak levels phase advanced by 4.5-6h, while the rhythm in monocyte and T lymphocyte numbers was not shifted. Conclusion: These results suggest distinct mechanisms of regulation between responsiveness to stimuli and cell numbers of the human immune system. Under a night-oriented schedule, only cytokine release was partially shifted in response to the abrupt change of the sleep-wake cycle. This led to a desynchronization of the different rhythmic immune parameters, which might contribute to the increased risk of infection, autoimmune diseases, cardiovascular and metabolic disorders, and cancer reported in night workers.

Research Funding: Canadian Institutes of Health Research

11:45 am SS28

Characterization of the Circadian Control of Human Circulating Neutrophils

Krisztina Ella[1], Roland Csépányi-Kömi[1], Krisztina Káldi[1]

[1]*Semmelweis University*

Abstract: Neutrophils are essential responders in bacterial and fungal infections, and they also contribute to tissue reactions in many autoimmune and inflammatory diseases. Although several immune responses linked to neutrophil functions have been described to be rhythmic, relatively few data are available about the impact of the circadian clock on the regulation of neutrophils. To get deeper insight into the operation of the molecular clock in these cells, we compared the time dependent expression of core clock components in human neutrophils and mononuclear cells. We found characteristic differences between the cell fractions in both the daily expression pattern and the relative expression of clock genes. BMAL1 showed low expression and the reduced nuclear accumulation in neutrophils, suggesting that the molecular oscillator is down-regulated in these cells. By following the expression of the maturation marker Cxcr4 and morphological characteristics (side-scattering properties and nuclear segmentation), we found that distribution of young and aged cells within the peripheral neutrophil pool displayed a daily rhythm. In addition, the plasma level of the CXCR4 ligand CXCL12- an important regulator of cell trafficking within the bone marrow - also showed synchronous fluctuations during the day. Expression of another maturation marker, the core component of the superoxide generating NADPH oxidase gp91phox, was also dependent on the time of the day, and changed parallel with the superoxide producing capacity of the cells. We suggest that maturation-dependent changes in neutrophil responsiveness rather than the cellular autonomous clock are involved in the daily regulation of human neutrophil functions.

Research Funding: NKFIH (K 108382, K115953)

12:00 pm SS29

Role of Inflammatory Signaling in the Mechanism by which the Saturated Fatty Acid, Palmitate, Modulates Circadian Clock Properties

Sam-Moon Kim[1], Nichole Neuendorff[1], Robert Chapkin[1], David Earnest[1]

[1]*Texas A&M University*

Abstract: Circadian clock dysregulation is a key factor in diet-induced metabolic disorders. Because palmitate (PAL) is a prevalent saturated fatty acid in high fat diet (HFD), which triggers proinflammatory responses contributing to metabolic disorders, we used in vitro and in vivo approaches to examine the relation between PAL effects on circadian clock properties and inflammatory signaling. During prolonged PAL treatment, fibroblast Bmal1-luc rhythms and mouse activity rhythms were marked by increases in circadian period (≈3h and 30min, respectively) relative to BSA controls. Interestingly, the effects of PAL treatment on circadian period in vivo were accompanied by increases in circulating levels of free fatty acids and increased activation of proinflammatory M1 macrophages with decreased alternative M2 activation. Acute PAL treatment induced time-dependent phase shifts of fibroblast Bmal1-luc rhythms, with maximal phase advances (2-4h) at hour 12, but only small shifts (<0.5h) at hour 6 and 24. This time-dependent variation in its phase-shifting effects on the clock mechanism was contemporaneous with the rhythmic pattern of PAL-induced inflammatory responses in fibroblasts; peak induction of NF-kB phosphorylation and IL-6 expression, which are key mediators of inflammatory signaling, occurred in response to acute PAL at hour 12, but these PAL-induced increases in inflammatory signaling were blunted at hour 6 and 24. To examine the role of inflammatory signaling in PAL-induced resetting of peripheral circadian clocks, we next determined whether the anti-inflammatory fatty acid DHA or other inhibitors of inflammatory signaling repress peak inflammatory and phase-shifting responses to PAL in Bmal1-dLuc fibroblasts. Treatment with DHA, AICAR (AMPK activator) or cardamonin (NF-κB inhibitor) significantly decreased PAL-induced NF-kB activation such that signaling activity was comparable to the basal levels found in BSA controls. Similarly, DHA, AICAR and cardamonin had significant effects in attenuating (by 70-80%) PAL-induced phase shifts of fibroblast Bmal1-dLuc rhythms. Therefore, inflammation through AMPK and the NF-κB signaling pathway may play a key role in the mechanism by which PAL induces circadian clock disturbances associated with HFD-mediated metabolic dysregulation.

Research Funding: None

12:15 pm SS30

A Novel Mechanism Links Inflammation to the Clock through REV-ERBα Protein Stability

Marie Pariollaud[1], Julie Gibbs[1], Baoqiang Guo[1], Nicholas Tomkinson[2], Dion Daniels[3], Yolanda Sanchez[3], Andrew Loudon[1], David Ray[1]

[1]*University of Manchester*, [2]*University of Strathclyde*, [3]*GlaxoSmithKline*

Abstract: The clock-controlled nuclear hormone receptor Rev-ERBα has emerged as a critical regulator of multiple pathways involved in metabolism, development and immunity. Recently, we discovered a major role for the clock in epithelial cells regulating lung inflammation, mediated by

control of neutrophil chemokine expression. In these new studies, we examined the role of REV-ERBα in pulmonary immunity. We used in-vivo gene targeting and nebulised lipopolysaccharide (LPS), a model for gram-negative bacterial infection, ex-vivo cell biology approaches and in vitro cell models.

Initial studies of Rev-erbα knock-out mice revealed an increase in pulmonary neutrophilia and inflammation upon aerosolised LPS challenge. Ex-vivo analysis revealed bronchial epithelial cells and macrophages both responded to selective REV-ERB agonist GSK1362 with a repression of inflammatory cytokines such as Il6 in macrophages and a repression of Cxcl5 in lung epithelial cells. Moreover, by selectively deleting the REV-ERBα DNA binding domain (DBD) in the mouse bronchial epithelium, we observed exaggerated inflammatory responses to LPS and augmented CXCL5 secretion; this is strikingly similar to the effects of BMAL1 deletion in these same cells (Nature Medicine 20, 2014), and implicates REV-ERBα as a major regulator of circadian inflammatory responses. Furthermore, a dual deletion of REV-ERBα DBD and REV-ERBβ in mouse bronchial epithelium has a more dramatic effect on neutrophil recruitment and chemokine secretion than deletion of just the REV-ERBα DBD; in both basal and bacterial challenged conditions.

We next tested whether expression of REV-ERBα protein is responsive to inflammatory stimuli. Using a novel monoclonal antibody, we observed a striking loss of REV-ERBα protein upon pro-inflammatory challenge. Further analysis revealed this degradation was dependent on 26S proteasome and driven by ubiquitination of REV-ERBα. Moreover, using novel REV-ERB-selective ligand GSK1362, ubiquitination was blocked and the protein protected from degradation.

Collectively, our results now propose a new model for a central role for REV-ERBα in conferring clock control to lung neutrophilic inflammation. We also identify a feed-forward circuit activated by inflammatory stimuli, leading to suppression of the endogenous anti-inflammatory REV-ERBα protein. Finally, we have discovered a novel mechanism for small-molecule regulation of REV-ERB as an anti-inflammatory molecule, operating via suppression of endogenous protein ubiquitinylation process. These observations implicate REV-ERB as a novel therapeutic target in human inflammatory disease.

Research Funding: Biotechnology and Biological Sciences Research Council, GlaxoSmithKline CASE studentship

11:00 am - 12:30 pm

Slide Session: ***Post-Transcriptional Regulation in the Clock***

Chair: Seung-Hee Yoo

11:00 am SS31

A Period2 Phosphoswitch Keeps the Beat in the Rising Heat

Jae Kyoung Kim[1], Zhou Min[2], Daniel Forger[3], David Virshup[2]

[1]*Korea Advanced Institute of Science and Technology*, [2]*Duke-NUS Graduate Medical School*, [3]*University of Michigan*

Abstract: Period (PER) protein phosphorylation is a critical regulator of circadian period, yet an integrated understanding of the role and interaction between phosphorylation sites that can both increase and decrease PER2 stability remains elusive. In this talk, we demonstrate a phosphoswitch model, where two competing phosphorylation sites determine whether PER2 has a fast or slow degradation rate. This mathematical model accurately reproduces the three-stage degradation kinetics of endogenous PER2. With the combination of mathematical modeling and biochemical experiments, we find that the phosphoswitch is intrinsically temperature sensitive, slowing down PER2 degradation as a result of faster reactions at higher temperatures. First envisioned nearly 60 years

ago, the phosphoswitch provides a biochemical mechanism for circadian temperature compensation. This phosphoswitch additionally explains how metabolic cues and timekeeping mutations regulate PER2 stability to control clock speed. The phosphoswitch provides a general mechanism to integrate diverse stimuli to regulate circadian period.

Research Funding: IRG10nov023 to D.M.V. from National Medical Research Council, Singapore; a program grant from the HFSP RPG 24/2012 to D.B.F.; and DMS-0931642 from NSF to J.K.K.

11:15 am SS32

CNOT1 Promotes Phosphorylation of Mammalian Clock Proteins via Pka

Yunfeng Zhang[2], Haitang Qin[2], Yongjie Feng[2], Peng Gao[1], Han Wang[2], Joseph Takahashi[3], Guocun Huang[1]

[1]*University of Texas, Southwestern Medical Center*, [2]*Soochow University*, [3]*University of Texas Southwestern, Howard Hughes Medical Institute*

Abstract: Protein kinase A (PKA) is a key component in the Neurospora circadian feedback loop, and the NOT1 protein is important for maintaining both WC-1 and WC-2 levels and promotes their phosphorylation. However, it remains unknown whether the related protein CNOT1 is associated with protein kinase A in the eukaryotic clockwork. In the present study we show that CNOT1 associates with both mammalian CLOCK and BMAL1, promotes their phosphorylation and stability, and inhibits the transcriptional activity of CLOCK/BMAL1. Expression of either CLOCK, BMAL1 or CNOT1 could interact with endogenous PKA as assessed by Co-immunoprecipitation. PKA can directly phosphorylate CLOCK and BMAL1 and is promoted by CNOT1. Genetic deletion of PKA by CRISPR/Cas9 results in longer periods of the circadian rhythm; while overexpression of PKA induces shorter periods. Furthermore, we found that CNOT1 associates with CLOCK and BMAL1 in liver and promotes their phosphorylation during the activation phase. PER2, but not CRY2, is also a PKA target. Our results suggest that CNOT1 and PKA play a critical role in the mammalian circadian clock.

Research Funding: National Natural Science Foundation Grant 31271281 (to G.H.)

11:30 am SS33

The E3-Ubiquitin Ligase Mdm2 Targets Period 2 for Degradation and Influences the Circadian Period Length

Jingjing Liu[1], Carla Finkielstein[1]

[1]*Virginia Tech*

Abstract: The mammalian circadian clock is sustained by the interplay of positive and negative transcriptional feedback loops, for which the turnover rate of CRY and PER proteins influences the clock's period length. A regulatory mechanism that controls protein stability is given by the proteasome pathway, which uses F-box β-TrCP1/2 E3 ligases to mark PER1 and PER2 substrates for degradation via ubiquitination. We know now that β-TrCP1/2 ligases selectively recognize phosphorylated degradation motifs (degron) in target substrates. Accordingly, overexpression of β-TrCP1/2 result in decreased phospho-PER2 levels; unexpectedly, overexpression of the dominant negative forms of the E3 ligases

led to a decrease of non-phosphorylated PER2. Our results indicate that PER2 ubiquitination engages two different types of E3 ligases that timely control PER2 accumulation in the nucleus. We report that the mouse double-minute 2 homolog (Mdm2) RING finger E3 ligase binds to PER2 in multiple regions including an overlapping site with β-TrCP. We determined that non-phosphorylated PER2 is a specific substrate for Mdm2 and that Mdm2-mediated PER2's polyubiquitination occurs both in vitro and in cells. Accordingly, overexpression of Mdm2 impacts PER2 half-life, whereas, down-regulation of this ligase by siRNA expression results in PER2 stability. Furthermore, we found Mdm2-dependent post-translational modification of PER2 directly influences circadian period length in MEF:LUC cells. Overall, our findings support a model in which, the degradation rate of PER2 during its accumulation phase in the nucleus is strictly controlled by the interplay between Mdm2 and β-TrCP E3 ligases; however, the balance is shifted toward β-TrCP-mediated PER2 degradation as its phosphorylated form accumulates over time. As a result, the levels and activity of these ligases play a determinant role and influence period length through a direct target on a key player of the negative feedback loop.

Research Funding: National Science Foundation

11:45 am SS34

Clock Transcription Factor CCA1 is Regulated through Sumoylation

Louise Hansen[1], Gerben van Ooijen[1]

[1] *University of Edinburgh*

Abstract: The circadian clock, or oscillator, is an endogenous timekeeper that synchronises biological processes with daily external rhythms such as light and temperature cycles. Post-translational regulation of clock proteins is essential for robust timekeeping. We now show that SUMO, a small ubiquitin-related post-translational modifier, contributes to oscillator function in Arabidopsis.

Mutant lines defective in SUMO machinery, including SUMO-ligase and -protease mutants, display aberrant, long circadian rhythms. Additionally, we observed sumoylation on the crucial plant clock transcription factor CCA1 in vivo. A fraction of the protein is sumoylated across the expression window of CCA1, with the phase of peak sumoylation in advance of peak total CCA1. In vitro experiments show that sumoylation negatively affects the affinity of CCA1 to its cognate promotor element, suggesting that SUMO could act as a reversible attenuator of CCA1 activity.

Research Funding: Louise Hansen and Gerben van Ooijen are funded by The Royal Society (UF110173 and RG120372).

12:00 pm SS35

Determining how CLK Promotes CYC Expression and Clock Function in Drosophila

Tianxin Liu[1], Guruswamy Mahesh[1], Wangjie Yu[1], Paul Hardin[1]

[1]*Texas A&M University*

Abstract: The identification and analysis of clock genes in Drosophila revealed that circadian timekeeping is based on transcriptional feedback loops in which CLOCK-CYCLE (CLK-CYC) heterodimers activate transcription of their feedback repressors PERIOD (PER) and TIMELESS (TIM). Clk is unique among clock genes because it is able to generate 'ectopic clocks' when expressed in non-clock cells. Like clocks within canonical clock cells, ectopic clocks also require cyc, which implies that cyc is broadly expressed. However, analysis of a transgene expressing GFP-CYC showed that CYC is present exclusively in canonical brain pacemaker neurons, suggesting that Clk somehow activates cyc when expressed in non-clock cells. If Clk activates cyc transcription, cyc mRNA should be enriched in clock cells. However, previous work suggests that cyc mRNA is not enriched in clock cells. Consistent with these studies, we find that cyc mRNA levels are not altered in Clkout null mutants, but CYC protein levels decrease dramatically in Clkout flies. These results indicate that Clk controls CYC protein synthesis or accumulation rather than cyc transcription. Since CLK binds to CYC directly, we hypothesize that CLK promotes CYC expression by stabilizing CYC protein. We tested this hypothesis in cultured S2 cells, and found that CYC protein is rapidly degraded when expressed alone but is stabilized when co-expressed with CLK. Likewise, when CLK was expressed in non-clock neurons in the fly brain, CYC levels increased specifically in the resulting ectopic clock cells. These results indicate that CLK promotes CYC accumulation by stabilizing CYC protein. Current experiments focus on defining the spatial pattern of cyc mRNA expression and determining the basic genetic requirements for a functional clock in ectopic cells. Successful completion of this project will reveal how Clk initiates clock function within and outside the normal clock cell pattern.

Research Funding: This research is funded by NIH grant R21NS094807.

12:15 pm SS36

Exploring the Connection Between Circadian Clock, Long Non-Coding RNA and Heterochromatin with Age

Jinhee Park[1], William Belden[1]

[1]*Rutgers University*

Abstract: The circadian clock is an endogenous, self-sustained oscillator that allows organisms to anticipate environmental changes. The circadian clock regulates greater than 40% of genes in a tissue-specific manner and governs timing of physiological and metabolic processes. The predominant regulatory mechanism of the clock is a transcriptional negative feedback loop, which requires timed chromatin structural changes. Recent studies ranging from Neurospora to mammals indicate one of the regulatory mechanisms is facultative heterochromatin. At least in Neurospora, the regulatory mechanism is dependent on long non-coding RNAs (lncRNAs) that originate from clock genes. The long-term physiological consequence of disrupted diurnal rhythm, or mutations in core clock leads to

accelerated aging and an increased incidence in age-related diseases. To understand the mechanisms underlying the circadian clock, circadian age-related decline and facultative heterochromatin, we perform a molecular and bioinformatics approach. We find that Bmal1 interacts with telomere and that there is a diurnal rhythm in the non-coding RNA TERRA (Telomere repeated RNA). Moreover, we detect a rhythm in histone H3 lysine 9 trimethylation (H3K9me3) at a subtelomere region of chromosome 7 and the rhythmic H3K9me3 is dampened as organisms age. In order to observe the genome-wide relationship between lncRNAs and the circadian clock, we analyze diurnal lncRNA transcriptome in aging. Analysis of diurnal lncRNA shows that numerous undefined lncRNAs have dynamic expression patterns that change with time and age. Taken together, our results suggest that diurnal lncRNAs change expression with age and this may cause genome-scale changes in heterochromatin that could potentially account for the age-related decline in circadian rhythm.

Research Funding: NIH/NIGMS GM101378

11:00 am - 12:30 pm

Slide Session: ***Photoreception and Physiology***

Chair: Samer Hattar

11:00 am SS37

Rhodopsin 7 Reduces Light Sensitivity of the Eyes and Affects Circadian Photoreception in Fruit Flies

Rudi Grebler[1], Christa Kistenpfennig[2], Matthias Schlichting[3], Christiane Hermann-Luibl[1], Joachim Bentrop[4], Stephan Schneuwly[5], Pingkalai Senthilan[1], Charlotte Helfrich-Förster[1]

[1]*University Wuerzburg*, [2]*Oxitec, Ltd.*, [3]*Brandeis University*, [4]*Karlsruhe Institute of Technology*, [5]*University Regensburg*

Abstract: Rhodopsin 7 (Rh7), a new invertebrate Rhodopsin gene, was discovered in the genome of Drosophila melanogaster in 2000, but, at the time, its ability to encode a functional Rhodopsin protein had not been determined. Here we show that Rh7 operates in receptor cell 8 (R8) of the fruit fly compound eyes. Unlike other Rhodopsins, Rh7 reduces the amplitude of the electroretinogram. This is true at all wavelengths and especially pronounced after dark-adaptation. Rh7 can neither activate the phototransduction cascade nor substitute for Rh1 in the outer receptor cells (R1-6). Rh7 rather seems to reduce light signaling in Rh6-positive R8 causing one major behavioral effect: it prevents dark-adapted flies from over responding to light in the morning. Our results indicate that Rh7 is crucial for fine-tuning light sensitivity of the flies' compound eyes by interacting with Rh6 and, therefore, point to a new mechanism of Rhodopsin function.

Research Funding: German Research Foundation (DFG) Collaborative Research Center "Insect Timing", SFB 1047, INST 93/784-1

11:15 am SS38

A Photoreceptor Clock is Required for Dorsal Suppression of S Opsin in the Mouse Retina

Onkar Sawant[1], Banumathi Tamilselvan[1], Amanda Horton[1], Sujata Rao[1]

[1]*Cleveland Clinic*

Abstract: Circadian clocks in the retina regulate multiple aspects of photoreceptor physiology and function. However the role of circadian clocks in photoreceptor development is relatively unknown. Recently we have shown a requirement for environmental light in regulating photoreceptor development. To determine if light signals are important to establish a circadian clock in the photoreceptors, we specifically deleted two of the core clock genes Bmal1 and Per2 from the photoreceptors. Surprisingly the rod photoreceptors are unaffected by Bmal1 or Per2 deletion, however there is a distinct effect on the cone photoreceptors. Loss of Bmal1 results in disruption of the S (short wavelength/blue) opsin expressing cones. This phenotype persists in the adults suggesting a continual requirement for the clock in maintaining the dorsal suppression of S opsin expression.

Since thyroid hormones can regulate cone opsin expression we investigated if local mediators of thyroid hormone signaling are controlled by the circadian clock. Our analysis shows a transcriptional regulation of the type2 deiodinase (Dio2) by Bmal1. Furthermore, Dio2 null animals have a similar phenotype as the Bmal1 loss of function mutants. Our data suggests that the circadian clock in the cone photoreceptors is required for the suppression of the S opsin expression in the dorsal retina by regulating thyroid hormone signaling. This is a novel finding showing an important role for the circadian clock in mediating thyroid hormone actions and could be a common mechanism to regulate thyroid hormone mediated signaling in other tissues.

Research Funding: Cleveland Clinic Foundation, Matilda Ziegler Award and Career Development Award from Research to Prevent Blindness (RPB).

11:30 am SS39

Opn5-Mediated Photoentrainment of Retinal Circadian Clocks

Ethan Buhr[1], Russell Van Gelder[1]

[1]*University of Washington*

Abstract: Cell populations within the mammalian retina have the ability to entrain local circadian rhythms of gene expression to light:dark cycles independently of behavioral or SCN phase. OPN5 is necessary for this photoentrainment in cultured mouse retina. We wished to assess the role of OPN5 on retinal circadian rhythms and photoreception in vivo. Mice without OPN5 (Opn5-/-) and wild-type littermates were behaviorally entrained to light:dark cycles for at least 2 weeks. Opn5-/- mice require longer for behavioral photoentrainment than wild-type mice, so behavioral cycles were monitored for full entrainment. Retinas and livers were then harvested at 3 hour intervals across a 24 hour cycle. RNA transcript levels of the clock genes Per1 and Per2 were analyzed by quantitative RT-PCR. The transcripts of Per1 and Per2 in wild-type retina displayed predictable differences across the 24 hour day as has been previously reported. However, these transcripts showed variable levels from retina to retina among Opn5-/- mice, and the circadian component of Per1 and Per2 transcript levels

was not observed in the averaged values of Opn5-/- retinas. The rhythms of transcript abundance of Per1 and Per2 were not different between the livers of wild-type and Opn5-/- mice. In addition, acute induction of both Per1 and Per2 was observed in retinas of wild-type animals exposed to an acute light pulse of 30 or 90 minutes, but neither of these genes was induced in retinas of Opn5-/- mice. However, induction of c-Fos was observed in the retinas of both cohorts of mice. In conclusion, Opn5-/- retinas are deficient in local photoentrainment both in vivo and in vitro while visual and behavioral photoreception through rods, cones, and melanopsin remains intact.

Research Funding: NIH/NEI; Research to Prevent Blindness

11:45 am SS40

Dichotomous Impact of Light Flashes on Circadian Phase Shifting and Melatonin Suppression in Humans

Jamie Zeitzer[1], Raymond Najjar[1]

[1]*Stanford University*

Abstract: Background: Examination of the effects of light on circadian rhythms in humans is typically done through monitoring responses of the timing of the rhythm of hormone concentrations or core body temperature over extended (days to weeks) protocols; these are difficult, time-consuming and laborious tasks. As such, proxies for measuring the impact of light on human circadian rhythms have been frequently used and include changes in both pupil size and melatonin concentrations. Recent rodent data, however, indicates that subsets of intrinsically photosensitive retinal ganglion cells (ipRGC) have different physiologic characteristics and likely innervate different areas of the brain. We examined whether this physiology contributes to separable effects in response to a sequence of brief light flashes.

Methods: In a series of two-day protocols, 39 participants were exposed (from hours 2-3 after habitual bed time) to 60 minutes of either continuous light (n = 8) or a sequence of 2-millisecond light flashes (n=31) that varied by interstimulus interval (ISI = 2.5 - 240 seconds). Melatonin phase shift and suppression, along with changes in alertness and sleepiness, were assessed.

Results: Melatonin phase shifted in an ISI-dependent manner, with peak response to an ISI = 7.6 ± 0.53 s. Melatonin suppression, objective alertness and subjective sleepiness, however, did not exhibit ISI-dependent changes. We also compared the eight participants exposed to continuous light to a subgroup of eight participants exposed to light flashes who exhibited similar light-induced phase shifts in melatonin. Compared to the group exposed to continuous light, those exposed to the flashes did not suppress melatonin, have an increase in alertness or reduction in sleepiness.

Conclusions: Circadian phase shifting responses to a sequence of light flashes were dependent on the time between flashes, while other non-image forming light responses thought also to be mediated by ipRGC, including melatonin suppression, increased alertness, and decreased sleepiness, did not exhibit a dependence on the time between flashes. Our data cautions the use of proxy measures of circadian responses to light as the physiologic mechanisms underlying these responses may not be the same.

Research Funding: National Heart Lung and Blood Institute (1R01HL108441-01A1) and Department of Veterans Affairs Sierra-Pacific MIRECC

12:00 pm SS41

Homeostatic Slow-Wave Sleep Response to Sleep Loss Depends on Age and Prior Light History

Virginie Gabel[1], Carolin F Reichert[1], Micheline Maire[1], Christian Cajochen[1], Antoine Viola[2]

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Abstract: BACKGROUND: The amount of human slow wave sleep (SWS) is homeostatically regulated by the duration of wakefulness prior to sleep onset. Compared to 16 hours of prior wakefulness, extended wakefulness of 40 hours prior sleep onset, usually leads to a 60% increase in SWS during the recovery night in young volunteers. Here we examined whether this increase is modulated by age and experienced illuminance levels during 40 hour of extended wakefulness (i.e. total sleep loss) prior recovery sleep.

METHODS: Twenty six young and twelve older participants underwent 40-hours of extended wakefulness once under dim light (DL: 8 lux), and once under either white light (WL: 250 lux) or blue-enriched white light (BL: 250 lux) exposure. Subjective sleepiness was assessed hourly and polysomnography was recorded continuously. Participants' sleep episodes were scheduled at their habitual bedtimes prior- [baseline night (BN)] - and after the 40-h episode of extended wakefulness [recovery night (RN)]

RESULTS: Overall, older participants felt significantly less sleepy than the young during 40 hours of extended wakefulness in all three light conditions (DL, WL, and BL). Despite lower sleepiness levels, the older participants responded with a higher SWS response to sleep loss in the recovery night than the young (177,32 % vs. 39,41 %) independent of light condition. Furthermore, compared to 40-hours of extended wakefulness under DL, the SWS response after 40-h under both WL and BL was significantly stronger in both age groups, particularly in the older (older: DL: 122,57%; WL: 231,38% BL 265,78% ; $p<0.04$, light x age interaction).

CONCLUSION: Our data indicate that besides the amount of prior wakefulness also experienced illuminance levels during wakefulness impact on homeostatic sleep regulation in humans in an age-dependent manner.

Research Funding: The Research was funded by Velux Foundations Switzerland and Philips.

12:15 pm SS42

Probing Entrainment of Ostreococcus Tauri Circadian Clock by Green and Blue Light through a Mathematical Modeling Approach

Quentin Thommen[1], Benjamin Pfeuty[2], Philippe Schatt[3], Amandine Bijoux[3], François-Yves Bouget[3], Marc Lefranc[1]

[1]*University of Lille*, [2]*National Center for Scientific Research*, [3]*University Pierre et Marie Curie*

Abstract: Most organisms anticipate daily environmental variations thanks to a circadian clock which entrains robustly to the day/night cycle, despite fluctuations in light intensity due to weather or seasonal variations. Marine organisms are also subjected to fluctuations in light spectral composition as their depth varies, due to differential absorption of different wavelengths by sea water. Studying how light

input pathways contribute to circadian clock robustness is therefore important. Ostreococcus tauri, a unicellular picoplanktonic marine green alga with low genomic complexity and simple cellular organization, has become a promising model organism for circadian biology. Functional and modeling approaches have shown that a core circadian oscillator based on orthologs of Arabidopsis TOC1 and CCA1 clock genes accounts for most experimental data acquired under a wide range of conditions. Some evidence points at putative light input pathway(s) consisting of a two-component signaling system (TCS) controlled by the only two histidine kinases (HK) of O. tauri. LOV-HK is a blue light photoreceptor under circadian control, that is required for circadian clock function. An involvement of Rhodopsin-HK (Rhod-HK) is also conceivable since rhodopsin photoreceptors mediate blue to green light input in animal circadian clocks. Here, we probe the role of LOV-HK and Rhod-HK in mediating light input to the TOC1-CCA1 oscillator using a mathematical model incorporating the TCS hypothesis. This model agrees with clock gene expression time series representative of multiple environmental conditions in blue or green light, characterizing entrainment by light/dark cycles, free-running in constant light, and resetting. Experimental and theoretical results indicate that both blue and green light can reset O. tauri circadian clock. Moreover, our mathematical analysis suggests that Rhod-HK is a blue-green light receptor and drives the clock together with LOV-HK.

Research Funding: French Ministry of Higher Education and Research, Nord-Pas de Calais Regional Council, CPER CIA, ERDF, LABEX CEMPI

11:00 am - 12:30 pm

Slide Session: ***Neurotransmitters, Channels and Neuronal Networks***

Chair: Hugh Piggins

11:00 am SS43

Calcium Circadian Rhythmicity in the Suprachiasmatic Nucleus: Cell Autonomy and Network Reinforcement

Takako Noguchi[1], Tanya Leise[2], Nate Kingsbury[3], Tanya Diemer[1], Lexi Wang[1], Michael Henson[3], David Welsh[1]

[1]*University of California, San Diego*, [2]*Amherst College*, [3]*University of Massachusetts Amherst*

Abstract: Circadian rhythms in mammalian physiology and behavior are coordinated by the suprachiasmatic nucleus (SCN) of the hypothalamus. Within SCN neurons, various aspects of cell physiology exhibit circadian oscillations, including circadian clock gene expression, levels of intracellular Ca2+ ([Ca2+]i) and neuronal firing rate. [Ca2+]i oscillates in SCN neurons even in the absence of neuronal firing. To determine the causal relationship between SCN circadian clock gene expression and [Ca2+]i rhythms, as well as the neuronal network-dependence of [Ca2+]i rhythms, we used viral vectors to introduce GCaMP3, a genetically encoded fluorescent Ca2+ indicator, into SCN neurons of PER2::LUC knockin reporter mice. Then, PER2 and [Ca2+]i circadian rhythms were imaged simultaneously in single neurons, in both dispersed and organotypic slice cultures. Of 87 dispersed SCN neurons, 91% had clear PER2 rhythms and 44% had clear [Ca2+]i rhythms. Peak [Ca2+]i preceded peak PER2 by an average of 7.1 h. Of 37 neurons in SCN slices, 100% had clear PER2 rhythms and 94% had clear [Ca2+]i rhythms. Both rhythms were weakened by blocking neuronal firing in SCN slices but not in dispersed cells which lack a synchronized circadian neuronal network. Mathematical simulations also predicted differential effects of blocking neuronal firing between multi- and single-

cellular models that were consistent with experimental results. Spatiotemporal analysis of relative peak times of [Ca2+]i and PER2 in SCN slices revealed relatively stable phase relationships. Both PER2 and [Ca2+]i rhythms were abolished in SCN cells deficient in the essential clock gene Bmal1. These results suggest that the circadian rhythm of [Ca2+]i in SCN neurons is cell autonomous but reinforced by a synchronized SCN neuronal network, and dependent on the canonical transcription-translation feedback loops of the molecular circadian clock but not upon neuronal firing in single cells.

Research Funding: Supported by NIH (R01 MH082945 to DKW) and a V.A. Career Development Award (DKW).

11:15 am SS44

Inferring the Functional Resynchronization Network in the Suprachiasmatic Nucleus

John Abel[1], Kirsten Meeker[2], Daniel Granados-Fuentes[3], Peter St. John[4], Thomas Wang[3], Benjamin Bales[2], Erik Herzog[4], Linda Petzold[2], Frank Doyle[1]

[1]*Harvard University*, [2]*University of California, Santa Barbara*, [3]*Washington University in St. Louis*, [4]*National Renewable Energy Laboratory*

Abstract: In the mammalian suprachiasmatic nucleus (SCN), a population of approximately 20,000 neuronal oscillators communicate within a network to coordinate precise circadian oscillations throughout the body. There has been significant recent effort in examining the intracellular genetic oscillator and intercellular biochemical coupling mechanisms, however, the network topology driving synchronization of the SCN has remained elusive. This network has been particularly challenging to probe at single-cell resolution, due to its oscillatory components and slow coupling timescale. In this work, we investigated the SCN network at a single-cell resolution through a tetrodotoxin-mediated (TTX) desynchronization and resynchronization. We then inferred functional connections in the SCN by applying the maximal information coefficient (MIC) statistic to bioluminescence reporter data from individual neurons while they resynchronized circadian cycling. Our results show that the functional network of circadian cells during resynchronization is small-world with an exponential node degree distribution. The network additionally exhibits spatial hierarchy, with two densely-connected ventral cores surrounded by sparsely-connected dorsal shells. These findings underscore the importance of the ventral SCN in mediating synchrony, even in the absence of light input from the retinohypothalamic tract. Finally, we performed simulations of the TTX-mediated resynchronization experiment using two circadian models to validate our predictions of network structure. Our results represent the first time this network has been examined at a single cell resolution, and present a new assay which may further be used to probe SCN connectivity.

Research Funding: NIH (NINDS grant 1R01GM096873-01 to F.J.D., L.R.P., and E.D.H.)
Institute for Collaborative Biotechnologies (grant W911NF-09-0001 from the U.S. Army Research Office)

11:30 am SS45

Inhibiting Matrix Metalloproteinases 2 and 9 Alters Circadian Neuronal Firing Patterns in the Suprachiasmatic Nucleus

Kathryn Abrahamsson[1], Rebecca Prosser[1]

[1]*University of Tennessee, Knoxville*

Abstract: Neurons in the master clock, or suprachiasmatic nucleus (SCN), of the brain exhibit near 24-hr, oscillatory circadian rhythms that synchronize mammalian behavior and physiology to the environment. It is known that molecular signaling pathways inside SCN neurons regulate circadian neuronal activity, but the involvement of extracellular-matrix (ECM) molecules in SCN clock phase regulation is unclear. Day vs. night differences in extracellular proteases and glial cell morphology in the SCN set precedent for the involvement of ECM proteins in regulating these changes. Two candidate proteins, matrix metalloprotease (MMP)-2 and MMP-9, may be the link between the ECM and circadian firing output of the SCN. Classically known as ECM remodelers, MMP-2/-9 can become activated when neuronal activity increases. MMP-2/-9 are also involved in regulating NMDA-receptor activity, a critical element of the photic phase shifting pathway. To examine the role of MMP-2/-9 in the SCN, acute SCN tissue slices (acquired from C57/BL6 mice housed in a 12:12 LD cycle) were treated with the MMP-2/-9 inhibitor BiPS at time-points when activation of NMDA-receptors can and cannot shift the clock, Zeitgeber time (ZT) 16 and ZT 6, respectively. During the day after drug treatment, single cell, extracellular activity recordings were taken from SCN neurons for 10 hours. Analysis of the peak firing rate showed that BiPS application at ZT 16 produced a robust phase delay. Additionally, application of BiPS at ZT 6 resulted in a significant phase advance in clock phase. Interestingly, co-application of BiPS and the NMDA receptor antagonist, AP5, at both time points (ZT 6 and ZT 16) blocked BiPS- induced phase shifts. Evaluation of total MMP-2/-9 expression in SCN tissue using Western blotting did not reveal day vs. night differences of either MMP. However, gelatin zymography (an assay of MMP-2/-9 enzymatic activity) of SCN protein extract shows that MMP-9 is more active at ZT 6 and ZT 16 than it is during the late night (ZT 23). Further studies investigating MMP-2/-9 and their relationship with NMDARs could reveal how these extracellular proteases participate in circadian phase modulation in the SCN.

Research Funding: Funded by the University of Tennessee, Knoxville

11:45 am SS46

Glial-Neuronal Signaling Controls Circuit-Level Coupling in the Suprachiasmatic Nucleus

Marco Brancaccio[1], Michael Hastings[1]

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Abstract: The specification of circadian time at the level of individual SCN neurons stems from well characterised transcription-translation feedback loops (TTFL), similar to the ones present in peripheral clocks. However, in the SCN, inter-neuronal communication in the form of paracrine and synaptic connectivity is also important to coordinate stereotyped cycles of circadian gene expression and electrophysiological activity, whose patterns are involved in the representation of essential circadian

properties, such as day length. On the other hand, the importance of glial cells and glial-neuronal coupling, if any, in circuit-level SCN timekeeping is still poorly understood. To address this, we followed circadian activation of neuronal and glial SCN populations by measuring intracellular calcium ([Ca2+]i) in SCN organotypic cultures. SCN slices were transduced with viral vectors expressing spectrally separated genetically encoded calcium reporters, whose expression was restricted by cell-type specific promoters to neurons or glia, respectively. Co-detection of [Ca2+]i in SCN glia and neurons revealed that not only SCN glia exhibit sustained [Ca2+]i circadian oscillations, but also that they are anti-phasic to the neuronal rhythms. To investigate this further we explored the circadian behaviour of putative gliotransmitters, with a focus on extracellular glutamate, a well characterised gliotransmitter in other brain regions. By using the genetically encoded glutamate sensor iGluSnFR, we revealed self-sustained circadian oscillations of extracellular glutamate in isolated SCN slices. Interestingly, these oscillations were also anti-phasic to neuronal [Ca2+]i and membrane potential, and therefore coherent to glial [Ca2+]i. By using pharmacological manipulations, as well as genetic models of SCN glial-neuronal temporal mis-alignment, we further investigated the role of glial released extracellular glutamate and found that glial input is required to shape spatio-temporal waves of clock gene expression of the SCN neuronal circuit. These results present a new level of temporal complexity within the SCN pacemaker, specifically antiphasic glial and neuronal oscillations, and also reveal a fundamental role for glia in SCN circadian time-keeping.

Research Funding: Medical Research Council of the United Kingdom

12:00 pm SS47

Scn Neurons of Cryptochrome-Deficient Mice Lack Circadian Timing in Intrinsic Excitability States and Do Not Gate Responses to Excitatory Input

Mino Belle[1], Beatriz Baño Otalora[1], Alok Joshi[1], Hugh Piggins[1]

[1]*University of Manchester*

Abstract: Intrinsic daily or circadian rhythms in physiology and behavior are generated by the synchronized activity of neurons within the brain's master circadian clock in the hypothalamic suprachiasmatic nuclei (SCN). Individual neurons in the SCN contain the molecular clockwork of which the Cryptochrome (Cry1-2) and Period (Per1-2) genes are important components. This intracellular clock drives changes in excitability, such that SCN neurons are more excited during the circadian day than at night. This enables the SCN to communicate clock phase information to the rest of the brain, and gate their responses to environmental cues, such as the light-dark cycle. The absence of the molecular clockwork abolishes SCN-driven circadian rhythms, but it is unclear how excitability of SCN neurons and their responses to neural input are affected. Here, using SCN slices from adult Per1::Luc animals lacking a functional molecular clock (Cry1-/-Cry2-/-) and their congenic wild-type (Cry1+/+Cry2+/+) littermates, we demonstrate that most SCN neurons from Cry1-/-Cry2-/- animals lack near 24h rhythms in Per1::Luc bioluminescence. Whole-cell recordings and intracellular calcium ([Ca2+]i) imaging show that although SCN neurons from Cry1-/-Cry2-/- mice express all the spontaneous excitability states that characterize such cells in Cry1+/+2+/+ animals, they did not show obvious day-night differences in these fundamental measures of excitability. Pharmacological manipulations identify altered receptor-dependent calcium release from intracellular stores as a key signaling pathway involved, and this conduit offers a tentative link between clock and membrane activities. Additionally, we found that SCN neurons from Cry1-/-Cry2-/- mice lacked appropriate gating to AMPA, a glutamatergic mimic of the light-input pathway. This is the first study to demonstrate how the absence of a functioning molecular clock affects excitability of SCN neurons to influence gating to photic input.

Research Funding: BBSRC (MDCB and HDP), SENECA 19701/PD/14 (BB-O) and HFSP (AJ)

12:15 pm SS48

BK Channel Inactivation Regulates Daytime SCN Excitability, Circuit and Behavioral Rhythmicity

Josh Whitt[1], Andrea Meredith[1]

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Abstract: Several ion channels have been identified that regulate the circadian pattern of action potential activity in SCN neurons, and for some, their window of effect on firing rate is correlated with day versus night differences in expression. However, the mechanisms that translate these diurnal expression differences into differential effects on firing during distinct time windows is not well understood. One such channel, the BK K+ channel, has a significant effect on nighttime firing in SCN neurons, but little effect during the day. Using whole-cell voltage-clamp recordings, we found that BK channels undergo N-type inactivation stemming from association with the beta2 subunit. Inactivating BK currents predominate during the day in the SCN, reducing steady-state current levels. At night inactivation is diminished, resulting in larger BK currents. Loss of beta2 eliminates BK channel inactivation, abolishing the diurnal variation in both BK current magnitude and SCN firing, and disrupts circadian behavioral rhythms. Selective restoration of inactivation via intracellular delivery of the beta2 N-terminal 'ball-and-chain' domain rescues BK current levels and firing rate, unexpectedly contributing to the subthreshold membrane properties that shift SCN neurons into the daytime 'upstate'. This data reveals inactivation gating comprises a biophysical switch that is required to change the contribution of BK channels to SCN excitability between day and night.

Research Funding: This work was supported by NHLBI Grant HL102758 (ALM) and NIAMS Training Grant T32-AR007592 (JPW).

Tuesday, May 24, 2016

11:00 am - 12:30 pm

Slide Session: ***Consequences of Circadian Disturbance***

Chair: Carolina Escobar

11:00 am SS49

"Of Islands and Pancakes": A Novel Method to Quantify and Visualize Mistimed Rhythms

Dorothee Fischer[1], Joana L. Matera[1], Katharina Wulff[2], Kirsten van Dycke[3], Gijsbertus Van der Horst[4], Harry van Steeg[5], Russell G. Foster[2], Lena K. Keller[1], Céline Vetter[1], Till Roenneberg[1]

[1]*Institute for Medical Psychology, Ludwig-Maximilian-University*, [2]*Nuffield Laboratory of Ophthalmology, University of Oxford, United Kingdom*, [3]*Centre for Health Protection, National Institute for Public Health and the Environment (RIVM); Erasmus University Medical Center*, [4]*DErasmus University Medical Center*, [5]*Centre for Health Protection, National Institute for Public Health and the Environment (RIVM), Leiden University Medical Center*

Abstract: Introduction. Sleeping at the 'wrong' internal time can have consequences for health and safety, particularly apparent in shift work, occupations with frequent jet lag, and psychiatric disorders featuring disrupted sleep-wake cycles. Desynchrony between sleep, circadian physiology, and external (social) demands –often called circadian disruption– are argued to underlie these adverse outcomes. Studies investigating its causes and consequences need a good quantification. Here, we propose a novel method to quantify the mistiming of behavioral and physiological rhythms, and demonstrate its versatility on diverse data sets, ranging from shift workers, day workers and school kids to patients with schizophrenia and jetlagged mice.

Methods. Our approach calculates the distance between actual and ideal time series represented by a vector length using Pythagoras' theorem. Actimetry and light data from rotational shift workers (n = 53, study period = 4 weeks), day workers (n = 24, 5 weeks), school kids (n = 20, 4 weeks), patients with schizophrenia and healthy controls (n = 36, 6 weeks) as well as breast-cancer prone mice in chronically altered or regular 12:12 light-dark conditions (n = 10, 10 weeks) were analyzed and compared with previously described measures of circadian disruption (i.e., Inter-daily Stability (IS) and 'Behavioral Entrainment' (BE)).

Results. Our method shows good congruence with IS and BE (bivariate correlations, r = -0.56IS – -0.48BE, P < 0.001), but offers unique, additional information. We unveil a distinctive geometry ("Islands and Pancakes") across subjects, work regimes and study protocols allowing for (i) statistical and visual comparison of different work and school schedules based on their timing and regularity, (ii) evaluation of activity-rest rhythms in psychiatric patients and controls associating disrupted rhythms with sleep problems in both groups, and (ii) identification of distinct behavioral response clusters ('shift worker', 'circular' and 're-entraining') to environmental changes in mice.

Conclusion. In view of a rising number of people exposed to disrupted sleep by unusual and early work hours altering daily routines and work-leisure balance, our method can help to systematically examine the role of mistimed rhythms in health and safety on an individual basis.

Research Funding: This research was funded by ThyssenKrupp Electrical Steel Europe and Siemens AG as well as a scholarship of Hanns-Seidel-Foundation to DF.

11:15 am SS50

Developmental Origin of Health and Disease (DOHaD) and the Circadian Clock: Later Life Health Effects of Gestational Circadian Rhythm Disturbance in Mice

Inês Chaves[1], Bram van der Eerden[1], Yanto Ridwan[1], Rutger Boers[1], Johannes van Leeuwen[1], Jeroen Essers[1], Joost Gribnau[1], Irwin Reiss[1], Gijsbertus van der Horst[1]

[1]*Erasmus University Medical Center*

Abstract: Recently, we provided causal evidence for circadian rhythm disturbance (CRD) as a breast cancer risk factor (van Dycke et al., 2015, Curr Biol 25:1932). In the present study, we investigated later life adverse health effects triggered by CRD during pregnancy.

Pregnant C57BL6 mice were subjected to either a constant 12hr light:12-hr dark LD cycle (control) or to repeated (i.e. once every 3 days) 8-hr phase advanced (eastbound jet lag, EJL) or delayed (westbound jet lag, WJL) LD cycles. At the day of delivery, dams and their offspring were kept again under a constant light-dark cycle. In the first weeks after birth, both EJL and WJL offspring showed a reduced weight gain, resulting in a life-long reduction in body weight.

At the age of 3 months, circadian performance was assessed in male offspring. EJL offspring displayed an increased tau in DD. Furthermore, EJL offspring adjusted faster to an 8 hr phase advance, while, oppositely, WJL offspring took less time to overcome an 8 hr phase delay.

Animals were followed in time for the occurrence of health effects. Strikingly, analysis of the bones (i.e. femur) of 6-month-old male offspring by microcomputed tomography revealed reduced trabecular and cortical bone mass in EJL offspring. Especially in the diaphysis, endocortical volume, perimeter and moment of inertia (a proxy for bone strength), were significantly reduced, whereas cortical thickness was elevated. Analysis of the cardiovascular system of 9-month-old female offspring revealed left ventricle hypertrophy in 50% of the EJL offspring.

In conclusion, we have shown that circadian rhythm disturbance in pregnant female mice by a chronic jet lag affects the development of the circadian system and predisposes to health effects in adult life. According to the Developmental Origins of Health and Disease (DOHaD) theory, fetal programming permanently shapes the body's structure, function, and metabolism. In this scenario gestational environmental factors (e.g. nutritional insults), interacting with the genes, contribute to later life disease. We are currently investigating whether gestational CRD leaves epigenetic marks on the fetal genome that remain present throughout life and that touch upon circadian performance, physiology and metabolism, and as a direct consequence, affect the vulnerability to later-life disease.

Research Funding: Research was funded by Erasmus University Medical Center.

11:30 am SS51

Metabolic Consequences of Internal Desynchrony

Vincent van der Vinne[1], Steven Swoap[2], Linh Vong[3], Bradford Lowell[3], David Weaver[1]

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Abstract: Disruption of the circadian system in humans doing shiftwork is associated with increased BMI and risk of metabolic syndrome. Internal desynchrony between the phase of the SCN and peripheral oscillators is believed to be a major contributor to the adverse consequences of shiftwork. Most animal models have both internal desynchrony and external desynchrony in which environmental rhythms reset the SCN repeatedly. To isolate the effect of internal desynchrony, we used a GABA-cell specific Cre-driver to lengthen the circadian period of the SCN but not the periphery (Vgat-Cre+; CK1Delta flox/flox; CK1Epsilon flox/+). In these mice, the SCN has an intrinsic period of ~27h while peripheral tissues maintain an intrinsic period of ~24h as determined by PER2::LUC bioluminescence recording in vitro. The long period of the SCN results in similarly long periods (~27h) in locomotor and body temperature rhythms in vivo, which do not entrain to 24h (12L:12D) lighting cycles but do entrain to 27h LD-cycles (13.5L:13.5D). In vivo measurement of PER2::LUC bioluminescence in anesthetized mice shows that peripheral oscillators are entrained to the long behavioral period with an advanced phase (5-6h), revealing internal desynchrony between the SCN and peripheral oscillations. Surprisingly, these mice have a 15% reduction in body mass relative to controls, when fed either normal chow or a high fat diet. These data are not consistent with the hypothesis that internal desynchrony leads to obesity.

Research Funding: National Institutes of Health R21 ES024684-01 to David Weaver

11:45 am SS52

Circadian Rhythm De-Synchronization Exacerbates Pathological Outcomes in an Animal Model of Ischemic Stroke

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Abstract: Circadian rhythm desynchronization in shift workers has been linked pathophysiology of chronic health disorders, especially vascular disease and related risk factors such as obesity and diabetes. The susceptibility of the vascular system to circadian rhythm disruption is illustrated by evidence indicating that stroke- and cardiovascular-related, but not overall, mortality is significantly increased among male shift workers. Thus, we examined the extent to which circadian desynchronization during chronic shifts of the light:dark (LD) cycle exacerbates ischemic stroke outcomes in rats and whether its detrimental impact on stroke impairments are further modified by biological sex.

Adult male and female rats were exposed for 8wks to either a fixed or shifted (12hr advance/5d) LD 12:12 cycle and then subjected to middle cerebral artery occlusion (MCAo). Pre and post sensorimotor testing was performed to assess functional deficits. Brains were collected at 5d post MCAo and processed for histological analysis of infarct volume.

Circadian entrainment of activity rhythms in all male and female rats exposed to the shifted LD cycle was severely disrupted. While regular estrous cycles were observed in females exposed to fixed

LD conditions, cyclicity was abolished and persistent estrous was evident in all shifted LD females. Disruption of estrous cyclicity in shifted LD females was associated with a significant increase in serum estradiol levels relative to that observed in fixed LD controls. Circadian rhythm disruption exacerbated stroke outcomes in both shifted LD male and female rats and potentiated sex differences in stroke impairments. In males, but not females, circadian disruption after exposure to the shifted LD cycle was marked by high rates mortality (80%). In surviving females on shifted LD cycles, circadian desynchronization produced significant increases in stroke-induced infarct volume and sensorimotor deficits with corresponding decreases in serum IGF-1 levels. These results suggest that circadian rhythm disruption associated with shift work schedules or the irregular nature of our everyday work and/or social environments may interact with other non-modifiable risk factors such as biological sex to modulate the pathological effects of stroke.

Research Funding: American Heart Association 14GRNT18370013 (DE)

12:00 pm SS53

Night Shift Work Disrupts Fractal Regulation of Human Motor Activity

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Abstract: Background and Objectives: Human motor activity possesses fractal activity fluctuations with similar temporal correlations across different time scales from minutes to hours. Fractal activity patterns can be a hallmark for health because the patterns are robust in healthy young under different environmental conditions but are altered with aging and in diseases such as dementia and mood disorders. Here we tested whether fractal activity patterns are disrupted during night shifts that are known to disturb circadian rhythms.

Methods and Results: We collected and examined ambulatory activity recordings of 13 permanent night shift workers (24-48 years old; 8 females and 5 males) and 14 controls who had 'normal' sleep-wake schedules and no night shifts (20-49 years old; 8 males and 6 females). We performed detrended fluctuation analysis (DFA) to quantify temporal correlations in activity at time scales from ~0.1-8h. Daytime activity fluctuations in controls showed strong correlations, as characterized by the DFA-derived exponent α~1.0 that was similar at different time scales, e.g., α1=1.00±0.02 (SE) at time scales<1.5h and α2=0.97±0.03 at >2h (p>0.2). Fractal activity patterns broke down in chronic shift workers during night shifts, i.e., correlations at time scales >2 h (α2=0.74±0.04) were weaker than those at smaller time scales (<1.5h) (α1=0.89±0.01; p =0.0007). The correlations at both small and large time scales were significantly weaker those in the control subjects (p=0.0005 for α1; p < 0.0001 for α2). In the same shift workers during days off after night shifts, the activity correlations became stronger (i.e., α1=0.97±0.02, p<0.0001; α2=0.88±0.04, p=0.02) but the fractal patterns were not fully restored as indicated by the slight but significant difference in correlations between the two time-scale regions (i.e., α1 > α2; p=0.02). Despite the changes in activity correlations, mean activity level during the wake-time of days off was similar to that of night shifts (p>0.1). The perturbed patterns at >2 hours resemble those observed in dementia patients while the perturbed patterns at <1.5 hours are reminiscent of those in patients with mood disorders.

Conclusion: Night shift work disrupts fractal activity regulation, which suggests detrimental health impacts.

Research Funding: NIH grants R00-HL102241, R01AG048108-01A1, P01AG009975, R01-HL094806, R01-DK099512, R01-HL118601, and NSBRI through NASA Grant NCC 9-58

12:15 pm SS54

Unravelling the Mechanisms of Chronic Circadian Rhythm Disturbance Using Transcriptomics and Metabolomics Approaches

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Abstract: Over the past years shiftwork has repeatedly been associated with negative health outcomes. Recently, we have shown that chronic circadian rhythm disturbance (CRD) increases breast cancer risk and body weight in cancer-prone p53R270Ha/+ WAPCre conditional mutant mice (Van Dycke et al., 2015). This study provided the first experimental proof that chronic CRD increases breast tumor development. Additionally, these data suggest that internal desynchronization and sleep disturbance are mechanisms linking shift work with cancer development and obesity.

In the current study, we further investigated the biological mechanisms underlying CRD-induced adverse health effects, in this unique mouse model as well as in female nurses participating in the KLOKWERK study. From the mouse study we used biomaterials (blood and liver biopsy) that have been collected at several time-points during the long-term study (1 year). These samples also included collected materials at different time-points during the day to investigate diurnal rhythmicity.

Analysis of liver transcriptomics in the mouse study showed a dramatic decrease in the number of liver genes with rhythmic expression due to CRD (LD: 549 rhythmic genes, CRD: 133 rhythmic genes). This was mainly due to a decrease in amplitude under prolonged CRD conditions (16 weeks exposure). These findings are indicative of a re-entrained, yet attenuated rhythmicity in gene expression upon prolonged exposure to weekly LD-inversions. Interestingly, enrichment analysis showed this attenuated rhythmicity occurs in genes involved in hormonal regulation, response to exogenous compounds and metabolism, comprising both lipid and glucose metabolism. These results indicate that the attenuated rhythmicity of liver genes might be related to the observed effect of chronic CRD on body weight.

Analysis of blood using targeted LC/MS metabolomics showed changes in serum metabolites in the mouse study. Interestingly, most changes occurred at a relatively early stage of the study (after 8 weeks of exposure, compared to 25 and 42 weeks of exposure). Additionally, most of the changes occurred in lipid metabolites.

Subsequently, metabolites will be analyzed in a study among female nurses (KLOKWERK study). In this study, biomaterials (blood, urine, feces) are being collected from 3 groups of female nurses: 1) working night shifts and recently started (<1 year, n = 100), 2) working night shifts for > 5 years (n = 100), and 3) age-matched nurses that do not work night shifts (controls, n = 100).

Combining 'omics' approaches with both rodent and human biomaterials from the KLOKWERK study will give us new tools for a better understanding of the underlying mechanisms of CRD-induced adverse health effects. Unraveling these molecular mechanisms is an important step forward in finding solutions to minimize adverse health risks associated with shift work.

Research Funding: This research is funded by the Dutch Ministry of Social Affairs and Employment.

11:00 am - 12:30 pm

Slide Session: ***Evolution, Synthetic Biology, Environment and Circadian Clocks***

Chair: Charalambos Kyriacou

11:00 am SS55

A Tunable Artificial Circadian Clock in Clock-Defective Mice

Choogon Lee[1], Matthew D'Alessandro[1], Jae Kyoung Kim[2], Stephen Beesley[1], Joseph Takahashi[3]

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Abstract: Self-sustaining oscillations are essential for diverse physiological functions such as the cell cycle, insulin secretion, and circadian rhythms. Synthetic oscillators using genetic feedback circuits have been generated in cell culture. These synthetic systems provide important insight into design principles for biological oscillators, but have limited similarity to physiological pathways. Here we report the generation of an artificial, mammalian circadian clock in vivo, capable of generating robust, tunable circadian rhythms. In mice deficient in Per1 and Per2 genes (thus lacking circadian rhythms), we artificially generated PER2 rhythms and restored circadian sleep/wake cycles with an inducible Per2 transgene. Our artificial clock was tunable as the period and phase of the rhythms can be modulated predictably.

A core feedback loop driving oscillations in the PER:CRY inhibitor complex is essential for rhythmicity, while other feedback loops that drive oscillations in the CLOCK:BMAL1 activator complex may contribute to robust rhythmicity. In the inhibitor complex, PER is the stoichiometrically rate-limiting component; its phosphorylation kinetics, balanced by kinases and phosphatases, functions as a circadian timer, determining period and phase. The tunable Per2 oscillator was generated in vivo by crossing tetracycline (Tet)-controlled transgenic Per2 mice (also responsive to doxycycline [Dox]) with Per1/2 double knockout mice. We showed that exogenous (Tet-driven or Dox-driven) oscillations of transgenic Per2 (tPer2) can restore molecular and behavioral rhythms to Per-knockout mice. Although we use the PER2 protein to interface with endogenous clock output pathways, this is not a simple rescue experiment because the endogenous clock feedback loop is no longer the main timekeeping mechanism in these mice; it is supplanted by an artificial mechanism driven by Tet or Dox. Our work shows that many of the endogenous genetic circuits in the current clock model are dispensable, and further study of our artificial clock may help to distinguish the truly critical mechanisms of clock regulation. Furthermore, the design principles of our approach may be applied to engineer other artificial oscillators for the study of other physiological processes.

Research Funding: This work was partially supported by NIH grant DK-090730 and a bridge grant from the Department of Biomedical Sciences, FSU (C.L.), and NSF grant DMS-0931642 to the MBI (J.K.K.).

11:15 am SS56

Circadian and Infradian Clocks in the Urochordate Botryllus Schlosseri

Rachel Ben-Shlomo[1], Mark Kowarsky[2], Debashis Sahoo[2], Benyamin Rosental[2], Katherine J. Ishizuka[2], Karla J. Palmeri[2], Stephen R. Quake[2], Irving L. Weissman[2], Ayelet Voskoboynik[2]

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Abstract: Urochordates are non-vertebrate members of the chordate phylum and are considered as the closest non-vertebrate living relative of vertebrates. The urochordate Botryllus schlosseri is a diurnal colonial marine organism that displays circadian oscillator and an infradian blastogenic cycle, in which the somatic constituent is replaced weekly. We are studying the molecular clockwork of B. schlosseri and the association between the circadian and the blastogenic oscillators.

The pace of the blastogenic cycle is affected by the daily light cycle. In natural 24 LD and DD lightening cycle and constant temperature conditions (19.5oC) the length of a blastogenic cycle is one week. In short LD cycles of 16 and 12 hours, the blastogenic cycle shortened fundamentally. Colonies conditions persisted to be good and there was no indication of stress of any kind.

We identified putative canonical circadian as well as known clock control genes and studied their circadian and blastogenic clockwork. We found at least four different paralogous of the transcription activators clock-Bmal1. RNA-seq analysis indicates that they are oscillating along the day, and show a corresponding cycling in short LD cycles. We also identified three different paralogous of timeless, cycling in different phases in natural and short LD cycles. At least twelve casein kinases paralogous have been detected, some are cycling in short LD cycle but not in a 24hr one, others cycle in different phases, while other transcripts cycle in similar phases. We also detected an orthologue of the vertebrate photoreceptor melanopsin (opsin 4). Its transcript oscillates in both short and natural LD cycles, yet in different phases. Various known clock controlled genes also have been revealed.

The effect of the daily light cycle on the B. schlosseri blastogenesis duration suggests a potential role for circadian cycles on the blastogenic infradian cycles as well as on early zooids and colonies development. We are now testing expression (RNA-seq and real-time PCR) of the identified clock genes along the day, along the blastogenic cycle and following light entrainment.

Research Funding: National Institutes of Health; Grantee: Ayelet Voskoboynik, Irving L Weissman, Stephen R Quake

11:30 am SS57

Pollutant Affects on the Circadian Rhythm of Daphnia Pulicaria

Kayla Coldsnow[1], Brian Mattes[1], Rick Relyea[1], Jennifer Hurley[1]

[1]*Renssealer Polytechnic Institute*

Abstract: Zooplankton are an essential component of aquatic food webs and a variety of organisms rely on these invertebrates as a food source. Therefore, changes in zooplankton abundance and behaviors, due to environmental factors such as toxins and pollutants, may trigger drastic consequences on aquatic systems. Perhaps the most substantial behavior of zooplankton is the Diel vertical migration, the mass migration from the epipelagic zone to the hypolimnion zone at the night/day transition and the inverse migration occurring at the day/night transition. This phenomenon is suggested to fall

under the control of the circadian clock. However, no functional molecular timekeeping mechanism has been established in zooplankton. In this work, we investigated the presence of a circadian rhythm in a representative zooplankton, Daphnia pulicaria. We established that per mRNA oscillated in a historic Daphnia population with a period approximating 24 hours. Next, we investigated the rhythms of per mRNA in strains of Daphnia that had previously been exposed to varying levels of sodium chloride over a four-month period. We found that Daphnia previously exposed to moderate levels of salt demonstrated a shortened circadian period, while Daphnia previously exposed to high levels of salt became arrhythmic. This work has great implications in ecosystem conservation as there is an increasing concern about road salt and its ability to enter an aquatic system. Based on our data, it appears that road salt effects on Daphnia circadian rhythms could cause a significant behavioral change, making Daphnia an unreliable food source and altering the food web. The implication of this research is that human pollutants could result in alterations in Diel vertical migration, the largest migration in biomass on the planet, thereby demonstrating unforeseen consequences of ecosystem contamination.

Research Funding: Rensselaer Polytechnic Institute, The Jefferson Project

11:45 am SS58

New Insights into the Genetics of Diurnal/Nocturnal Preference

Mirko Pegoraro[1], Eran Tauber[1]

[1] *University of Leicester*

Abstract: Most animals are active during specific times of the day, either diurnal or nocturnal. In addition, some species, referred to as crepuscular, are active mainly during dawn and dusk. Although it is clear that diurnal preference is hardwired into the species' genes, the genetic basis underlying this trait is largely unknown.

Under laboratory conditions, Drosophila is crepuscular, showing a bi-modal activity profile. However, recent experiments in our lab indicated that high variability among individuals exist, particularly in strains that derive from different wild populations. By assembling together flies from different populations, we have generated a highly diverse population whose progeny exhibited extreme diurnal preference, including diurnal and nocturnal flies. We have used this population as a starting point for an artificial selection experiment in which we selected males that show the most extreme diurnal preference and mated them to their sisters. The response to selection was strong, and after 10 selection cycles we obtained highly diurnal (D) and nocturnal (N) strains. Another strain that was not selected and showed intermediate behaviour (crepuscular) served as a control (C). These strains provide us with a unique opportunity to understand the genetics of diurnal preference.

We have profiled gene expression in these lines using RNAseq and identified 209 differentially-expressed genes. In another set of experiments, we tested for genetic correlation of diurnal preference with other trait phenotypes, including those related to the circadian clock, and life history traits. We also explored the visual system in the selected lines and found a significant difference in their electroretinograms (ERG).

Overall our study encompasses a broad range of approaches including quantitative genetics, microbiology and electrophysiology within a whole-organism context, and provide the first step for understanding the impact of variation in diurnal preference in a wide range of animals, including humans.

Research Funding: This study was supported by the Biotechnology and Biological Sciences Research Council, UK (BBSRC; www.bbsrc.ac.uk) grant BB/K001922/1 to ET.

12:00 pm SS59

Codon Usage Affects Drosophila Period Protein Structure and Function

Jingjing Fu[1], Katherine Murphy[2], Mian Zhou[3], Ying Li[2], Vu Lam[2], Christine Tabuloc[2], Joanna Chiu[2], Yi Liu[1]

[1]*University of Texas, Southwestern Medical Center*, [2]*University of California, Davis*, [3]*East China University of Science and Technology*

Abstract: Most amino acids are coded by more than one synonymous codon. Although codon usage bias is a universal feature of all genomes, its biological functions, especially in animal systems, are not clear. To determine the role of codon usage in animals, we take advantage of the sensitivity and robustness of the Drosophila circadian system. Bioinformatics analyses indicate that Drosophila genome has a strong codon bias for G/C at the wobble positions. Without changing amino acids, we codon-optimized parts of the open reading frame of period, a core clock gene involved in oscillator function, and showed that per codon usage is important for circadian clock function. Optimization of per codon usage resulted in conformational changes of PER protein, altered PER phosphorylation profile and stability, and impaired PER function in the circadian negative feedback loop, which manifests into impaired molecular rhythmicity and abnormal circadian behavioral output. This study provides the first in vivo example that demonstrates the role of codon usage in determining protein structure and function in an animal system. Together with our previous studies in Neurospora and cyanobacteria, these results suggest a universal mechanism across all kingdoms of life that uses a codon usage "code" within genetic codons to regulate co-translational protein folding.

Research Funding: This work is supported by grants from the National Institutes of Health and the Welch Foundation (I-1560) to Yi Liu.

12:15 pm SS60

Circadian Genes, Photoperiodic Clock and Diapause in Insect, Pyrrhocoris Apterus

David Dolezel[1], Joana Kotwica-Rolinska[1], Lenka Pivarciova[1], Veronika Urbanova[1], Bulah Chia-Hsiang Wu[1]

[1]*Institute of Entomology*

Abstract: Background: Organisms living in temperate regions synchronize their behavior, physiology and development with 24-hour cycles and alternating seasonal environment. The endogenous circadian clock orchestrates daily periodic activity even under constant conditions with a characteristic free running period close to 24 hrs. Photoperiodic timer measures the ratio of light-to-dark duration (the photoperiod). Under long photoperiods, organism enters a summer program, while short photoperiods trigger a winter response, which usually stops development (the diapause). Connection between circadian clocks and photoperiodic timer is suggested by some authors and rejected by others.

Methods: To test a possible relation of circadian clock and photoperiodic timer, we used Pyrrhocoris apterus, a heteropteran bug with a robust photoperiodically-regulated diapause response and a solid circadian rhythmicity. Importantly, both these phenotypes are manifested in the same developmental stage, in the adult. We hypothesize, that circadian clock and photoperiodic timer are connected if a specific change of the free running period results in a shifted photoperiodic response.

Results/Conclusion: We performed a functional screen of circadian gene candidates and identified a specific manipulation leading to slower circadian clock. Females were still able to diapause under short days and fully reproduced under long photoperiods. However, the critical photoperiod, a condition when 50% of the tested females reproduce and 50% undergo the diapause, was significantly lengthened. These data suggest shared genetic components between circadian clocks and photoperiodic timer. Interestingly, northern P. apterus populations possess long free running periods and longer critical day length, while populations from central Europe have faster clock and shorter critical photoperiod.

Research Funding: Czech Science Foundation # 14-32654J (D.D.); INsecTIME (to L.P.)

11:00 am - 12:30 pm

Slide Session: *Clocks and Neuropeptides*

Chair: Christopher Colwell

11:00 am SS61

Synchronous Drosophila Circadian Pacemakers Display Non-Synchronous Ca2+ Rhythms in Vivo

Xitong Liang[1], Timothy Holy[1], Paul Taghert[1]

[1]*Washington University, St. Louis*

Abstract: In Drosophila, molecular clocks control circadian rhythmic behavior through a network of ~150 pacemaker neurons. To explain how the network's neuronal properties encode time, we performed brain-wide calcium imaging of groups of pacemaker neurons in vivo for 24 hours. Pacemakers exhibited daily rhythmic changes in intracellular Ca2+ that were entrained by environmental cues and timed by molecular clocks. However, these rhythms were not synchronous, as each group exhibited its own phase of activation. Ca2+ rhythms displayed by pacemaker groups that were associated with the morning or evening locomotor activities occurred ~4 hours before their respective behaviors. Loss of receptor for neuropeptide PDF promoted synchrony of Ca2+ waves. Thus neuropeptide modulation is required to sequentially time outputs from a network of synchronous molecular pacemakers.

Research Funding: This research was supported by the Washington University McDonnell Center for Cellular and Molecular Neurobiology and by grants from the National Institutes of Health.

11:15 am SS62

The Small Gtpase RHO1 is Required in a Dosage-Dependent Manner to Align Peptidergic Control of Behavioural Rhythms with Clock-Controlled Gene Expression

Miguel Ramírez Moreno[1], Neethi Rao[2], Herman Wijnen[3]

[1]*University of Southampton*, [2]*University of Virginia*, [3]*Centre for Biological Sciences and Institute for Life Sciences*

Abstract: In a genetic screen for mutations affecting daily locomotor activity rhythms in the fruit fly Drosophila melanogaster we uncovered a deficiency on chromosome 2R encompassing a number of genetic loci including the gene encoding the small GTPase RHO1. When heterozygous, this deficiency affected locomotor behaviour in constant dark conditions both by weakening rhythms at constant temperature and by accelerating temperature-mediated resetting. Chromosomal mutations and transgenic knockdown in clock cells targeting Rho1 produced similar behavioural deficits or even complete loss of circadian locomotor activity in constant dark (DD) conditions. Spatio-temporal mapping of these phenotypes identified contributions from both the peptidergic ventral lateral neurons expressing the neuropeptide PIGMENT DISPERSING FACTOR (PDF) as well as PDF-negative dorsolateral neurons clock neurons. Transgenic knockdown of Rho1 did not appreciably affect the survival, gross morphology, or core clock expression rhythms of relevant lateral and dorsal clock neuron subsets. This genetic manipulation did, however, blunt the diurnal and circadian rhythms of remodelling of the dorsal PDF-secreting projections of the small ventral lateral neurons (s-LNvs). Yet, the behavioural phenotype of clock-specific Rho1 knockdown was different from that of complete loss of PDF signalling in terms of the daily locomotor activity profile in light-dark cycles and the degree of arrhythmia in DD conditions. Instead, reduced RHO1 levels in clock neurons appear to trigger behavioural arrhythmia in DD due to misregulated PDF signalling. This conclusion is supported by our observation that (1) the chronobehavioural phenotypes of Rho1 knockdown are fully rescued in constant red light (RR), a condition when circadian pacemaker function does not require PDF signalling and (2) loss of function mutation of the PDF receptor gene Pdfr was epistatic to and partially rescued the behavioural deficits resulting from Rho1 knockdown. Taken together, our results indicate that RHO1 acts in both the PDF-expressing s-LNvs as well as other PDFR-expressing cells to permit clock-controlled PDF signalling and behavioural output.

Research Funding: This PhD project is funded by The Gerald Kerkut Charitable Trust and the University of Southampton.

11:30 am SS63

Reciprocal Communications of Clock Neurons via PDF and CCHa1 Neuropeptides in Drosophila

Taishi Yoshii[1], Yuri Fujiwara[1], Christiane Hermann-Luibl[2], Takanori Ida[3], Charlotte Helfrich-Förster[2]

[1]*Okayama University*, [2]*University of Wuerzburg*, [3]*University of Miyazaki*

Abstract: In the fruit fly Drosophila melanogaster, clock genes/proteins are expressed in about 150 brain neurons that control circadian activity rhythms. Most of the clock neurons have a self-sustained molecular oscillator and are able to synchronize to light-dark cycles. However, they need to communicate with each other to avoid internal desynchronization under free-running conditions. Pigment-dispersing factor (PDF) is a neuropeptide that is expressed in the s-LNv and l-LNv clusters of about 16 clock neurons and plays a role in the internal communication from the PDF-positive clock neurons to other clock neurons that express the PDF-receptor. In this study, we identified the neuropeptide CCHamide1 (CCHa1) as a new communication factor in the Drosophila circadian network. CCHa1 is expressed in the DN1a cluster and its knockdown increases the percentage of arrhythmic flies and phase-shifts the clock protein cycling in the s-LNv under prolonged constant dark conditions. We also found that the ccha1 receptor gene is expressed in the s-LNv and l-LNv clusters and we could reproduce the behavioral phenotype of the ccha1 knockdown by down-regulating ccha1 receptor gene expression in the clock neurons. Thus, our results suggest that the DN1a clock neurons communicate with the PDF-positive s-LNv neurons via CCHa1, which in turn allows the s-LNv cluster to synchronize with other clock neurons even under prolonged free-running conditions.

Research Funding: Our work is funded by the Japan Society for the Promotion of Science.

11:45 am SS64

The Ptth Neuropeptide Couples Central and Peripheral Clocks in Drosophila

John Ewer[1], Mareike Selcho[2], Carola Millan[3], Angelina Palacios-Munoz[4], Franziska Ruf[2], Lilian Ubillo[1], Jiangtian Chen[2], Chihiro Ito[5], Valeria Silva[1], Christian Wegener[2]

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Abstract: Circadian clocks impose daily periodicities to many behaviors and physiological processes in a wide variety of organisms. In animals, many tissues are known to contain circadian pacemakers, and whereas much is currently known about the molecular mechanisms that produce rhythmicity within circadian pacemakers, less is known about how the activity of these pacemakers is coordinated. In insects, most known peripheral pacemakers are autonomous, and their synchronization is accomplished through exposure to common entraining signals such as light, which can penetrate the translucid exoskeleton. A notable exception is the clock of the prothoracic gland (PG), the endocrine tissue that produces the steroid molting hormone, ecdysone. In Drosophila and other insects, the activity of the PG clock depends on the brain clock, and together these two clocks restrict the time of adult emergence to a species-specific window within the day.

We used genetic techniques, neuroanatomy, and imaging, in conjunction with an eclosion assay, to investigate the pathway that couples the central brain clock to the peripheral PG clock.

We report that the PTTH- (prothoracicotropic hormone) producing neurons, which regulate the timing of every insect molt by controlling the production of ecdysone from the PG, couple the central and peripheral clocks. Thus, either silencing PTTH neurons or knocking down PTTH expression in PTTH neurons causes a loss of the circadian rhythmicity of emergence. The same phenotype is observed when the PTTH receptor, TORSO, and other elements of the PTTH transduction pathway, are knocked down specifically in the PG. The PTTH neurons contact neurites of the PDF-expressing sLNv clock neurons, and imaging results show that these sLNvs inhibit the activity of PTTH neurons in a PDF-independent manner. By speeding up and slowing down the brain vs. PG clock we show that the brain clock is the dominant ("Master") clock as compared to the PG ("Slave") in regulating the circadian rhythmicity of emergence.

The PTTH neurons and PTTH signaling couple the central clock and the peripheral PG clock, which is essential for the expression of a circadian rhythm of emergence. In this coupling the brain clock exerts a dominant effect over the PG clock.

Research Funding: FONDEQUIP, ONR Global (USA), CINV Millennium Institute grants (to JE); CONICYT Postdoc #3160177 (To AP); German Science Foundation: SFB 1047 Insect timing, project B2 (to CW).

12:00 pm SS65

Decoding the Firing Patterns of Scn Vip Neurons

Cristina Mazuski[1], Samantha Chen[1], Tracey Hermanstyne[1], Erik Herzog[1]

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Abstract: Release of VIP (vasoactive intestinal polypeptide) and GABA from VIP neurons in the suprachiasmatic nucleus (SCN) plays a key role in entraining and synchronizing circadian rhythms. Comprising 10-15% of SCN neurons, VIP neurons are arguably the best characterized subset of SCN neurons; however little is known about what firing patterns underlie the release of VIP and/or GABA. To characterize these firing patterns, we identified the spontaneous firing activity of VIP neurons across multiple days through 'optical tagging'. To do so, we plated SCN cell cultures from mice expressing channelrhodopsin2 in VIP neurons (VIP-ChR2) on multielectrode arrays and cultured the SCN for approximately three weeks. We then recorded spontaneous extracellular firing for three days before activating the ChR2 with light flashes (10Hz for 1h). We identified VIP+ neurons by their reliable increase in firing within 10ms of the stimulation and then analyzed the previous days of spontaneous firing for characteristic firing patterns. Our results indicate that VIP+ neurons exhibit characteristic circadian and instantaneous firing patterns. Across the day, VIP+ neurons fire for longer per day and are more phase clustered compared to VIP- neurons. On shorter time scales, VIP+ neurons exhibit either tonic or 'burst-like' firing patterns. Although these neurons showed circadian variation in their firing, their tonic or 'burst-like' firing pattern remained stable throughout the day. We found similar patterns from VIP neurons in daytime SCN slice whole-cell recordings. We next tested these two firing patterns on VIP-ChR2 SCN slices in vitro for 1 h every 24 h for 5 days. Preliminary results suggest that stimulating VIP neurons with different firing patterns yielded different phase shifting and entraining effects on PER2 circadian rhythms in the cultured SCN slice. Overall, our results suggest that specific firing frequencies and patterns modulate neurotransmitter release with different consequences for the circadian system.

Research Funding: This work was supported by NIGMS F31 grant #116517 and NINDS grant #095367.

12:15 pm SS66

Doublecortin-Like Regulates Circadian Rhythms of Locomotor Activity by Controlling Vasopressin Signaling in the SupraChiasmatic Nucleus.

Erno Vreugdenhil[1], Dirk-Jan Saaltink[1], Boris Polm[1], Emilie van der Sande[1], Buddy Boerkoel[1], Johanna Meijer[1], Claudia Coomans[1]

[1]*Leiden University Medical Center*

Abstract: The biological clock in mammals is characterized by high levels of structural plasticity. We have recently identified a novel plasticity protein, Doublecortin-like (DCL), that is highly and specifically expressed in the shell of the mouse suprachiasmatic nuclei (SCN) but not in any other hypothalamic nuclei (Saaltink et al, 2012). DCL is a n neurogenesis, dendritic plicated fically expressed in the shell of the suprerous plasticity genes microtubule-associated protein, which has been implicated in neurogenesis, dendritic outgrowth and structural re-arrangements of the microtubule cytoskeleton enabling dynamic movements of cell bodies and dendrites. In addition, DCL is necessary for oxidative phosphorylation and ATP synthesis (Verissimo et al, 2013).

We have inspected DCL expression in the SCN by confocal microscopy and found that DCL expressing cells overlap with vasopressin(VP)-expressing cells. However, instead of co-localizing in the same cells, DCL expressing cells envelop VP cells. To further investigate the role of DCL in the SCN, we generated transgenic mice expressing doxycycline-induced short-hairpin RNA's targeting DCL mRNA (sh-DCL mice). Compared with littermate wild type (WT) controls, sh-DCL mice exhibit significant shorter periods of their endogenous circadian behavioural activity when analysed by passive infrared registration (PIR) sensors (23.7 hours versus 23.9 hours for WT mice, $p<0.01$). As VP receptor knockout mice exhibit similar shorter periods and as DCL expression overlaps with that of VP in the SCN, we analysed VP expression in sh-DCL mice and in their littermate controls. In line with a feedback role of VP, we observed an intricate VP+ neuronal network in WT mice with numerous varicosities in the core of the SCN. Surprisingly, compared with littermate WT mice, VP fluorescence was mainly observed in cell bodies with a disrupted VP+ neuronal network in the SCN core in sh-DCL mice. Remarkably, 3D reconstructions of VP cell bodies show a six-fold increase in volume. Together, our data points to the existence of a DCL+ cellular and plastic network in the shell that controls circadian rhythmicity by directing VP transport and feedback to the core of the SCN.

Saaltink et al (2012). The J. Comp. Neurol. 520(13), 2805–2823.

Verissimo et al, (2013). PloS One, 8(9), e75752.

Research Funding: Funded by the Leiden University Medical Center, Dutch Diabetes Research Foundation Grant 2013.81.1663 (to C.P.C.).

11:00 am - 12:30 pm

Slide Session: *Sleep*

Chair: Martha Vitaterna

11:00 am SS67

Cerebral Underpinnings of Human Circadian Performance Modulations During Sleep Loss

Carolin F. Reichert[1], Micheline Maire[1], Virginie Gabel[1], Antoine Viola[1], Eric Salmon[2], Christina Schmidt[2], Christian Cajochen[1]

[1]*University of Basel*, [2]*University of Liège*

Abstract: Background: Sleep-loss-related deteriorations in cognitive performance are particularly pronounced during night- and recover during daytime due to circadian mechanisms. Cerebral correlates of these circadian variations are largely unknown.

Objectives/Methods: In a within-subjects design, 31 healthy adults participated in a 40-h sleep deprivation (SD) and a 40-h nap protocol (NP, 10 cycles of 80-min naps / 160-min wakefulness). Blood-oxygen-level dependent (BOLD) activity during working memory (WM) performance was assessed by the n-back task every 8 h.

Results: Under SD, performance deteriorated during nighttime, but recovered during the next day ($p<0.05$). BOLD activity decreased under SD from day- to nighttime in frontal regions ($pFWE<0.05$), but did not further decline during the remainder of the SD. Superior frontal BOLD activity was positively associated with performance during SD at night and day ($pall<0.05$), and functionally connected to a hypothalamic and brainstem regions during daytime after sleep-loss ($ps.v.c.<0.05$). When assessed during the first day of SD, under normal sleep pressure conditions, activity in the postero-lateral part of the hypothalamus covaried with a marker of circadian wake-promotion (evening nap sleep efficiency; $ps.v.c.<0.05$), while this link was not observed under disproportionally low or high sleep pressure conditions. BOLD activity in the same hypothalamic region, assessed under normal sleep pressure, predicted the ability to perform well at the same circadian phase after a night of sleep-loss.

Conclusions: We have evidence for specific hypothalamic correlates of circadian wake-promotion during WM performance. These correlates vary according to sleep pressure, and might thus be involved in neurobehavioral vulnerability to sleep loss.

Research Funding: This work was supported by the Swiss National Science Foundation (# 310030_130689) and by La Roche-Foundation, and by the Niklaus and Bertha Burkhardt-Buergin Foundation.

11:15 am SS68

Social Regulation of Naturally Occurring Plasticity in Sleep and Circadian Rhythms in Bees

Guy Bloch[1], Moshe Nagari[1]

[1]*Hebrew University of Jerusalem*

Abstract: Many animals show considerable periods of activity with no circadian rhythms. In social insects such as honeybees and bumblebees natural plasticity in circadian rhythms is associated with division of labor, one of the organization principles of insect societies. Forager bees typically have strong circadian rhythms whereas "nurse" bees care for the brood around the clock with attenuated or no circadian rhythms. There is profound plasticity in this system allowing bees to switch between activity with and without circadian rhythms along with the colony needs. Little is known however about the social factors and sensory pathways that mediate plasticity in circadian rhythms in bees or other animals. Given that around the clock activity is associated with brood care behavior, we first tested the hypothesis that the need to feed the brood induces around the clock brood tending. We developed an assay allowing high resolution monitoring of locomotor activity for individually isolated nurse bees in the presence of brood. Using this system we found that both larvae, that require feeding, and pupae that do not, modulate circadian rhythms /or and sleep in the two species of bees. Surprisingly, in bumblebees, the influence of pupae and pre-pupae on sleep was stronger compared to that of larvae. Empty pupal cocoons also affected sleep, but this effect weakened with time. This transitionary influence is consisted with the involvement of volatile chemical signals that are released from the pupae. We next tested the hypothesis that the antennae are needed for brood modulated plasticity in circadian rhythms. We amputated the flagella of nurse bees and recorded their circadian rhythms in isolation or in observation hives. Flagella removal did not affect circadian rhythms in individually isolated bees but in the hive flagella-less bees showed diurnal patterns of activity whereas sham treated control bees were active around the clock similar to nurses in typical colonies. Finally, we tested the hypothesis that the brood influence on the nurse locomotor activity rhythms is mediated by pheromones. We found no consistent influence for either brood extracts or synthetic brood pheromone on the strength of circadian rhythmicity in honeybees. Taken together our studies suggest that the regulation of circadian rhythms by the brood is complex and not limited to feeding behavior. At least some of the brood signals are perceived by the antennae, but additional studies are needed to determine to what extant brood pheromones are involved.

Research Funding: Israel Science Foundation

11:30 am SS69

Light-Dependent Regulation of Sleep/Wake States by Prokineticin 2 in Zebrafish

Shijia Chen[1], Sabine Reichert[2], Jason Rihel[2], David Prober[1]

[1]*California Institute of Technology*, [2]*University College London*

Abstract: Light affects sleep/wake behaviors indirectly by providing a cue that entrains circadian rhythms but also via direct regulation of behavior through a phenomenon known as masking. While circadian entrainment by light is well characterized at the molecular level, genes that underlie masking are largely unknown. Using zebrafish, a diurnal vertebrate, we found that both overexpression and

mutation of the neuropeptide prokineticin 2 (Prok2) affect sleep/wake behaviors in a light-dependent but circadian-independent manner. We also found that the Prok2 overexpression phenotype requires prokineticin receptor 2 and induces expression of galanin, a sleep-inducing peptide, in the hypothalamus in a light-specific manner. These results suggest that Prok2 regulates the direct effect of light on sleep/wake behaviors in zebrafish, and that it may do so in part by inducing expression of galanin in the hypothalamus.

Research Funding: National Institutes of Health, Rita Allen Foundation, Sir Henry Wellcome Trust, UCL Excellence Fellowship, European Research Council Starting Grant.

11:45 am SS70

The Lateral Line Confers Evolutionarily Derived Sleep Loss in the Mexican Cavefish

James Jaggard[1], Beatriz Robinson[1], Ian Oh[2], Bethany Stahl[1], Pavel Masek[3], Masato Yoshizawa[4], Alex Keene[1]

[1]*Florida Atlantic University*, [2]*Davidson Academy, Reno*, [3]*State University of New York, Binghamton*, [4]*University of Hawaii*

Abstract: Sleep is defined by altered sensory processing including an elevated arousal threshold, yet, little is known about the interactions between sleep and sensory processing. The blind Mexican cavefish is comprised of an extant surface dwelling form and 29 cave morphs that have independently evolved numerous sensory and behavioral changes, including the proliferation of mechanoreceptive lateral line neuromasts and the convergent evolution of sleep loss. In this study, we investigate the relationship between the highly sensitive lateral line system and sleep loss in cave-dwelling fish. Ablation of the lateral line system enhanced sleep in the Pachón cavefish population suggesting that heightened sensory input from the lateral line underlies evolutionarily derived sleep loss. Spatially targeted lateral line ablations and behavioral analysis in surface-cave hybrids localized the wake-promoting neuromasts in Pachón cavefish to superficial neuromasts of the trunk and cranial regions. Strikingly, there was no effect of lateral line ablation on sleep in four different cavefish populations tested, suggesting distinct neural mechanisms regulate the evolution of sleep loss in independently derived cavefish populations. The wake-promoting neuropeptide Hypocretin/Orexin (HCRT) is dramatically over-expressed in Pachón cavefish and pharmacological inhibition of HCRT signaling restores the surface fish sleep phenotype in Pachón cavefish. Removal of the lateral line significantly reduces hcrt expression in Pachón cavefish, suggesting the mechanoreceptive neuromasts suppress sleep by enhancing hcrt expression. Taken together, these findings demonstrate that evolutionarily derived changes in sensory processing regulate sleep and provide a model for examining the integration of these fundamental processes.

Research Funding: NSF IOS-1426265, NIH R01 NS085252

12:00 pm SS71

Sexually Dimorphic Regulation of Sleep in Drosophila

Daniel Machado[1], Dinis Afonso[1], Kyunghee Koh[1]

[1]*Thomas Jefferson University*

Abstract: Studies of sleep regulation have focused on two main mechanisms: circadian and homeostatic. However, since sleep is incompatible with other critical behaviors, such as searching for mates, additional factors (e.g., sex drive) also influence sleep. Here, we report the identification of a Drosophila neural circuit involved in male-specific regulation of sleep. Activation of a small number of neurons in the central brain, defined by the MS1-Gal4 driver, leads to markedly reduced sleep in male flies. Notably, activation of MS1 neurons has little effect on female sleep. We find that a subset of MS1 neurons express TDC2, an enzyme required for synthesis of octopamine, which is analogous to norepinephrine. Genetic and pharmacological experiments show that male sleep suppression by MS1 activation requires octopamine signaling.

A previous study showed that a male fly paired with a female fly exhibits a pattern of locomotor activity distinct from a male paired with a female [1]. We find that whereas wild-type male-female (MF) pairs show reduced sleep compared with male-male (MM) pairs, male flies in which MS1 neurons are inhibited through expression of tetanus toxin exhibit little female-induced sleep loss. These data suggest that MS1 neurons allow males to stay awake so they can search for and court females. Since the male-specific transcription factor FruitlessM (FruM) is required for male reproductive behavior [2], we examined the relationship between MS1 neurons and fruM-expressing neurons. Activation of fruM neurons result in reduced sleep specifically in males, mimicking the effect of MS1 activation. Further, our data show that MS1 and fruM neurons do not overlap, but instead form sexually dimorphic synaptic contacts. Robust GRASP (GFP Reconstitution Across Synaptic Partners) signals between MS1 and fruM neurons are observed in males, but not in females.

Collectively, our data suggest that octopaminergic MS1 neurons define a novel arousal center that forms a circuit with fruM neurons for sexually dimorphic regulation of sleep. This circuit may serve as a valuable model for investigating the neural basis of how the brain balances competing needs to arrive at appropriate behaviors.

1. Fujii S et al., Curr Biol, 2007. 17: 244-51.

2. Yamamoto D and Koganezawa M, Nat Rev Neurosci, 2014. 14: 681-92.

Research Funding: NIH/ R21NS094782

12:15 pm SS72

Dissection of the Downstream Circadian Circuitry Involved in Sleep Regulation

Fang Guo[1], Michael Rosbash[1]

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Abstract: Drosophila sleep like mammalian sleep is modulated by two systems, a homeostat influenced by experience and a circadian process controlled by the clock. The role of specific clock neurons in either sleep system is largely unknown. Using optogenetic activation and inhibition, we identified a sleep-promoting subgroup of circadian DN1 neurons. Characterization of these cells

was aided by video recording as well as by a novel reporter system, which assays calcium within small numbers of discrete neurons in wake- and sleep-behaving flies. The calcium patterns generally reflect circadian regulation as well as neuronal firing and are specific for different groups of circadian neurons. The sleep-promoting DN1 cells contact and suppress M and E cell activity via glutamate-mGluRA signaling. DN1 neuronal activity also manifests sexually dimorphic features, with parallel sexually dimorphic effects on sleep. Other DN1 subgroups have very different projection/connectivity patterns and are functionally distinct from the sleep-promoting DN1s. We will report more generally on sleep-wake-relevant connectivity within the circadian network as well as between circadian neurons and other regions of the fly brain.

Research Funding: HHMI

11:00 am - 12:30 pm

Slide Session: ***Micro-Organisms, Cancer and Cell Cycle***

Chair: Carla Finkielstein

11:00 am SS73

A Human Gut Bacterium Express Circadian Rhythms and Swarming Response to Melatonin

Jiffin Paulose[1], Meenu Krishnasamy[1], Vincent Cassone[1]

[1]*University of Kentucky*

Abstract: Recent studies have shown that the gut microbiome varies with respect to microbial abundance as well as diversity in a circadian fashion. Although dietary variations (timed restricted feeding, high fat diet, etc) have been shown to shift these rhythms, only a few signaling pathways between host and commensal bacteria have been identified. We hypothesized that one such pathway involves gut-secreted melatonin. Protein BLAST analysis querying human melatonin receptors against the collected amino acid sequences from the Human Microbiome Project yielded several proteins sharing 24-42% identity with either hMEL1A or hMEL1B sequences. A well-represented species, Enterobacter aerogenes, exhibited increased swarming in the presence of physiological levels of melatonin in semi-solid agar. The swarming effect was specific to melatonin and not found in other, related clinical strains. The swarming presented as a bulls-eye pattern, with a periodicity of ~24 hours per ring. A lux-encoding reporter plasmid under the promoter of the flagellar stator protein MOTA revealed circadian expression of pMotA independent of melatonin administration. These temperature-compensated rhythms were synchronized under 1nM melatonin. Further proteomics analysis revealed proteins orthologous to the Kai complex of Synechococcus. These data provide the first evidence of circadian rhythms in a non-cyanobacterial microbe and a novel pathway by which circadian timing information is passed from host to commensal bacteria. Current investigations are centered around potential input pathways through which melatonin interacts with these and other gut microbes. This study is funded by NIH R01 AG045833-01.

Research Funding: NIH R01 AG045833-01 awarded to Vincent Cassone.

11:15 am SS74

Trypanosoma Brucei Infection Accelerates the Mouse Circadian Clock

Filipa Rijo-Ferreira[1], Margarida Vaz[2], Luisa Figueiredo[2], Joseph Takahashi[3]

[1]*University of Texas, Southwestern / IMM*, [2]*Instituto de Medicina Molecular*, [3]*University of Texas Southwestern, Howard Hughes Medical Institute*

Abstract: Sleeping sickness is an infectious disease that, if left untreated, leads to coma and eventually death. It is caused by Trypanosoma brucei - a unicellular parasite that lives in the bloodstream and interstitial spaces of several organs, including the brain. Patients with sleeping sickness show alterations of sleep/wake cycle, body temperature and endocrine secretion, which have led to the hypothesis that sleeping sickness may be a circadian rhythm disorder. To test this, we recorded the circadian running-wheel activity of T. brucei-infected mice. We observed that even in LD conditions, despite running 2-fold less than control mice, infected mice run 7-fold more during the rest phase. After 60 days post-infection, some animals run ~50% of their daily activity during the rest phase. In the same manner, during infection the core body temperature peak shifted from the dark to the light phase. These observations show that as infection progresses the circadian rhythm of the host is disrupted. In DD conditions, we detected a gradually shorter period of running-wheel activity a few days after infection. This is surprising because parasites take >15 days to accumulate in the brain, suggesting that the early effects in mice behavior may be a consequence of a peripheral signal, metabolite or hormone that feedback on the master clock. When we assessed PERIOD2::LUC expression ex vivo of several organs of T. brucei-infected mice, we observed that, although all organs have robust circadian rhythms, those with higher parasite load have ~2 h shorter period than non-infected organs. However, this doesn't appear to be a common feature of all parasitic infections, as Plasmodium (malaria) infected mice showed no differences in PER2::LUC expression in a similar assay. Finally, we co-cultured T. brucei parasites with PER2::LUC fibroblasts and observed a shortening of the circadian period, suggesting that the presence of T. brucei parasites could directly modulate the circadian clock of their host. Overall, these results show that Trypanosoma brucei infection disrupts the circadian rhythm of its host by accelerating the period of the circadian clock.

Research Funding: Howard Hughes Medical Institute

11:30 am SS75

Activating Circadian Clock Function in Cancer Cells Inhibits Tumor Growth

Silke Kiessling[1], Lou Beaulieu-Laroche[1], Ian D. Blum[1], Dominic Landgraf[2], David Welsh[2], Kai-Florian Storch[1], Nathalie Labrecque[3], Nicolas Cermakian[1]

[1]*McGill University, Douglas Mental Health University Institute*, [2]*University of California, San Diego*, [3]*University of Montreal*

Abstract: Cancer cells exhibit uncontrolled fast cell division. Circadian clocks control tumor suppressor and key cell cycle genes, and circadian disruption is known to promote cancer. Here we addressed the hypothesis that enhancing circadian rhythmicity in tumor cells controls their cell cycle and thereby reduces proliferation.

To this end, we compared clock gene expression, proliferation, apoptosis and cell cycle phase entry of B16 melanoma cells with either a functional or a dysfunctional clock. We found that circadian

gene expression is suppressed in B16 cells, but treatments inducing circadian rhythmicity, such as dexamethasone, forskolin and heat shock, triggered rhythmic clock gene expression in B16 cells. Activation of the tumor clock induced rhythmic cell cycle mRNA and protein expression, and rhythmic entry into cell cycle phases. Interestingly, this resulted in fewer cells in S phase and more in G1 phase, indicating less DNA replication and proliferation. Thus we tested whether B16 cell growth is regulated by their intrinsic circadian clock: two days after DEX treatment we counted about 50% fewer cells than without treatment and no change in apoptosis.

When B16 cells were injected into mice to generate subcutaneous tumors, clock gene expression was low and arrhythmic, but DEX treatment induced circadian rhythms. Accordingly, DEX treatment slowed down B16 tumor growth in vivo. Similar effects were observed in human colon carcinoma HCT-116 cells in vitro and tumors in vivo, showing that the effect extends to other cancer types and to human cells.

The host circadian system did not seem to be involved as the effect of DEX was recapitulated in immune-deficient NSG mice, and immune cell infiltration in tumors was not significantly affected by DEX. Knockdown of Bmal1 in B16 cells using shRNA prevented the effects of DEX on proliferation, tumor growth and cell cycle in vitro and in vivo, demonstrating that B16 cell proliferation is regulated by their intrinsic circadian clock rather than being a clock-unrelated action of DEX.

Altogether, we demonstrate that strengthening circadian rhythmicity in tumor cells slows down the cell cycle and tumor growth. Thus, our work reveals that enhancing clock function might represent a novel strategy to control cancer progression and thereby improve the outcome of anti-cancer therapy.

Research Funding: CIHR, Natural Sciences and Engineering Research Council, Canadian Foundation for Innovation, Veterans Affairs Merit Award, Fonds de recherche du Québec

11:45 am SS76

Real-Time Bioluminescence Reporters of Circadian Rhythms and Signaling Pathways in Solid Tumours in Vitro and in Vivo

Robert Dallmann[1], Ludmila Gaspar[2], Ermanno Moriggi[2], Steven A. Brown[2]

[1]*University of Warwick*, [2]*University of Zurich*

Abstract: Circadian clocks modulate most mammalian physiological processes in a time-of-day dependent manner. In reverse, pathophysiological maladaptations like cancer can either disrupt the temporal structure of physiology and metabolism, or are caused by such a disruption. On the mechanistic level, these processes are not fully elucidated yet.

Here, we used bioluminescence reporter assays to assay the clock status of a number of established cell lines and human primary tumors cells in vitro. We found that while some cell lines are robustly rhythmic, others are arrhythmic. We then confirmed this interesting result in cells that were obtained from patient tumor biopsies and confirmed these results. However, using exemplary arrhythmic and rhythmic mouse cancer cell lines, we found that both, clock containing and clockless tumor cell lines were rhythmic in vivo. Subsequently, we wanted to if this "rescue" effect happens other cell signalling pathways controlling physiology and metabolism in developing tumors using reporter system developed in the lab. Again, closely related clockless and one clock containing murine colorectal adenocarcinoma lines, i.e., C51 and C26 were used. Like circadian clock function, we found that some signaling pathways exhibit a highly rhythmic pattern in vivo, but not in vitro. This is highly interesting because, it suggests that tumor physiology might be – at least – partially driven by the host clock, and, thus, giving an enticing possible rational chronotherapy. Especially the results from

our in vivo experiments will be valuable in understanding which cellular pathways are rhythmically regulated in tumors by the host clock.

Research Funding: Krebsliga Schweiz, Alexander von Humboldt Foundation, Swiss National Science Foundation

12:00 pm SS77

Intercellular Coupling of Cell Cycle and Circadian Clock in Adult Stem Cell Cultures

Toru Matsu-ura[1], Andrey Dovzhenok[1], Eitaro Aihara[1], Jill Rood[2], Yan Ren[1], Tongli Zhang[1], Marshall Montrose[1], Sookkyung Lim[1], Sean Moore[2], Christian Hong[3]

[1]*University of Cincinnati*, [2]*Cincinnati Children's Hospital Medical Center*, [3]*University of Cincinnati College of Medicine*

Abstract: Circadian clock-gated cell division cycles are observed from cyanobacteria to mammals through intra-cellular molecular connections between the two oscillators. Here, we demonstrate Wnt signaling-mediated inter-cellular coupling between the cell cycle and the circadian clock in 3D murine intestinal organoids (enteroids). We observe synchronized circadian rhythms and cell division cycles in enteroids with 1:2 coupling ratio of circadian rhythms (24-h) and the cell cycle (12-h). Knockdown of a core circadian clock gene, Bmal1, dramatically reduced the synchronized cell division cycles, suggesting that the circadian clock is necessary for the synchronization of cell cycle progression. Remarkably, we observed reduced amplitude oscillations of circadian rhythms in intestinal stem cells and progenitor cells, indicating that intracellular molecular connections are unable to mediate circadian clock-dependent cell division cycles. Instead of the intracellular coupling, we discovered that circadian clock in Paneth cells and cell cycle in intestinal stem cells and progenitor cells are intercellularly coupled through periodic secretion of Wnt from Paneth cells. Our stochastic simulations reproduced 1:2 coupling of circadian clock and cell cycle with Wnt as the key coupling component. Our finding revealed a new role of Paneth cells as a local pacemaker which regulate the timing of cell divisions in intestinal stem cells and progenitor cells in the context of circadian rhythmicity.

Research Funding: DARPA

12:15 pm SS78

Cry2 and Fbxl3 Promote Circadian Destruction of c-Myc

Anne-Laure Huber[1], Stephanie Papp[1], Emma Henriksson[1], Sabine Jordan[1], Anna Kriebs[1], Madelena Nguyen[1], Martina Wallace[2], Zhizhong Li[3], Christian Metallo[2], Katja Lamia[1]

[1]*The Scripps Research Institute*, [2]*University of California, San Diego*, [3]*The Genomics Institute of the Novartis Research Foundation*

Abstract: Circadian disruption due to shift work and other aspects of modern life profoundly impacts physiology and increases risk of disease, including cancer. Here, we describe an unexpected biochemical function for the circadian repressor Cry2 as a required component of an Fbxl3-containing E3 ligase complex that recruits phosphorylated c-MYC for ubiquitylation. c-MYC is a critical regulator of cell proliferation; the phosphorylation site in c-MYC that interacts with the Cry2-Fbxl3 dimer (T58)

has long been recognized as a hotspot for mutation in cancer. T58 is the central residue in a c-MYC phospho-degron, though the full machinery responsible for its turnover has remained obscure. Cry1 cannot substitute for Cry2 in this complex; we show that their unique functions explain previously conflicting reports regarding their role in tumorigenesis that has fueled controversy about the relationship between circadian clocks and cancer. Genetic deletion of Cry2 increases cell growth and tumorigenesis, while Cry1 can sustain circadian rhythms of behavior and transcription. Thus, increased cancer risk caused by circadian disruption does not require global disruption of rhythmic gene expression.

Research Funding: Supported by grants from the NIH (DK090188 and DK097164), the Kinship Foundation, the Sidney Kimmel Cancer Research Foundation, and the Lung Cancer Research Foundation.

Wednesday, May 25, 2016

11:00 am - 12:30 pm

Slide Session: *Clock Outputs*

Chair: Han Wang

11:00 am SS79

Loss of ZBTB20 Causes Unimodal Behavioral Rhythms and Impairs Circadian Output

Zhipeng Qu[1], Hui Li[2], Guangsen Shi[1], Zhiwei Liu[3], Pancheng Xie[1], Guocun Huang[4], Joseph Takahashi[4], Weiping J Zhang[2], Ying Xu[1]

[1]*Nanjing University to Soochow University*, [2]*Second Military Medical University*, [3]*Soochow University*, [4]*University of Texas Southwestern, Howard Hughes Medical Institute*

Abstract: Many animals display morning and evening bimodal activities in the 24 h day/night cycle. However, little is known regarding the potential components involved in the regulation of morning and evening behavioral rhythms in mammals. Here, we found that the loss of zinc finger protein gene Zbtb20 led not only to a loss of evening activity but also to a burst in morning activity. The change in bimodal to unimodal activity was also found to downstream rhythms, including body temperature, metabolism and light responsiveness. We further showed that ZBTB20 participated in the regulation of Prokr2 expression, and the injection of adeno-associated virus (AAV) expressing PROKR2 could partly restore evening activity in Nestin-Cre; Zbtb20fl/fl mice, demonstrating that ZBTB20-mediated PROKR2 signaling was critical for the maintenance of evening behavioral rhythms. Using a combination of RNA sequencing and mass spectra identification, we furthermore revealed that the transcriptional factor ZBTB20 targeted multiple metabolic and neurological processes, providing a potential mouse model that integrates circadian output signals in neurodegenerative disorders.

Research Funding: This work is supported by National Science Foundation of China (No. 31230047).

11:15 am SS80

How Does the Mammalian Circadian Clock Generate Tissue-Specific Rhythmic Outputs?

Joshua Beytebiere[1], Helene Vitet[1], Jerome Menet[1]

[1]*Texas A&M University*

Abstract: Circadian clocks in mammals rely on the heterodimeric transcription factors CLOCK:BMAL1 to coordinate the rhythmic expression of 10-15% of the genome, and enable biological functions to perform optimally at the most appropriate time of the day. Characterization of clock-controlled genes in different tissues revealed that rhythmically expressed genes are largely tissue-specific, and many genes that are rhythmically expressed in one tissue are constitutively expressed in others. In this project, we aimed at characterizing the mechanisms by which the circadian clock, which is identical in every cell, controls a tissue-specific circadian transcriptional program. To this end, we first identified the genes targeted by the circadian clock in three mouse tissues (liver, heart, and kidneys) by performing BMAL1 chromatin immunoprecipitation (ChIP) at the genome-wide level. We found that BMAL1 DNA binding sites are mostly tissue-specific and the few BMAL1 sites common to all three tissues lie in the core clock genes promoter. Further analysis indicate that tissue-specific enhancers, i.e. chromatin regions that are open and accessible to transcription factors in a specific tissue, largely contribute to the tissue-specificity of BMAL1 DNA binding. In addition, BMAL1 binding sites are significantly enriched for the consensus sequences of tissue-specific transcription factors, suggesting that cooperative DNA binding also affect the binding of CLOCK:BMAL1 in a tissue-specific manner. Thus, our results suggest that both chromatin accessibility and cooperation of BMAL1 with tissue-specific transcription factors enable the molecular clock to coordinate a tissue-specific circadian transcriptional program that rhythmically regulates tissue-specific biological functions.

Research Funding: Texas A&M University start up funds.

11:30 am SS81

Integrating Functional Genomics Data Reveals Tissue-Dependent Mechanisms Underlying Circadian Gene Expression

Jake Yeung[1], Saeed Omidi[1], Felix Naef[1]

[1]*EPFL*

Abstract: Mammalian organs have evolved to schedule distinct functions at specific times throughout the day. This synchrony with the daily fluctuations in the external environment uses the circadian clock, which ticks in nearly all cells of the body. Although distinct tissues share a common core clock circuitry, studies have shown that circadian oscillations in gene expression are highly tissue-specific, the regulation of which is poorly understood.

Here, we integrated large-scale functional genomics data to identify regulatory mechanisms orchestrating distinct oscillations in different tissues. To delineate the possible combinations of transcriptional rhythms across tissues, we developed a model selection approach that systematically identified modules of genes sharing similar rhythmic combinations across tissues. We applied this model to an atlas of temporal mRNA expression in mouse tissues and found that our modules

spanned a diverse breadth of combinations including tissue-wide and tissue-specific rhythms as well as rhythms with discordant phases in several tissues.

To identify transcriptional regulatory mechanisms underlying these gene modules, we developed a penalized linear regression model. By combining transcription factor (TF) binding site predictions, chromatin accessibility information, and gene expression data, our model can infer temporal activities of TFs across tissues. We predicted a tissue-wide module of TFs whose oscillating activities were phase coherent across tissues. Moreover, this module significantly extended the expected repertoire of known core clock regulators. Next, we predicted TFs with distinct temporal activities in different tissues, suggesting that certain tissue-specific rhythms and rhythms with discordant phases could be generated in part from single TFs. For liver-specific rhythms, however, combinatorial analysis of TF binding sites predicted that concerted action between core clock and liver-specific TFs underlie rhythms in the liver, suggesting there may be multiple ways to generate tissue-specific rhythms. Taken together, our integrative analysis highlights the capability of data modeling in disentangling tissue-specific and circadian regulatory landscapes.

Research Funding: Swiss National Science Foundation grant, Natural Sciences and Engineering Research Council fellowship

11:45 am SS82

Mining for Novel Candidate Clock Genes in the Circadian Regulatory Network

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Abstract: Background: Most physiological processes in mammals are temporally regulated by means of a master circadian clock in the brain and peripheral oscillators in most other tissues. A transcriptional-translation feedback network of clock genes produces near 24 h oscillations in clock gene and protein expression. Here, we aim to identify novel additions to the clock network using a meta-analysis of public chromatin immunoprecipitation sequencing (ChIP-seq), proteomics and protein-protein interaction data starting from a published list of 1000 genes with robust transcriptional rhythms and circadian phenotypes of knockdowns.

Results: We identified 20 candidate genes including nine known clock genes that received significantly high scores and were also robust to the relative weights assigned to different data types. Our scoring was consistent with the original ranking of the 1000 genes, but also provided novel complementary insights. Candidate genes were enriched for genes expressed in a circadian manner in multiple tissues with regulation driven mainly by transcription factors BMAL1 and REV-ERB α,β. Moreover, peak transcription of candidate genes was remarkably consistent across tissues. While peaks of the 1000 genes were distributed uniformly throughout the day, candidate gene peaks were strongly concentrated around dusk. Finally, we showed that binding of specific transcription factors to a gene promoter was predictive of peak transcription at a certain time of day and discuss combinatorial phase regulation.

Conclusions: Combining complementary publicly-available data targeting different levels of regulation within the circadian network, we filtered the original list and found 11 novel robust candidate clock genes. Using the criteria of circadian proteomic expression, circadian expression in multiple tissues and independent gene knockdown data, we propose six genes (Por, Mtss1, Dgat2, Pim3, Ppp1r3b, Upp2) involved in metabolism and cancer for further experimental investigation. The availability of public high-throughput databases makes such meta-analysis a promising approach to test consistency between sources and tap their entire potential.

Research Funding: BMBF (T-Sys, 0316164G, 01GQ1503), DFG (SPP InKomBio) and Bernstein Center for Computational Neuroscience Berlin (grant 01GQ1001C)

12:00 pm SS83

Transcriptional Variation across SCN Subregions

Eric Mintz[1], Ghada Nusair[1], Jessica Vespoli[1]

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Abstract: The suprachiasmatic nucleus (SCN) of the hypothalamus acts as an endogenous circadian clock. Individual neurons of the SCN are capable of acting as independent circadian oscillators, but the SCN exhibits a specific structural organization, with subregions that can be defined by a variety of criteria, including neuropeptide expression and cellular responses to stimuli. SCN subregions vary in their rhythms of gene expression as a function of their location in the SCN, and differences between subregions can be influenced by external inputs such as the ambient photoperiod. One question is whether SCN neurons are fundamentally homogeneous, with separation of functions derived from location in the nucleus or small differences in phenotype such as neuropeptide expression, or whether neurons that express different neuropeptides also show other substantial differences in underlying gene expression. To address this question, we selected three regions of the mouse SCN for transcriptional analysis, based on the location of major cell groups expressing vasoactive intestinal polypeptide (ventral), gastrin-releasing peptide (central), or vasopressin (dorsal). Using laser capture microscopy, we extracted three tissue samples from the SCN corresponding to these three regions from each of 38 male C57BL/6J mice. Samples were taken in constant dark at 12 circadian time points, with circadian time determined using onset of wheel-running activity at CT 12. RNA from these samples was extracted, amplified, and hybridized to Affymetrix whole genome arrays. Results suggest that SCN neurons in the three subregions are fundamentally similar and show limited differences in transcriptome. The three neuropeptides that were used to define the subregions were correctly associated with their subregions, along with the neuropeptides somatostatin and preproenkephalin. A larger group of genes was identified as elevated in the ventral region of the SCN, but are largely expressed in oligodendrocytes. These data suggest that differences in phase and function among SCN subregions may largely be due to properties arising from neurons' location in the nucleus rather than on fundamental differences in cell function.

Research Funding: This research was supported by National Science Foundation grant IOS-1021957 and the Kent State Department of Biological Sciences.

12:15 pm SS84

Altered Bile Acid Dynamics in Mice Lacking Nocturnin

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Abstract: Cyclic processes in both behavior and physiology are aligned to the external environment through the circadian clock. Nocturnin (Noc) is a rhythmically expressed gene regulated in part by the circadian clock and intimately linked to the metabolic state of an organism. Encoding a deadenylase, NOC protein is thought to regulate mRNA turnover through its ability to remove poly(A) tails from mRNA transcripts. Loss of Nocturnin (Noc-/-) in mice results in resistance to diet-induced obesity. In this study we set out to explore Nocturnin's responsiveness to various nutrient challenges (diet, fasting, refeeding). We found that Noc mRNA expression is up-regulated in conditions of nutrient

excess (high fat diet, refeeding following a fast) and down-regulated during nutrient deprivation (fasting). We next measured changes in the hepatic transcriptome of WT and Noc-/- mice fed a high fat diet by RNA-seq and found broad up-regulation of genes along the cholesterol biosynthetic pathway in Noc-/- mice in the fed, fasted and refed states. Systemic levels of cholesterol in Noc-/- mice were not changed, however, and so we examined cholesterol degradation via bile acid synthesis. We found a significant increase in gene expression of bile acid synthesis genes which corresponded to increased gallbladder volumes in Noc-/- mice in both the fasted and refed state. Lastly, we investigated the contribution of NOC to the rhythmic production of cholesterol and bile acids by performing a 24h metabolic profile of WT and Noc-/- mice. Noc-/- mice show a significant increase in gallbladder volume during the light phase and dysregulation of the phasing of expression of bile acid synthesis genes primarily over the light:dark transition, when Noc expression is normally peaking in WT animals. The hepatic cholesterol profile shows a reduction early in the light phase of Noc-/- mice, when bile acid production is presumably increased, followed by an increase prior to the light:dark transition which could be a homeostatic compensation for cholesterol being used for bile acid synthesis. As bile acids' role has broadened to include intracellular metabolic signaling, NOC's regulation of bile acid synthesis by acting as a "gain control" provides a novel mechanistic layer to the circadian regulation of metabolism.

Research Funding: NIGMS

11:00 am - 12:30 pm

Slide Session: *Light and Neuronal Networks*

Chair: François Rouyer

11:00 am SS85

Drosophila Clockwork Dynamics: Functional Contributions of Strong and Weak Neuronal Oscillators to Circadian Synchrony and Light Response

Todd Holmes[1], Logan Roberts[1], Tanya Leise[2], David Welsh[1], Ceazar Nave[1]

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Abstract: Light is the primary signal that calibrates circadian neural circuits and thus coordinates daily physiological and behavioral rhythms with solar entrainment cues. How heterogeneous circadian circuits can generate robust physiological rhythms while remaining flexible enough to respond to synchronizing stimuli has long remained enigmatic. Our study describes how the Drosophila circadian neural circuit dynamically reorganizes spatial and temporal patterns of Period oscillator activity to facilitate circadian synchrony and light response. We find that circadian networks express a mix of strong and weak components critical for robust yet flexible responses to external cues for phase advance. A longstanding challenge for investigators of the mammalian suprachiasmatic nucleus is physiologically activating retina-mediated photic responses while monitoring large ensembles of deep brain individual oscillators. However, circuit-wide responses to light can be monitored in cultured Drosophila whole brain explants due to expression of the primary photoreceptor Cryptochrome in circadian neurons. Using custom analysis of real-time bioluminescence datasets, we provide a detailed spatiotemporal map of whole dynamic network phase ensembles, at single neuron resolution, in free-running and light-shifting conditions. By combining ex vivo bioluminescence imaging data with computational modeling of complementary oscillator types in the Drosophila circadian neural network, we show that transiently dampening the amplitude and synchrony of certain neuron oscillators appears to be a key adaptive feature of circadian systems. We postulate that a light

evoked shift of most oscillators towards a transiently dampened state facilitates large phase shifts so that stronger oscillators can pull the network into a new state of phase-shifted synchrony. These results suggest that the dynamic coupling between weak and strong oscillators underlies seasonal adaptability and could be deliberately directed and amplified as treatment for conditions such as jet lag and seasonal affective disorder.

Research Funding: This work was funded by NIH grants GM102965, and GM107405 to TCH and an NSF Graduate Research Fellowship DGE-1321846 to LR.

11:15 am SS86

Dual-Mode Control of Network Flexibility in the Drosophila Clock Circuit

Abhishek Chatterjee[1], Angelique Lamaze[1], Elisabeth Chelot[1], Joydeep De[1], Beatrice Martin[1], Rossana Serpe[1], Sebastian Kadener[2], Paul Hardin[3], Patrick Emery[4], Francois Rouyer[5]

[1]*NeuroPSI, National Center for Scientific Research,* [2]*The Hebrew University of Jerusalem*, [3]*Texas A&M University*, [4]*University of Massachusetts Medical School*, [5]*National Center for Scientific Research*

Abstract: A multioscillator clock network drives circadian rhythms in locomotor activity. Across animal orders, the clock-driven daily activity pattern is reformatted by ambient light. However little is known about the circuit mechanisms that govern light-dependent network flexibility. Our data in Drosophila indicate that the hierarchical relationship between the master clock in the PDF(+) LNv neurons and the two major groups of downstream oscillators in the LNd and the DN1p neurons, is vetted by light. Light strengthens coupling between LNv and LNd while weakening the coupling between LNv and DN1p. This conditional slave-choice allows the PDF(+) LNv oscillator to keep control of the behavior since LNds phase the main activity peak in presence of light, whereas the DN1ps do so in absence of light. Such flexibility in interoscillator coupling by the LNv is layered on its clock-independent role of directly gating the output of the downstream oscillators through PDF signaling. By transcriptionally tuning the expression of the pdf gene in accordance with light-intensity, the LNv neurons calibrate the output of the light-inhibited DN1ps. Therefore, accompanying external changes in the fly's photic environment, the interactions between oscillator neurons are readjusted at two separable scales to achieve network flexibility. Our work on how the Drosophila circadian network in the central brain reorganizes in response to light availability could reveal general principles of achieving state-dependent adaptive flexibility in other multioscillator clock systems.

Research Funding: ANR, EMBO

11:30 am SS87

Optogenetic Investigation of SCN Communication and Photoperiodicity

Michael Tackenberg[1], Jeffrey Jones[2], Douglas McMahon[1]

[1]*Vanderbilt University*, [2]*Stanford University*

Abstract: The ways in which the retinorecipient SCN neurons that express vasoactive intestinal polypeptide (VIP) communicate with non-retinorecipient regions of the nucleus, and how that communication results in coordinated output, remain unclear despite considerable advancements in

recent years. Previous interrogations have been limited technically by the lack of suitable methods for evoking electrical activity in specific SCN cell sub-populations. Using optogenetics in vivo, with channelrhodopsin targeted to VIP neurons using a VIP Cre driver mouse line, we have induced electrical activity in VIPergic neurons in the SCN, mostly located in the ventrolateral region, with precise temporal and spatial resolution. Using this system, we have explored the role of different durations of high firing rate within the SCN on photoperiodic behavior. Here we show that extending the high-firing phase of the rodent SCN into the subjective night results in long-photoperiod-like behavior phenotypes. This demonstration of the dependence of photoperiodic behavior on the electrical activity of VIP neurons in the SCN has implications for future research into photoperiodic and seasonal influences on physiology.

Research Funding: NSF GRFP 0909667 to MCT, NIH R01 EY015815 to DGM, NIH R01 GM117650 to DMG, VU Discovery Grant to DGM, F31 NS082213 to JRJ.

11:45 am SS88

Polarity of GABAA Signaling Influences the Dynamics of SCN Coupling

Jennifer Evans[1]

[1]*Marquette University*

Abstract: Suprachiasmatic nucleus (SCN) neurons are cellular clocks that interact (i.e., couple) with one another to synchronize at the network level. Nearly all SCN neurons express GABA, but the role of GABA in network synchronization has been difficult to define. Using a quantitative coupling assay, we have revealed that the role of GABAA signaling in SCN coupling is dependent on the state of the network. Specifically, when SCN neurons are tightly coupled under standard lighting conditions, GABAA signaling acts to inhibit synchronization. In contrast, when SCN neurons are desynchronized by long day photoperiods, GABAA signaling facilitates re-synchronization. Because synchronization is critical for SCN clock function, understanding the bases of this neuroplasticity is fundamentally important. Here we test the mechanistic bases of plasticity in the functional role of GABAA signaling. First, we find that long day photoperiods do not potentiate GABA-induced resetting in the SCN, but instead fundamentally alter the nature of this response. Next, we reveal that the SCN displays daily rhythms in the protein expression of chloride co-transporters that determine the polarity of GABAergic responses. Further, we find that chloride co-transporter rhythms are region-specific and modulated by light. Lastly, we demonstrate that the dynamics of SCN re-synchronization are influenced by light-induced changes in the function of chloride cotransporters. These results provide insight into the mechanisms underlying plasticity in the role of GABAA signaling in SCN coupling. As in other neuronal networks, plasticity in GABAA signaling appears to modulate SCN network activity to encode individual experience and influence behavior in a changing environment.

Research Funding: NIH grant R01NS091234; Whitehall Foundation Grant 2014-12-65

12:00 pm SS89

Geniculohypothalamic Gabaergic Signalling Modulates Suprachiasmatic Nuclei Responses to Retinal Input

Lydia Hanna[1], Lauren Walmsley[1], Michael Howarth[1], Timothy Brown[1]

[1]*University of Manchester*

Abstract: The suprachiasmatic nuclei (SCN) form part of a reciprocally interconnected network of visual nuclei including portions of the thalamus and pretectum, collectively termed the non-image forming (NIF) visual system. While direct retinal input to the SCN is essential for circadian photoentrainment, the roles of other NIF nuclei in shaping photic SCN responses are only poorly understood. In particular, it is unclear how/whether thalamic input via the geniculohypothalamic tract (GHT) directly modulates SCN neuronal responses to retinal signals. To address this, we established an in vitro slice preparation which retains all NIF nuclei and their connections largely intact. Multi-electrode recording/stimulation confirmed that this preparation maintains circadian rhythmicity for >24h in vitro, optic tract (OT) input to the SCN, thalamus and pretectum and a functional GHT. We next show that optogenetic activation of GABAergic GHT neurons exerts relatively little effect on spontaneous SCN neuronal activity but induces a long-lasting suppression of responses to OT-input. This GHT-driven modulation of SCN responsiveness was almost completely abolished by pharmacological blockade of GABAA and GABAB receptors and exhibited a pronounced day-night variation, with more substantial suppressions during the projected day. Of note, long-term monitoring of optogenetically-evoked activity in the thalamus indicated that this action was not associated with overt circadian rhythmicity in GHT output. Finally, in vivo electrophysiological recordings revealed that inhibition of GHT signalling attenuated specific features of SCN-driven light responses, suggesting that GHT cells are primarily activated by illumination of the contralateral retina. Together these data provide new insight into the mechanisms by which GHT-signalling contributes to circadian visual processing, providing a mechanism by which thalamic activity can gate retinal input to the clock according to time of day or arousal state.

Research Funding: BBSRC

12:15 pm SS90

Atypical Opsins in Photoentrainment and Development

Minh-Thanh Nguyen[2], Shruti Vemaraju[2], Ethan Buhr[3], Sujata Rao[4], Michael Iuvone[5], Russell Van Gelder[3], Richard Lang[1]

[1]*Cincinnati Children's Hospital Medical Center,* [2]*Visual Systems Group, Abrahamson Pediatric Eye Institute, Division of Pediatric Ophthalmology and Center for Chronobiology, Cincinnati Children's Hospital Medical Center,* [3]*University of Washington Medical School,* [4]*Cole Eye Institute,* [5]*Emory University School of Medicine*

Abstract: The non-canonical opsin OPN5 (Neuropsin) is known to be expressed in multiple tissues and to mediate light-dependent signaling. We have recently shown that OPN5 is expressed in a subset of retinal ganglion cells and is required for photoentrainment of the local circadian clock. Our ongoing analysis shows that Opn5 mutant mice show vascular development anomalies in the eye. These include a precocious regression of the hyaloid vessels and a mild overgrowth of the retinal vascular network. We also identified changes in the regulation of dopamine levels in the Opn5 mutant mouse.

At day 5, Opn5 null mice show tyrosine hydoxylase expression anomalies higher-than-normal levels in the vitreous. To test the possibility that dopamine might directly regulate vascular development, we performed a conditional deletion of Drd2 in vascular endothelial cells. This produced a persistent hyaloid vessel network and suggested that dopamine is a direct regulator if vascular development in the eye. In adult mice, dopamine levels in the eye are regulated both by light and by the circadian clock machinery. We are currently testing whether at developmental stages, the circadian clock is an intermediate in the regulation of dopamine levels and vascular development. We are also currently testing the activity of Opsin 5 and Opsin 3 in regulating photoentrainment in the many non-retinal tissues in which they are expressed.

Research Funding: Abrahamson Pediatric Eye Institute, National Institutes of Health

11:00 am - 12:30 pm

Slide Session: ***Human Health, Behavior and Society***

Chair: Phyllis Zee

11:00 am SS91

Genome-Wide Association Analysis and Functional Follow-Up Identifies Novel Loci for Chronotype in 100,420 Individuals from the UK Biobank.

Jacqueline Lane[1], Irma Vlasac[1], David Bechtold[2], Simon Kyle[3], Debbie Lawlor[4], Andrew Loudon[2], Susan Redline[5], Frank Scheer[5], Martin Rutter[2], Richa Saxena[1]

[1]*Massachusetts General Hospital*, [2]*University of Manchester*, [3]*University of Oxford*, [4]*University of Bristol*, [5]*Brigham and Women's Hospital, Harvard Medical School*

Abstract: Background: Chronotype is a natural manifestation of our internal biological clock and is influenced by age, sex, environment, genetics, and other biological factors. Chronotype is associated with sleep disorders, cognitive and physical performance, chronic metabolic and neurologic disease, particularly when there is circadian desynchrony between internal and external timing. Although chronotype is highly heritable, there have been few large scale studies that have examined the genetic and environmental variants that influence inter-individual variation in human chronotype and which have interrogated causal pathways.

Methods: Using self-reported chronotype and genetic information from 100,420 subjects of European ancestry from the UK Biobank, we performed a genome-wide association study. Chronotype was reported as "definite morning", "more morning than evening", "more evening than morning" and "definite evening" preference. We measured heritability and performed single variant association tests adjusting for age, sex, ancestry and genotyping array (n= 39,025,120 SNPs). Follow up analyses included gene-based association tests, gene-set analysis, heritability partitioning across tissues and functional classes, pair-wise genetic correlation to 19 traits. Circadian luciferase assays on cells bearing candidate gene knock-downs were also performed.

Results: We identified 12 genetic loci, of which 9 are novel and 5 are in or near a gene with an established role in circadian rhythms. The 12 loci account for 4.3% of chronotype variation, and genome-wide genetic variation accounts for 19.8% of chronotype variation. Pathway analysis implicates a role for central nervous and ocular systems and fear-response related pathways. Heritability of chronotype is enriched in transcriptional enhancer and conserved genomic regions, as well as the CNS and adrenal gland/pancreas. In cross-trait analyses, significant genetic correlation was observed between chronotype and education, schizophrenia, and BMI, pointing towards shared biology, with a relationship between eveningness chronotype and increased educational attainment.

Conclusion: These results expand our knowledge of the human circadian system, and expose the influence of circadian characteristics on health-related outcomes.

Research Funding: Supported by NIH grants, The University of Manchester (Regional Innovation Funding) and UK Medical Research Council.

11:15 am SS92

Differential Dna Methylation at Circadian Clock (related) Gene Loci in Pre-Eclampsia

Inês Chaves[1], Caroline van den Berg[1], Emelie Herzog[1], Eric Steegers[1], Sten Willemsen[1], Bert van der Horst[1], Regine Steegers-Theunissen[1]

[1]*Erasmus University Medical Center, Rotterdam*

Abstract: The circadian clock significantly impacts health during the life course. Human and animal studies showed that genetic defects in clock genes and circadian disruption by chronic jet lag or long-term shiftwork increase the risk of disease, such as metabolic syndrome, cardiovascular disease and cancer. Preeclampsia (PE) is the main cause of maternal mortality and often complicated with premature birth and low birth weight. Especially these children are at risk of non-communicable disease during the life course. PE is a placental-related disease associated with cardiovascular, neurological and metabolic complications. Importantly, disruption of light/dark cycle during pregnancy has been associated with PE, suggesting a link with the circadian clock. It is unlikely that a genetic defect is at the basis of the connection between the circadian clock and PE. We hypothesize that PE is associated with alterations in DNA methylation at circadian clock (related) gene loci.

In this nested case-control study, patients with pre-eclampsia (early- and late-onset), fetal growth restriction, preterm birth and uncomplicated controls were compared. Genome-wide DNA methylation was determined in umbilical cord white blood cells, endothelial cells, and placental biopsies of 29 pre-eclampsia pregnancies, 27 fetal growth restriction, 20 spontaneous preterm birth cases and 36 uncomplicated controls were investigated. In order to specifically focus on circadian clock (related) genes, we took a pathway driven approach where methylation levels of 939 CpGs of 39 circadian clock (related) genes were analyzed. The data show that DNA methylation in early-onset PE is significantly different form the control groups: we have identified 6 differentially methylated CpGs in placental tissue and 21 differentially methylated CpGs in umbilical cord white blood cells. Oppositely, no differentially methylated CpGs were identified in late-onset PE.

In this study we identified differential DNA methylation of circadian clock (related) gene loci in placental tissue and umbilical cord white blood cells from pregnancies with early-onset pre-eclampsia. Future research should address whether these DNA methylation markers can be used as early predictors of health in the offspring.

Research Funding: This research project was funded by internal grants.

11:30 am SS93

Ultradian Rhythms of Locomotor (In)Activity in a Real-World Sample of 120,000 Hours of Human Sleep

Eva Winnebeck[1], Dorothee Fischer[2], Maria P Hidalgo[3], Thomas Kantermann[4], Lena K Keller[1], Rosa Levandovski[3], Joana Matera[1], Luisa K Pilz[3], Celine Vetter[5], Till Roenneberg[1]

[1]*Ludwig Maximilian University, Munich,* [2]*Harvard T.H. Chan School of Public Health,* [3]*Federal University of Rio Grande do Sul,* [4]*University of Groningen,* [5]*Brigham and Women's Hospital, Harvard Medical School*

Abstract: In most animals including humans, locomotor activity is greatly reduced during sleep. Although this relative inactivity is one of the behavioral hallmarks of sleep and routinely used to estimate times of sleep and wake from activity recordings, any information contained in the relative inactivity itself has been largely ignored.

To assess the potential of this untapped resource, we turned to our actimetry database comprising >20,000 days of wrist-actimetry from 574 human subjects "in their natural habitat". In these actimetry records, we identified 16,517 sleep bouts based on relative inactivity that fulfilled our analysis criteria. From these 120,000 h of sleep, we converted all locomotor activity to 'Locomotor Inactivity During Sleep' (LIDS) using a simple transformation that emphasizes times of inactivity over times of activity.

LIDS showed clear ultradian oscillations, with 50% of rhythms displaying a period between 95-130 min (median: 110 min). LIDS levels declined gradually with time asleep (25 % over 4 cycles) and the 1st LIDS acrophase occurred at median 0.2 cycles after sleep onset. We did not find any evidence for an ongoing LIDS rhythm between two adjacent sleep bouts.

Analyses across groups in our database (incl. shift workers and psychiatric patients) as well as across age and gender provided insights on parameters that influence LIDS characteristics. Whereas LIDS period proved astonishingly stable, LIDS Gestalt showed specific differences between groups, gender and age: e.g. levels were higher in females than in males and the rate of decline decreased with age.

Comparison of LIDS with polysomnographic sleep measures in a dedicated dataset revealed that LIDS oscillated in synchrony with the NREM-REM sleep cycle. LIDS also corresponded well with common sleep parameters such as slow wave sleep, REMs, or sleep stages.

Given the wealth of information that can be extracted from LIDS and its intriguing link with basic sleep physiology, we postulate that locomotor (in)activity during sleep is a valuable resource for the study of sleep. Especially since locomotor activity can be easily recorded longitudinally in the most diverse real life conditions (in humans as well as animals) and provides information also about wake-time activity, we predict that it will significantly advance our understanding of sleep.

Research Funding: Friedrich-Baur-Stiftung

11:45 am SS94

The Effect of Chronotype and Time of Year on School Attendance and Performance

Giulia Zerbini[1], Vincent van der Vinne[2], Lana Otto[1], Till Roenneberg[3], Thomas Kantermann[1], Martha Merrow[3]

[1]*University of Groningen*, [2]*UMass Med School*, [3]*Institute of Medical Psychology, Ludwig-Maximilians-Universität München*

Abstract: There is accumulating evidence that both sleep timing and duration influence school performance. Late chronotypes and shorter sleep duration correlate with lower grades. Recently, we showed that early chronotypes outperform late chronotypes in the morning, but not in the early afternoon. In the current study, we investigated the effect of school attendance on grades, and how school attendance varies with chronotype and time of year. We hypothesized late chronotypes would have more absenteeism, resulting in lower school performance. We assessed chronotype (an estimation of an individual's phase of entrainment) via the Munich ChronoType Questionnaire (MCTQ), using the midpoint of sleep on school-free days corrected for oversleep due to sleep debt accumulated on school days (MSFsc). We collected grades, indicators of school attendance (i.e. late arrivals, dismissals from class, sick leaves, and sick leave duration) and MCTQ data for a Dutch high school in Coevorden, the Netherlands (52° 40' N / 6° 45' E) between August 2013 and June 2015. We found that absenteeism peaked in winter (late arrivals $F(2,75)=40.51$, $p<0.0001$; dismissals from class $F(2,74)=6.183$, $p=0.0033$; sick leaves $F(2,75)=49.50$, $p<0.0001$; sick leave duration $F(2,75)=60.57$, $p<0.0001$). Although the winter peak in absenteeism was observed in all chronotypes, late chronotypes were more likely to be absent from class throughout the year (academic year 2013-2014: late arrivals $Chi2(1, N=735)=14.11$, $p=0.0002$; dismissals from class $Chi2(1, N=502)=18.90$, $p<0.0001$; sick leaves $Chi2(1, N=1300)=5.887$, $p=0.0153$; sick leave duration $Chi2(1, N=1300)=5.478$, $p=0.0193$). Absenteeism, in turn, significantly negatively impacted student's grades (academic year 2013-2014: late arrivals $F(1,481.4)=29.27$, $p<0.0001$; dismissals from class $F(1,486.7)=44.21$, $p<0.0001$; sick leaves $F(1,481.1)=13.96$, $p=0.0002$; sick leave duration $F(1,484.9)=13.02$, $p=0.0003$). Results from academic year 2014-2015 resemble results from academic year 2013-2014. Taken together, our findings suggest that sleep duration, time of day of testing, and absenteeism all contribute to school performance. Later school starting times would allow increased sleep duration and may lead to improved performance in adolescents, especially those with a late chronotype.

Research Funding: Our work is supported by the Technology Foundation STW grant P10-18/12186 and the University of Groningen.

12:00 pm SS95

Sleep Hygiene and Academic Performance in College Undergraduates

Gideon Dunster[1], Isabelle Hua[1], Horacio de la Iglesia[1]

[1]*University of Washington*

Abstract: Previous work has established a relationship between sleep and academic performance. While this link is supported by several studies much of the historical data we have on sleep in students comes from self-reported sleep information in the form of sleep diaries or post-hoc surveys which can be unreliable and of limited quantitative value. On the other hand, intervention studies that investigate the role of specific sleep patterns or variables focus on single-night or short term memory changes that can be hard to translate into real-life behaviors. The goal of the present study is to assess the association between specific sleep parameters and academic performance in college students

using objective and quantitative measures of sleep in a large sample of undergraduate students. We outfitted 92 college students (46 Female) at the University of Washington with wristwatch activity monitors in order to track sleep patterns for two weeks. Sleep parameters analyzed included sleep onset, offset, duration, efficiency, and social jet lag. Contrary to previous studies, average sleep duration during the school week was not significantly correlated with academic performance. In contrast, the standard deviation of sleep duration and sleep onset during school days were negatively correlated with academic performance. None of the other parameters had predictive value in terms of academic performance. These results suggest that while the amount of daily sleep may not affect academic performance in college students, a regular and consistent sleep schedule may be more important.

Research Funding: Materials are all owned by the lab, graduate student salary provided through a Teaching Assistant position in the Biology Department at the University of Washington.

12:15 pm SS96

Long Weekly Work Hours Increase the Risk of Adverse Health and Safety Outcomes in First-Year and More Experienced Resident Physicians

Celine Vetter[1], Charles A. Czeisler[1], Jason P. Sullivan[1], Christopher P. Landrigan[1], Laura K. Barger[1]

[1]*Brigham and Women's Hospital, Harvard Medical School*

Abstract: Background. Previous work has shown that extended-duration shifts are associated with adverse performance and safety outcomes in first-year residents. Whether seniority is protective when examining the association between work hours characteristics and performance and safety in resident physicians is unclear.

Methods: From 2002-2007, a total of 4,796 medical residents (68% first-year residents) responded to monthly online questionnaires and reported their work schedule, demographic and lifestyle information, together with reports of motor-vehicle crashes (MVCs), near-crashes, medical errors and occupational exposures. We used Poisson Regression (Proc Genmod) to estimate the relative risk (RR) with 95% confidence interval (CI) across categories of weekly work hours (≤47h, 48-64h, 65-79h, 80-99h, ≥100h) and adverse performance and safety outcomes. GEE accounted for the correlated data structure, and interaction terms were included to examine potential interactions.

Results: 10,087 motor vehicle incidents (MVCs and near-crashes), 8,476 medical errors and 2,959 occupational exposures were reported during follow-up. In multi-variable adjusted models, we observed a significant dose-response relationship between weekly work hours and all outcomes (Ptrend all <0.0001). While experienced residents overall had a lower risk of any adverse outcomes as compared to first year residents (RR=0.37-0.57), an association with long work hours was seen within both groups (Ptrend all <0.0001). The interaction between seniority and weekly work hours was significant for motor-vehicle incidents and medical errors (motor-vehicle incident: first year residents RR≥100h=6.58, CI=5.29-8.18; experienced residents: RR≥100h=3.64, CI=2.08-6.35, Pinteraction=0.007; medical errors: first year residents: RR≥100h=12.45, CI=8.83-17.55; experienced residents: RR≥100h=5.73, CI=2.62-12.53, Pinteraction=0.008), respectively, but not occupational exposures (first year residents RR≥100h=4.65, CI=3.15-6.88; experienced residents: RR≥100h=4.89, CI=3.24-10.66, Pinteraction>0.5).

Conclusions: Our results demonstrate that long work hours are associated with increased risk medical errors, motor vehicle incidents and occupational exposures among resident physicians at all levels of experience.

Research Funding: This research was funded by a postdoctoral scholarship of the German Research Foundation (CV) and R01 OH 010300 (NIOSH, LKB).

Poster Session Abstracts

8:30 pm - 10:30 pm

Poster Session I

S1

Constant Light Promotes Tumor Development via Insulin Resistance and Altered Inflammatory Response

Natalí N Guerrero-Vargas[1], Raful Navarro-Espindola[1], Mara Guzman-Ruiz[1], Estefanía Espitia[1], Adrián Báez-Ruiz[1], Ruud Buijs[2], Carolina Escobar[1]

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Abstract: Nowadays people are more active during their normal sleeping period either for work, study or fun (nocturnal lifestyle). As a result of this extended or sometimes inverted active period, the exposure to light at night is a common practice and could even be considered a feature of modern lifestyle.

In rodents, light at night induces metabolic disturbance, such as body weight gain, fat accumulation and increased blood glucose levels. Also light at night promotes increased tumor development. A possible cause is the decrease in melatonin levels due to light exposure.

We are studying the interaction between host and tumor cells, we hypothesized that constant light exposure in rats could promote the growth of implanted tumors via an increased inflammatory response and a disrupted metabolism, characterized by glucose intolerance in the host.

In male Wistar rats exposed to 3 weeks of constant light conditions (LL) and rats exposed to light-dark cycle (LD), we investigated the growth of subcutaneously inoculated glioma cells (C6). We observed increased tumor volume in LL rats on days 12 and 13 after tumor induction as compared to LD tumor volume. Tumor growth decreased serum triglycerides and induced higher glucose levels. Both correlated with tumor size at day 13.

With a glucose tolerance test (GTT) we determined that LL rats were already glucose intolerant and had increased insulin levels, before C6 cells inoculation, providing a favorable metabolic environment for tumor growth.

Since inflammation is a recognized hallmark of cancer, we decided to evaluate the inflammatory response to LPS in LL and LD rats. In LD and LL rats we tested TNF-α and IL-6 plasma levels after lipopolysaccharide (LPS) administration and demonstrated that LPS administration to LL rats, triggered higher pro-inflammatory cytokines levels as compared to LD rats.

Together these results suggest that in LL conditions an increased inflammatory response and a disrupted metabolism may provide propitious conditions for tumor development.

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S2

Circadian Rhythm of Proteins in Breast Cancer Tissue Cultured Cells

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Abstract: Cancer cells have broken circadian clocks when compared to their normal tissue counterparts. Breast cancer is a heterogeneous disease which 12% of women in the United States contract representing 25% of all cancers in women. There are cell lines which represent various types of non-tumorous and tumorous epithelial breast tissue. We previously showed that these cell lines, both non-tumorous and tumorous, experience circadian-like oscillations at the mRNA level when serum shocked. At the mRNA level, tumorous cells do not show circadian rhythm in canonical genes but do express other genes which have circadian-like oscillations. These circadian-like genes seem to be different than the genes which oscillate in non-tumorous breast tissue. Here, we look at the protein expression of cell lines to see how it relates to the mRNA expression. We serum shocked non-tumorous MCF-10A and tumorous MCF-7 breast tissue cell lines with 50% horse serum. We followed protein production for 48 hours taking 4-hour time points and running them on 2-dimensional electrophoresis in duplicate. We scanned the gels and analyzed them using PDQuest software. The profiles of the proteins from both, MCF-10A and MCF-7, cell lines where compared and we identified an average of 452 spots for MCF-10A and 334 spots for MCF-7. We found that MCF-7 shares 228 spots with MCF-10A and 106 spots are unique of MCF-7. The resulting spots were digitized, the intensity quantified, and oscillating proteins identified using the JTK package in R software. Our analysis showed 15 and 41 circadian-like proteins (<0.05 p-value) over 48-hours in MCF-10A and MCF-7 cells, respectively. However, we did not identify any common proteins among cell lines. We will determine the protein identity of these rhythmic spots by MALDI-TOF. Additionally, we will contrast the identified proteins with circadian clock function in breast cell lines. Our results suggest that proteins might represent rhythmic oscillation in the regulation of molecular changes of breast non-tumorigenic and tumorigenic cells. These differences in rhythmic oscillations may represent possible targets for chronotherapy.

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S3

Disruption of the Cardiomyocyte Circadian Clock Influences Myocardial Insulin Signaling

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Abstract: Background: In humans and animals, whole body insulin sensitivity oscillates over the day. Aberrant insulin sensitivity and circadian dyssynchrony exacerbate cardiometabolic disease states. Molecular links between the circadian clock and myocardial insulin signaling/sensitivity are currently unknown.

Hypothesis: The cardiomyocyte circadian clock regulates insulin signaling in the heart, and disruption of this clock leads to aberrant insulin signaling.

Methods: Microarray and qRT-PCR analyses were performed on ventricles isolated at different times of day from cardiomyocyte-specific BMAL1 knockout (CBK) and control (CON) mice to investigate the influence of the cardiomyocyte circadian clock on insulin signaling components. Western blotting was performed to interrogate the activation status of known insulin signaling components in the basal state. To gain greater insight regarding insulin sensitivity, 6-hour fasted CON and CBK mice were challenged with a submaximal insulin dose in vivo (0.167 U/kg body weight, 5 min) at ZT12. Subsequently, ex vivo perfused working hearts from CON and CBK mice were challenged with insulin (100μU/mL, 5 min) at ZT12. Activation of insulin signaling was assessed by western blotting for in vivo and ex vivo insulin challenges.

Results: Pik3r1, encoding the regulatory subunit of the PI3K complex (p85a), was identified as being regulated by the cardiomyocyte circadian clock, and was suppressed in CBK hearts. Accordingly, Akt phosphorylation (Ser-473) was increased in CBK hearts. Importantly, in vivo and ex vivo insulin challenges lead to a greater increase in p-Akt in CBK versus CON hearts, consistent with greater insulin sensitivity. This was associated with increased p-mTOR (downstream target of Akt), as well as the mTOR targets, S6 and 4EBP1, in CBK hearts. In contrast, p-AS160 and p-GSK3b (additional Akt targets) were decreased in CBK hearts.

Conclusions: The circadian clock regulates myocardial p85a and Akt expression, which was associated with differential activation of downstream Akt targets. The findings are suggestive of decreased glucose utilization and increased protein synthesis, consistent with the metabolic phenotype of CBK hearts. Collectively, these data highlight the cardiomyocyte circadian clock as a novel modulator of myocardial insulin signaling.

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S4

Environmental Circadian Disruption Increases Ischemic Brain Damage

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Abstract: Background: Stroke is the fifth leading cause of death and the leading cause of long term disability in the United States. Shift work is associated with an increased risk of both ischemic heart disease and stroke, but the mechanisms for these relationships are unknown. Shift workers experience sleep/wake patterns that become misaligned with the circadian rhythms of the brain and body. In mice, such circadian disruption can alter inflammatory responses in ways that may affect stroke outcome.

Hypothesis: Environmental circadian disruption increases brain damage associated with an ischemic event in a schedule-dependent manner.

Methods: Male C57BL/6 mice were subjected to one of 5 lighting schedules involving single or repeated phase delays or phase advances of the light cycle, mimicking some aspects of rotating shift work schedules. Mice were then subjected to middle cerebral artery occlusion surgery to induce a transient ischemic event. Mice were sacrificed 3 days post-surgery and cryostat sections were evaluated to determine the volume of damage in each brain.

Results: Four weekly 6-hour phase advances of the light cycle induced an increase of median infarct volume compared with controls. The effects of the schedule appeared to subside by 3 weeks after cessation of the lighting manipulation. Neither a single phase advance nor four weekly phase delays induced such changes in stroke volume.

Conclusion: Environmental circadian disruption does increase infarct volume following an ischemic stroke in mice in a schedule-dependent manner. These findings could lead to recommendations on scheduling or preventative medical strategies for reducing the impact of lifestyles that are disruptive to circadian timing.

Research Funding: This work was supported by grant G12MD007602 to Craig Bond and by grant SC1GM112567 to Alec Davidson.

S5

Developmental Regulation of the Narrow Abdomen Ion Channel in the Drosophila Circadian Pacemaker

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Abstract: The sodium leak channel NARROW ABDOMEN (NA)/ NALCN contributes to rhythmic neuronal output in the circadian pacemaker. In Drosophila, na is broadly required in the pacemaker network to promote rhythmic behavior, and rhythmic expression of the channel regulator Nlf1 is implicated as a potential mechanism for regulating channel expression and/or activity. To evaluate the developmental vs. adult requirements for NA and its regulators in the Drosophila circadian system, we have combined transgenic RNA interference with the temperature inducible tubulin-GAL80[ts] system. Surprisingly, we find that developmental expression of endogenous na is both necessary and sufficient to promote rhythmic behavior in adults. Moreover, the auxiliary channel subunit unc79 and the regulatory gene Nlf1 exhibit similar developmental requirements. We find that developmentally restricted expression of channel components or regulatory genes promotes robust expression of channel complex proteins that largely persists into adulthood. In contrast, limiting channel gene expression to adult stages fails to promote substantial expression of channel complex proteins. Taken together, our data suggest that adult expression of the NA channel complex in pacemaker neurons is established primarily during development. Notably, we find that transgenic overexpression of Drosophila NA in adult pacemaker neurons can restore rhythmic behavior to na mutants. Thus, adult driven channel expression may contribute to pacemaker neuron function and behavioral output in some contexts. Nevertheless, it is unlikely that rhythms in NA channel complex expression levels contribute substantially to rhythmic output in adult clock neurons. Instead, we propose that post-translational mechanisms, including mechanisms independent of Nlf1, are most important for clock regulation of NA function.

Research Funding: University of Iowa

S6

CRTC Potentiates Light-Independent timeless Transcription to Sustain Circadian Rhythms in Drosophila

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Abstract: Light is one of the strongest environmental time cues for entraining endogenous circadian rhythms. Emerging evidence suggests that CREB-regulated transcription co-activator 1 (CRTC1) is a key player in this pathway, stimulating light-induced Period1 (Per1) transcription in mammalian clocks. Here, we demonstrate a light-independent role of Drosophila CRTC in timely activating circadian transcription to sustain free-running circadian behaviors. Genomic deletion of the crtc locus causes long but poor locomotor rhythms in constant darkness (DD). Overexpression or RNA interference-mediated depletion of CRTC in circadian pacemaker neurons similarly impairs the DD rhythmicity, indicating that Drosophila circadian clocks are sensitive to the dosage of CRTC. The crtc null mutation delays the overall phase of circadian gene expression yet crtc effects on the oscillating levels ofTIMELESS (TIM) proteins are most evident in the clock neurons. In fact, CRTC overexpression enhances CLOCK/CYCLE-activated transcription of tim but not per in clock-less S2 cells whereas CRTC depletion suppresses it. Consistently, TIM overexpression partially but significantly rescues the behavioral rhythmicity in crtc mutants. Taken together, our data demonstrate that Drosophila CRTC potentiates tim transcription to coordinate molecular rhythms with circadian behaviors, implicating CRTC-dependent clock mechanisms have co-evolved with selective clock targets among different species.

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S7

Neuropeptide-F and Acetylcholine Mediate Photic Phase Resetting of Drosophila Circadian Behavior

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Abstract: Light is a critical zeitgeber for circadian clocks. The Drosophila clock is very sensitive to light, and a brief light pulse can shift the phase of circadian behavior by several hours. The model for this resetting posits that circadian photoreception is cell-autonomous: CRYPTOCHROME (CRY) senses light, binds to TIMELESS (TIM) and promotes its degradation, mediated by JETLAG (JET). However, we and others have previously shown that circadian photoreception requires cooperation between groups of neurons. In particular we have shown that the Morning (M) and the Evening (E) oscillators work synergistically to reset rhythmic behavior. Moreover, recent studies suggest a hierarchy between these two groups with E-oscillators leading the phase shift of the network. We found that ablation or attenuation of firing from the E-oscillators abrogated phase resetting mediated by short pulses of light. Unexpectedly, ablation of the M-oscillators or loss of its key neuropeptide PDF did not compromise this photoresponse. To establish the neurochemical identity of the signals

transmitted from the E-oscillators during photoreception, we inhibited their known neurotransmitters by RNA interference (RNAi). Knocking down Neuropeptide F (NPF) and a vesicular transporter for acetylcholine (VACHT) resulted in a partial decrease in phase delay responses. Conversely, over expression of the NPF receptor increased phase delays. Down regulating both NPF and VACHT further reduced delaying phase shifts, indicating that release of NPF and acetylcholine by the E-oscillators have additive effects on photic phase resetting. In summary, our results further emphasize the importance of the E-oscillators for circadian light responses, and identify NPF and acetylcholine as important neurotransmitters mediating photic entrainment.

Research Funding: NIH

S8

Grooming Behavior of Drosophila is Under Circadian Regulation

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Abstract: Grooming consists of all forms of body surface care and, as a behavior, is seen in varying degrees in all terrestrial vertebrates. But how grooming fits into an animal's daily schedule remains unknown. We sought to determine whether grooming is under circadian regulation, and if so, what are the underlying mechanisms. We have developed a method employing video tracking and machine learning algorithm to address this question in Drosophila melanogaster. Our apparatus is able to track 20 flies for more than 10 days and automatically interpret their behavioral data with minimal user input. Our results suggest that flies invest a significant portion of their waking time in grooming and the time spent in this behavior varies weakly across individuals and with changes in light-dark conditions. Analysis of recordings in constant darkness reveals strong circadian oscillations of average grooming behavior in phase with the more widely studied locomotion rhythms. Mutations in the clock gene period alter or abrogate oscillations in daily grooming in accordance with their effects on fly locomotion patterns. Although these concordant changes in grooming and locomotion may suggest that the subtle grooming movements are simply a subset of the more robust locomotion activities, close inspection of temporal patterns in the data indicate that grooming is independently controlled by the clock. Our results now offer a platform for long-term and unbiased analysis of fruit fly grooming and are likely to accelerate fundamental neurobiological discoveries of this elusive yet ubiquitous animal behavior.

Research Funding: This work was supported by funds from University of Miami.

S9

Igf-Ii mRNA-Binding Protein Regulates Night Sleep in Drosophila

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Abstract: Sleep is an essential behavior found in most species of the animal kingdom. However, the underlying molecular mechanisms of sleep regulation remain largely unknown. Here we identified that an RNA binding protein-IMP (IGF-II mRNA-binding protein) regulates sleep in Drosophila.

Imp-deficient flies exhibit significant decreases of night sleep with decreases of sleep bout durations and lengthened sleep latency. IMP is expressed in a large population of neurons in fly brain, including mushroom bodies and circadian neurons. We further tested in which neurons Imp is required to regulate sleep by using multiple GAL4 drivers expressed in known sleep circuitry. Knocking down of Imp in circadian neurons recapitulated the sleep phenotype observed in Imp mutants. Interestingly, over-expression of Imp in circadian neurons significantly shortened the sleep latency without affecting total amount of sleep. Taken together our results indicated that IMP is required in circadian neurons for sleep regulation.

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S10

Light-Induced Plasticity of Drosophila Clock Function

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Abstract: Light entrainment of the circadian clock of the model organism Drosophila melanogaster involves input from the visual organs as well as CRYPTOCHROME (CRY), a cell-autonomous circadian photoreceptor. CRY undergoes a conformational change in response to blue light, allowing it to bind the core clock component TIMELESS (TIM) and target it for proteasomal degradation via the F-box protein JETLAG (JET), thus impacting the negative feedback function of TIM and its regulatory target PERIOD (PER). We investigated the plasticity of circadian clock function in the brain and peripheral tissues of Drosophila in response to various environmental light treatments and made the following observations:

1) Unlike mammals Drosophila does not exhibit after-effects on free-running behavioural period length in constant dark (DD) following entrainment to white Light:Dark equinox photocycles (LD) of varying length (5 to 10 cycles/week).
2) Drosophila locomotor behaviour and peripheral molecular rhythms entrain to a remarkably wide range of LD periods (5 to 10 cycles/week).
3) The range of entrainable LD periods for circadian behaviour does not depend on PIGMENT DISPERSING FACTOR-expressing cells or their synaptic or peptidergic signalling, but it does require CRYPTOCHROME and the JETLAG-mediated photo-entrainment.
4) Constant red light (RR), which stimulates RHODOPSIN1 and 6 in the compound eyes but not CRY, represents a free-running condition for clocks, in which circadian pacemaker function shifts from the PDF-expressing small ventral lateral neurons to CRY-expressing dorsolateral neurons in a gender-dependent manner.

The absence of canonical DNA methyltransferases in Drosophila may be responsible for its lack of (epigenetically-driven) after-effects of photocycle period, whereas the presence of the cell-autonomous circadian blue light photoreceptor CRY in fruit flies but not mammals accounts for the wider range of photocycle entrainment of both behaviour and molecular rhythms in the former. Comparison of neural clock function in DD versus RR conditions has uncovered a remarkable gender-dependent environmentally-determined plasticity in circadian pacemaker function that we will discuss in more detail.

Research Funding: The Gerald Kerkut Charitable Trust and the University of Southampton

S11

Abnormal PDF Expression Leads to Arrhythmicity in Vrille Mutants

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Abstract: A wide array of organisms use endogenous circadian clocks to drive daily rhythms in physiology, metabolism and behavior. These clocks keep circadian time under constant environmental conditions via core and interlocked transcriptional feedback loops. In the core loop from Drosophila, CLOCK-CYCLE (CLK-CYC) activate transcription of their own feedback repressors, PERIOD (PER) and TIMELESS (TIM), which control rhythmic transcription peaking near dusk. An interlocked feedback loop is also activated by CLK-CYC, but in this loop the activator PAR Domain Protein 1 (PDP1) and the repressor VRILLE (VRI) feedback to drive Clk and other genes whose transcription peaks near dawn. Since vri null mutants cause embryonic lethality, the impact of vri function on circadian timekeeping is yet to be determined. Here we report on a FRT-FLP based strategy undertaken to generate conditional vri adult mutants and their behavioral & molecular analysis. We engineered a ~80kb vri Bac transgene, called vri70kb-FRT, that rescues the developmental lethality of vri null mutants, but then can be conditionally inactivated to generate vri null adults. These mutant adults have been validated by demonstrating that VRI protein is lacking in the FLP targeted brain neurons via immunostaining. vri null mutants exhibit arrhythmic locomotor activity, yet core feedback loop function persists, suggesting that VRI impairs locomotor activity through genes of output pathways. Indeed, expression of the neuropeptide PIGMENT DISPERSION FACTOR (PDF), which is required for activity rhythms, is aberrant in key brain pacemaker neurons that lack VRI. We will report on our current studies to decipher the link between VRI and PDF expression.

Research Funding: The research is funded by Texas A&M University.

S12

Nucleotide Variation in Drosophila Cryptochrome Linked to Circadian Clock Function: An Association Analysis

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Abstract: Genetic variations in circadian clock genes may serve as molecular adaptations, allowing populations to adapt to different local environments. Our previous survey of genetic variation of Drosophila cryptochrome (cry) suggested that this locus is maintained by natural selection in a North American population. Here, we extended our analysis to 33 congenic strains carrying European cry alleles. Neutrality tests of variation in the cry locus indicated a signature of natural selection, similar to our previous findings in North-American alleles. The results also suggested that two parts of the gene are largely targeted by different selective forces.

We used the new panel for high resolution mapping of the association between allelic variation in CRY and circadian clock traits. Association was assessed using Trait Analysis by the aSSociation, Evolution, and Linkage (TASSEL) software. We recorded the locomotor activity of multiple individuals from each line (minimising the environmental variation) and carried the association mapping using the line

mean values. We identified a cluster of six linked SNPs in the second intron that were significantly associated with the acrophase of the free-run activity (in DD). This cluster defines two haplotypes (All1, All2,), which exhibit small but highly significant differences in various circadian phenotypes. Importantly, CRY expression was also dramatically affected by these alleles suggesting that the All1/All2 variation is important for the transcriptional regulation of cry.

Research Funding: This study was partly supported by a Biotechnology and Biological Sciences Research Council (BBSRC) award to Eran Tauber (BB/K001922/1).

S13

Identification and Characterization of Genes Controlling Development of PDF-Positive Clock Neurons in the Fruit Fly Drosophila Melanogaster

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Abstract: Pigment-dispersing factor (PDF) is the neuropeptide indispensable for regulating circadian rhythm in the fruit fly Drosophila melanogaster. pdf gene is specifically expressed in the small ventral lateral neurons (s-LNvs) and large ventral lateral neurons (l-LNvs).

While it is well known that the pdf-positive neurons play an essential role in maintaining behavioral rhythmicity under free-running conditions, little is known about how development of the clock cells are regulated at the molecular level. To answer this issue, we conducted an RNAi screen for genes controlling development of the clock cells.

Here, we report 4 genes that influence development of the s-LNvs and the expression of PDF. The genes include longitudinals lacking (lola), which is a Zn-finger transcriptional factor, and Phosphoinositide-dependent kinase 1 (pdk1). The adult brain of lolaRNAi and pdk1RNAi flies showed abnormal development of the s-LNvs. The numbers of s-LNvs in either lola or pdk1 knockdown flies decreased, and their cell sizes also became smaller. Furthermore, the RNAi flies for these 4 genes displayed arrhythmic behavior. We therefore hypothesize that lola and pdk1 are essential for development of the s-LNvs of the s-LNvs in Drosophila.

Research Funding: Grant-in-Aid for JSPS Fellows

S14

Photoperiod Interacts With Running Wheel Availability to Modulate Circadian Food Anticipatory Activity in Mice

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Abstract: Rats and mice fed once daily exhibit food anticipatory activity (FAA) rhythms generated by food-entrainable circadian oscillators (FEOs) located outside of the suprachiasmatic nucleus (SCN). When mealtime is restricted to the light period, FEOs in nocturnal rodents must compete with output

from the light-entrained SCN pacemaker, which promotes sleep in the day. The amplitude of the SCN pacemaker is believed to be modified by photoperiod. This is suggested in part by the increased magnitude of light-induced phase shifts following exposure to short-day compared to long-day photoperiods, an effect interpreted as evidence that pacemaker amplitude is either decreased (limit-cycle oscillator model) or increased (oscillator population synchrony model) by short days, and the reverse by long days. In either case, if photoperiod modifies SCN amplitude, and amplitude regulates the strength of pacemaker output on behavioral state, then photoperiod can be expected to modify FAA magnitude, in nocturnal rodents fed in the light period. Results from one study conform to this general prediction, by showing increased FAA under long-day photoperiods (Pendergast et al, 2009). Here we confirm and extend (and complicate) this finding in a study of C57BL/6j mice entrained to 16:8 (long-day, N=20) and 8:16 (short-day, N=20) photoperiods (white LED, ~70lux). Half of the mice in each group were housed with running wheels. Activity in the other mice was recorded using motion sensors. As expected, the duration of nocturnal activity was significantly compressed in long-day mice compared to short-day mice, and this difference persisted in a skeleton photoperiod (to equate total daily light exposure) and in subsequent DD. Phase delay shifts to light pulses at CT15 on day 5 of DD were significantly larger in short-day mice, consistent with an effect on pacemaker amplitude. After re-entrainment to long and short days, all mice were gradually limited to a 4h daily meal beginning 4h prior to lights-off. Mice in the long-day group with running wheels exhibited enhanced FAA compared to short-day mice with wheels. Unexpectedly, mice without wheels showed the opposite effect. Following a 6h delay of mealtime, FAA shifted more slowly in long-day mice with wheels. Photoperiod and wheel running together modulate FAA, via mechanisms yet to be determined.

Research Funding: Supported by an operating grant (REM), graduate fellowship (MJM) and undergraduate research award (SP) from NSERC, Canada.

S15

Temporal Restricted Feeding Induces Time-Dependent Behavioral Changes in Mice

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Abstract: Food is a potent zeitgeber of peripheral tissues such as liver. Temporal restricted (TR) feeding is able to uncouple circadian peripheral oscillators from the central pacemaker in the suprachiasmatic nucleus. The timing of food administration has a critical health toll, yet the mechanism underlying such effects remains unclear. To further understand the physiological impact of feeding, we investigated the behavioral and metabolic consequences of different feeding paradigms. To this end, we developed an automated feeder system that controls the timing and duration of food availability, and that also precisely records both feeding and locomotor activities for individual mice. Using this system, mice were maintained on different feeding schedules: ad libitum food access (AL) and temporally restricted feeding during either the dark (TR-dark) or the light (TR-light) phase.

We found that both TR fed groups modify their feeding pattern when compared to AL fed mice; however, the feeding pattern in the TR-light condition was the most affected. This indicates that both the duration and timing of food availability affect feeding behavior. Interestingly, the wheel running activity of TR-light fed mice remains limited to the dark phase even though their food intake occurs entirely during the light phase. Since desynchronization between feeding and locomotor activities is associated with metabolic dysfunction, we investigated the consequences of each feeding condition

on body weight and blood glucose levels. Notably, despite the fact that TR-light fed mice consume less food compared with other groups, there was no difference in body weight gain. In addition, TR-dark fed mice showed similar blood glucose profiles to AL fed mice; however, the profile was altered in mice in the TR-light condition. This suggests that the altered feeding pattern displayed by mice fed only in the light phase affects the capacity to maintain blood glucose homeostasis.

Overall, we developed a system that allows us to uncover detailed individual behavioral changes that mice undergo when exposed to conflicting environmental signals (food and light/dark cycles). Understanding these feeding-induced behavioral changes will help elucidate the as yet unknown mechanism underlying the beneficial effects of temporal restricted feeding.

Research Funding: UTSW Medical Center, NIH

S16

Neural Correlates of Food Anticipatory Activity in Mice Subjected to Once or Twice-Daily Feeding Periods

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Abstract: In mice, the presence of food for only a few hours a day (1 meal) results in the appearance of food anticipatory activity (FAA). Mice can also anticipate 2 meals / day, with FAA split between meals. The underlying neural mechanism of FAA and its behavioral splitting with 2 meals may differ from 1 meal, with the possible involvement of two different neuronal populations. To study this, 2 experiments were performed using memory deficient tissue-type plasminogen activator knock out (tPA-/-) male mice and wild type (tPA+/+, C57BL/6J) males. tPA-/- mice are severely deficient in long-term potentiation, long-term depression, and hippocampal-based learning and memory tasks. Mice were individually maintained in 18L:6D photoperiod with ad libitum (AL) food. After entraining to LD conditions, in the 1st experiment, mice received either 1 meal of 4h at ZT5 (ZT0: lights onset) or at ZT12, and perfused after 1 week at ZT4 or ZT11, respectively. The other group was maintained in AL and perfused at similar ZTs to serve as controls. In the 2nd experiment, mice received 2 meals (separating from 5h) of 2h each, at ZT5 and ZT12. After a week, mice were perfused either at ZT4 or at ZT11. Mice lost more weight under one feeding period per day than under two per day. With 1 meal, FAA developed in all mice with higher activity and longer duration at the later feeding period. Similarly, in 2 meals study, with the 1st meal, all mice developed FAA, although with the 2nd meal, 58% tPA+/+ and 75% tPA-/- developed FAA. Fos-immunohistochemistry data suggests that in response to 1 meal, Fos expression in dorsomedial hypothalamus (DMH) and arcuate nucleus (ARC) is higher in tPA-/- mice than tPA+/+. Contrary to this, with 2 meals, Fos dampens in ARC in both meals, although in the DMH Fos increases in tPA-/- mice in 1st meal. These data suggest that the mechanisms underlying FAA differ between the one and two-meal situations, and that the loss of tPA may limit adaptation to multiple periods of food availability per day.

Research Funding: Postdoctoral financial assistance from Kent State University and Kent State Department of Biological Sciences are greatly acknowledged.

S17

Time of Feeding Regulates Circadian Gene Expression in Mouse Peripheral Tissues

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Abstract: Animals have been adapted to the daily cycles of food availability; therefore energy metabolism has evolved to be circadian. The aim of the present study is to determine how time restriction (TR) of feeding during a specific time of day changes circadian behavior and gene expression in mouse peripheral tissues. We developed automated feeders to regulate timing of food availability and accurately record real-time feeding behavior as well as running wheel activity 24 hours a day. With these feeders we observed that ad libitum (AL) mice eat ~80% of their food at night. In accordance with this, mice fed either AL or TR-dark show similar patterns of circadian gene expression in peripheral tissues. However, when mice were fed only during the light phase (TR-light) gene expression patterns shifted according to the feeding time. Interestingly the magnitude of the phase shift was dependent on the gene. Both core clock genes, Bmal1 and Per2 shift their phase ~12h matching the 12h shift in food-intake. However, Rev-Erbα and Dbp, which are under direct control of core clock genes, show smaller phase shifts in their rhythmic expression. Other metabolic genes, such as Nampt, show intermediate patterns. Amplitude of gene expression was also dependent on feeding time. Rev-Erbα and Dbp amplitudes were increased under TR-dark feeding while decreased under TR-light feeding, suggesting that it would be optimal to eat only during the active phase. Our results suggest that the integration of the feeding signals are complex and affect core clock and metabolic genes differently. Consequently, desynchrony of circadian gene expression within the same peripheral tissue occurs when food intake is restricted to the resting phase. Since adaptation of circadian physiology to meal timing is important to optimize energy and nutrient utilization, this study helps to unravel the contributing molecular mechanisms behind the beneficial effects of time restricted feeding in order to improve treatments for metabolic diseases in the future.

Research Funding: NIH, University of Groningen, MSc fellowship

S18

Phase Shifts in Circadian Peripheral Clocks Caused by Exercise are Dependent on the Feeding Schedule in PER2::LUC Mice

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Abstract: Circadian rhythms are regulated by the suprachiasmatic nucleus (SCN) clock, a main oscillator and peripheral clock. The SCN clock can be entrained by photic and non-photic stimulations, and an interaction exists between photic and non-photic entrainment. Moreover, peripheral circadian clocks are entrained not only by scheduled feeding but also by scheduled exercise. Thus, the entrainment of peripheral circadian clocks may be due to an interaction of entrainment ability between feeding and exercise. Here, we examined the effect of wheel-running exercise on the phase of peripheral clocks in

PER2::LUC mice under various feeding schedules. The phase and waveforms of peripheral clocks were not affected by the wheel-running exercise. Exercise for 4 h during the early dark period (morning) and late dark period (evening) delayed and advanced the peripheral clocks, respectively. The feeding phase was advanced and delayed by evening and morning exercise, respectively, suggesting that the feeding pattern elicited by the scheduled exercise may entrain the peripheral clocks. Exercise did not change the phase of the peripheral clock under a one-meal per day schedule. When the phase of the peripheral clocks was advanced by the feeding schedule of giving 2 or 4 meals per day during the light and dark periods, wheel-running exercise during the morning period significantly moved the phase back to the original positions observed in the mice maintained under free-feeding conditions and without exercise. When the phase of peripheral clock was unaffected by the schedule of 2 meals per day during the dark period, morning exercise did not affect the phase. Wheel-running exercise increased the serum level of corticosterone, and dexamethasone injection instead of exercise changed phase advance to normal phase through the feeding schedule of 2 meals per day back. In adrenalectomy mice, exercise-induced normalization of phase advance through a feeding schedule of 2 meals per day was abolished. In summary, scheduled exercise-induced phase shift was weaker than scheduled feeding-induced phase shift. Phase advance through a feeding schedule of 2 or 4 meals per day was attenuated by wheel running, treadmill exercise, or dexamethasone injection in the early dark period (morning). Corticosterone release may be involved in exercise-induced phase shift of peripheral clocks.

These results suggest the strong interaction between feeding and exercise for phase shifts in peripheral clocks.

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S19

Levofloxacin-Induced QT Prolongation Depends on the Time of Drug Administration

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Abstract: Many physiological processes exhibit diurnal rhythmicity. As a result, the exposure, effectiveness and the severity of side effects of a drug may vary with the time of the day. In this study, we investigated the effect of dosing time on drug-induced delayed ventricular repolarization. Delayed ventricular repolarization is manifested as a prolonged heart-rate corrected QT (QTc) interval on the electrocardiogram (ECG) and is a common side effect of many therapeutic drugs. A randomized, cross-over, clinical trial was conducted in which 12 healthy male subjects received the QTc-prolonging drug levofloxacin at 02:00, 06:00, 10:00, 14:00, 18:00 and 22:00. Using a pharmacokinetic-pharmacodynamic modelling approach to account for variations in drug exposure, heart rate and daily variation in baseline QTc, we found that the relationship between the concentration of levofloxacin and the QTc interval shows a 24-hour sinusoidal rhythm. Simulations show that the extent of levofloxacin-induced QTc prolongation depends on dosing time, with the largest effect at 14:00 (1.73 [95% prediction interval: 1.56-1.90] ms per mg/L) and the smallest effect at 06:00 (-0.04 [-0.19-0.12] ms per mg/L). These results suggest that the extent of drug-induced QTc prolongation depends on the time of day, introducing a bias in the assessment of drug-induced QTc prolongation and potentially resulting in a misjudgment of the risk to patients.

S20

Automatic Scoring of Heart Rate and Wrist Movements to Assess Sleep Architecture

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Abstract: The unique way to establish sleep stage classification remains, until now, the manual or automatic analysis of polysomnographic recordings. Sleep scoring is a major output in a number of settings, such as preoperative testing, clinical research, and evaluation for referral to a sleep center. The aim of our study is to assess a new approach to characterize sleep architecture parameters based on heart rate and body movement recordings instead of polysomnography.

60 sleep nights have been recorded in 12 young healthy men and women volunteers, combining traditional PSGs manually analyzed by two independent and well-trained visual scorers (PSG A and PSG B) according to the rules of the AASM, and off-the-shelf system (Holter ECG and actimetry) for recording heart rate and body movements then analyzed by this new approach named SOMNO-ART.

Over 48 artifact-free nights, the intra-class correlation coefficient (ICC), used to assess the consistency or reproducibility of quantitative measurements made by different observers measuring the same quantity, was classified as excellent for 9 and good for 2 of the 11 sleep descriptors measured.

In conclusion, the automatic analysis of heart rate and body movements during sleep leads to an equivalent evaluation of sleep architecture to the ones obtained from manual scoring of polysomnography. This new scoring technique presents valuable advantages given the fact that the simplicity and ease of the recording system make sleep evaluation possible anywhere and for any number of successive nights.

Research Funding: PPRS Research

S21

Circadian Misalignment and Risk-Taking in Night Shift Workers

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Abstract: Introduction: Circadian misalignment can impact health and performance, and is of particular concern for shift workers. Impairments in cognition can result from circadian misalignment, and may impact decision making and risk-taking. Previous studies in day workers have demonstrated that sleep loss can lead to increased appetitive and risk-taking behaviors; however, fewer studies have examined this in permanent night shift workers despite the high prevalence of excessive sleepiness and sleep disruption. This study examined the relationship between risk-taking behavior and circadian misalignment in a sample of permanent night shift workers.

Methods: Thirty permanent night shift workers (8 male) participated in a larger study examining the health consequences of circadian misalignment. Circadian phase was evaluated using dim-light salivary melatonin onset (DLMO), and DLMO at or after 6am was considered full circadian alignment.

Risk-taking behavior was evaluated using a computerized Stop-Light paradigm, which was completed at 7am. This paradigm mimics the context of a traffic light, where a go/no-go decision must be made at onset of the yellow light, which varies randomly. Successful go trials were rewarded with 25 points, and a percentage of unsuccessful trials were punished with loss of 25 points. Punishment rates varies across a total of 6 blocks.

Results: Workers with larger circadian misalignment earned less total points ($r=.46$, $p<.05$). While participants were more conservative on higher risk trials, this effect was not moderated by degree of circadian misalignment. However, risk-taking behavior did decrease with sleepiness prior to the task ($r=-.51$, $p<.01$), perhaps due to increased risk aversion or decreased appetitive drive. Finally, workers with better circadian alignment achieved higher success rates on go trials ($r=.42$, $p<.05$), suggesting that circadian alignment is associated with improved nocturnal ability for decision making in the context of risk.

Conclusion: Increased circadian alignment in shift work may be associated with improved performance in decisions involving risk. This offers further insight into the cognitive vulnerabilities related to circadian misalignment that may impact risk for errors, accidents, and injuries in night shift workers.

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S22

Evaluation of Biomathematical Models in Predicting Cognitive Impairment among Short-Haul Airline Pilots

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Abstract: The aviation industry relies heavily on bio-mathematical models to aid in schedule design and to guide duty hour limits. There are numerous bio-mathematical models that have been developed to predict performance impairment arising from acute and chronic sleep loss, circadian misalignment, and sleep inertia. It is unclear how well these models predict alertness and performance in an operational environment. The aim of this project was to evaluate the effectiveness of four models in predicting cognitive impairment including sleep loss and modest circadian misalignment in a short-haul airline operation.

We collected sleep and performance measures from 44 (4F) pilots over a four-week period that included early, daytime, and night flight periods. We collected sleep data to use as an input to the models via actigraphy and sleep logs. We compared the model predictions to performance on the psychomotor vigilance test (PVT), which was collected before, during and after each flight, upon waking and before bed. A subset of pilots provided urine samples for the assessment of circadian phase ($n = 13$).

Our preliminary findings suggest that the application of models to predict sleep and circadian phase in an operational environment may be appropriate in some, but not all situations.

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S23

Resetting of Human Peripheral Clocks by Phototherapy During Simulated Night Shift Work

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Abstract: Introduction: Using a simulated night shift experiment, we previously showed that 3-cycle of 8-h bright light exposure at night can fully reset the central clock whereas it took 10 days to observe resetting of clocks in peripheral blood mononuclear cells (PBMCs). The aim of the present study was to better quantify the resetting effects of bright light exposure on both central and peripheral clocks in subjects living at night. Methods: Eighteen healthy subjects (23.7±4.2 years old; 2 women) were enrolled in a simulated night shift experiment. They were assigned to either a control (dim light conditions <10 lux) or bright light (10,000 lux for 8 h at night) group (n=9/group). Hourly blood samples were obtained for 24 h during a constant posture scheduled before (baseline) and after 3 days under a night-oriented schedule. Both central (plasma cortisol and melatonin) and peripheral clock markers (PER1-3, BMAL1 and REV-ERBα expression in PBMCs) were measured. Significant rhythms were determined with harmonic regressions applied to group data using a nonlinear mixed-effect model. F-statistics provided by the model were used to asses significant phase shifts. Results: In both groups, significant rhythms ($P<0.05$) were found at baseline and under the night-oriented schedule for central and peripheral markers (except PER3 in the bright light group at baseline, $P=0.07$), with phases similar to those previously reported. No significant phase shifts were observed in the control group. Bright light induced significant phase delays ($P<0.05$) of ~7-9h for central (melatonin: -6h52; cortisol: -9h16) and peripheral markers (PER1: -7h36; PER2: -9h13; PER3: -8h52; REV-ERBα: -7h33). A trend for an advanced BMAL1 rhythm was also observed (+4h05; $P=0.06$). Conclusion: We have shown faster resetting of peripheral clocks than we previously reported. To our knowledge, this is the first demonstration, in humans, that various clock components are sensitive to night-induced circadian disruption and can be reset at a different rate by bright light exposure at night.

Research Funding: Canadian Institutes of Health Research

S24

Chrono-Typing and Political Orientation: Evidence of Left-Leaning Owls and Right- Leaning Larks

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Abstract: Background: Ideological opinions have been classified most often in terms of a single left-right dimension. The two core aspects of the left-right dimension comprise attitudes such as "change" versus "stability" and "equality" versus "inequality".

Objective: We investigated whether political ideology via a single left-right dimension correlates with chronotype, assessed by a single morning-evening type dimension and phase of entrainment.

Methods: More than 4000 participants online-filled in the Munich Chronotype Questionnaire and the Morningness-Eveningness Questionnaire (MEQ) along with a simple question: "how do you judge

your political orientation: left, rather left, rather right, or right?" We only included responders with German as mother tongue residing in Switzerland, since the definition and the response attitude to a single political left-right dimension differs between countries and cultures.

Results: The remaining 3500 participants judged themselves as follows: 12.8% left, 52.1% rather left, 31.7% rather right, and 3.4% right. The age and gender distribution as well as sleep duration during work and free-days did not significantly differ among political classifications. However, left-leaning people's phase of entrainment was significantly later than in right-leaning people, based on local time on free days (MSFsc), along the left-right dimension: left: 4.2h; rather left: 4.07h; rather right: 3.8h; right: 3.66h. Furthermore, the daily preference (MEQ) was significantly more morning oriented in right-leaning people, and they felt significantly more refreshed in the morning than left-leaning people.

Conclusions: Our data indicate that besides circadian period and environmental light exposure, ideological opinions varies predictably with human phase of entrainment and diurnal preference.

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S25

Gestational Day Length and Risk of Depression in Adulthood in Women

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Abstract: Background: Longer photoperiod during gestation can produce more stable circadian clocks and less depressive-like behavior in rodents; however, epidemiologic studies have not explored this association in humans.

Objective: We examined the association of gestational day length (as a proxy for light exposure) and depression among U.S. women in the Nurses' Health Studies.

Methods: Women reported their birth date (assumed to be the 280th day of gestation) and birth state (used to derive birth longitude based on the state's population center) on initial study questionnaires; this information was used to estimate the length of daylight for each gestational day, based on equations published by the National Oceanic and Atmospheric Administration. Women also reported antidepressant use and clinician-diagnosed depression on study questionnaires at ages 37-79 years. Logistic regression was used to estimate odds ratios [OR] (and 95% confidence intervals [CI]) for depression across quintiles of total gestational day length, the difference between minimum/maximum gestational day length (to capture variation in day length across gestation), and each of these exposures by trimester of pregnancy.

Results: Total gestational day length was not associated with depression in these cohorts (multivariable-adjusted p-trend=0.7 across quintiles of total gestational day length, and OR: 1.00, 95% CI: 0.96-1.04 comparing highest vs. lowest quintiles), and results were similar by trimester (p-trends=1.0, 0.9, and 1.0 for the three trimesters, respectively). However, greater differences between minimum/maximum gestational day lengths were related to reduced odds of depression (multivariable-adjusted p-trend<0.0001, and OR: 0.85, 95% CI: 0.82, 0.89 comparing extreme quintiles). Moreover, this association was specific to the first and second trimesters of pregnancy (multivariable-adjusted p-trends=0.02, <0.0001, and 0.5 in the three trimesters, respectively).

Conclusions: These findings may be consistent with a novel hypothesis suggesting that gestational light exposure might influence the risk of depression in humans. Greater variation in gestational day

length was associated with less depression in these women, which may reflect enhanced adaptation to environmental influences during early and middle life.

Research Funding: NIH R21

S26

Association of Allostatic Load and Shift Work Among US Adults

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Abstract: Background: Allostatic load is a multidimensional indicator theorized to quantify experience- and lifestyle- induced physiological changes using markers from multiple biological systems. An increased allostatic load suggests prolonged or poorly regulated responses to internal and external stressors and has been associated with cardiovascular disease, declines in physical and cognitive function, and all cause mortality.

Objectives: To determine whether shift work is associated with allostatic load and associated health-damaging behaviors such as unhealthy eating.

Methods: This cross-sectional study used data from the American National Health and Nutrition Examination Survey 2005-2010. Shift work included an evening, night, or rotating schedule. Allostatic load was measured using nine biomarkers representing metabolic, cardiovascular, and inflammatory systems. A total of 7663 working adults aged 18 and older were included in the analysis.

Results: Shift work, collective measure of evening, night, and rotating work, was associated with a high allostatic load (allostatic load score ≥3). Stratified analysis suggests that female shift workers have an increased risk for developing a high allostatic load (Odds ratio, OR, 1.82; 95% Confidence interval, CI, 1.23-2.69) compared to men (OR, 1.30; 95% CI, 0.91-1.85). When adjusted for age, sex, race, income, and diet quality-measured in part using the Healthy Eating Index-2005, rotating and night workers had a positive correlation with allostatic load.

Conclusions: This study suggests shift work to be a risk factor for increased allostatic load and associated disorders.

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S27

Achilles, a Clock Controlled Gene, Regulates Innate Immunity in Drosophila

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Abstract: Circadian rhythms are daily oscillations of metabolism, physiology and behavior through a sophisticated system composed of a core clock regulatory feedback loop as well as thousands of tissue-specific clock-driven cycling transcripts. The mechanism of circadian rhythms is essential for most organisms, including Drosophila, to adapt to predictable environmental changes.

Fruit flies are known to behave in a rhythmic manner. Since fruit flies feed largely on rotting fruits, they have developed vigorous immune defense against pathogens. Because of their rhythmic activities, the immune system also acts in a circadian manner. However, it is not totally clear how this rhythmic immune response is regulated. Here we propose that Achl (Achilles), an uncharacterized rhythmic gene that is regulated by the core clock acts as the link between circadian clocks and downstream rhythmic immune responsive pathways in Drosophila. RNA-sequencing data indicates that knock down Achl in neurons causes dramatic activation of immune responsive genes. As a result, a functionally increased pathogen-resistance is found in Achl knock down flies when they are challenged with both gram-negative and gram-positive bacteria, P. aeruginosa and S.aureus using survival assay, colony forming unit assay and qPCR assay on immune responsive genes after inoculation. Meanwhile, no significant change in core clock gene expression and locomotor activity is observed, suggesting that Achl plays the role in mediating rhythmic immune response downstream of core clock genes in Drosophila. More interestingly, Achl RNAi flies have a decreased lifespan comparing to control flies, suggesting a trade-off for having an elevated immune response all the time. Overall, our data suggest that core clock genes drives rhythmic expression of Achl in certain neuron cells, which regulates the immune response in the fat body through neuronal or secretary pathways.

Research Funding: University of Missouri Research Board

S28

Chronotoxicity of Everolimus on the Immune System

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Abstract: Circadian rhythms are endogenous 24-h variations found in nearly all physiological processes. These rhythms are generated by circadian clocks, located in most cell types, including cells of the immune system. mTOR (mammalian target of rapamycin) inhibitor everolimus is an anticancer agent effective against many cancers and also using as immunosuppressant. Everolimus causes toxicities mostly in lymphoid organs including thymus, spleen, lymph nodes and leads to decreased circulating lymphocytes and leukocytes. It is known that drug toxicity and tolerability may be modified depending on the administration time. In our study, we aimed to investigate the chronotoxicity of everolimus on immune system.

C57BL/6 male mice were kept in light-dark (LD) 12h: 12h cycle for synchronization (ZT0 represents the light onset). After synchronization mice were treated orally with 5 mg/kg everolimus or vehicle at ZT1(day) or ZT13 (night) for 28 days. Following the treatment period, mice were sacrificed at ZT1 and ZT13. Blood was collected and flow cytometry was performed to determine the amount of peripheral blood immunocytes. Histopathological damage was investigated on thymus tissues according to the area of cortex and medulla. The thymus/body indexes were calculated as well. The differences between groups were analyzed with ANOVA.

Cortical atrophy was observed in the thymus upon everolimus administration at both ZT1 and ZT13. Strikingly, cortical atrophy in the thymus was more evident when mice treated at ZT1 as compared to ZT13. Thymus organ weights were decreased as compared to controls in each group (ZT1 vs control $p<0.001$; ZT13 vs control $p<0.001$). However, changes in thymus weights were more significant in mice receiving everolimus at ZT1 than ZT13 ($p<0.05$). The amount of immunocytes decreased at ZT1 as compared to control whereas no significant difference observed at ZT13. Total leukocytes

(p<0.01), total lymphocytes (p<0.05), absolute values of T helper (+CD4) (p<0.01) and B cells (+CD19) (p<0.01) were decreased significantly as compared to control at ZT1.

The circadian timing system may play a crucial role in immunological toxicity of everolimus. Chronotherapy of this drug is an effective way to decrease toxicity on the components of immune system.

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S29

Time of Day-Dependent Sensitivity to LPS: A Sensory Role for the Autonomic Nervous System

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Abstract: The nervous system exerts a very important influence on the immune response by means of hypothalamus-pituitary-adrenal (HPA) axis activation, fever induction or sickness behavior (anorexia, sleep disturbance, pain). Additionally, now it is well known that the intensity of the immune response is influenced by the autonomic nervous system.

Since lipopolysaccharide (LPS) minimally passes the blood brain barrier (BBB), the way by which the information about the presence of LPS reaches the brain remains unclear. We hypothesized that the sensory part of the autonomic nervous system is responsible for the immediate sensing of LPS, in order to modify its output to the immune system and mount an adequate response to the inflammatory stimulus.

In the current study, male Wistar rats were intravenously injected with LPS (2ug/kg) and sacrificed 50 minutes later to study neuronal activity by c-Fos immunohistochemistry. Upregulation of c-Fos was observed in sensory autonomic centers such as the nucleus of the solitary tract (NTS) and the dorsal horn (DH) of the spinal cord.

Since the biological clock, the suprachiasmatic nucleus (SCN) can influence the autonomic nervous system, we evaluated day/night differences in the sensing of LPS. The level of activation in these sensory structures depends on the time of LPS administration, with higher c-Fos counts at ZT2 than at ZT14. Plasma levels of cytokines such as tumor necrosis factor alpha (TNFα) and interleukin 6 (IL6) are higher at ZT2 than at ZT14. The present data shows that the sensory part of the autonomic nervous system can detect the presence of circulating LPS. Furthermore, it suggests that the SCN could modify the sensitivity of the autonomic sensory nerve terminals to LPS promoting a day/night difference in brain activation and thus, a differential control of cytokine release.

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S30

The Relationship Between Light Exposure and Subsequent Sleep: What Happens Outside of the Lab?

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Abstract: Background: Timing and intensity of light exposure impacts entrainment of the circadian clock. Light exposure during sleep influences sleep consolidation, sleep architecture and subjective sleep quality. Exposing individuals to either blue or green light in the hours before bedtime sleep alters sleep structure with reduced REM sleep after blue light. High intensity light before sleep appears to affect sleep latencies, whereas light in the morning appears to reduce sleep duration at the expense of REM sleep. It appears that light expected to affect the clock in the hours preceding sleep alters sleep structure and timing. Light exposure at other times of day show differing results. Here we assess in a field study whether an individual's light exposure has a relationship to subsequent sleep. Methods: Young, healthy individuals (21 individuals (9 men) mean age: 23.4±2.1 yrs) wore a wrist actigraph (detecting per minute both photopic lux and activity counts) for 6 days as well as two nights of home EEG recordings. Participants also completed a dim-light melatonin saliva collection in our facilities under <10lux light directly after the actigraph data was collected. Results: sleep onset time appeared to be related to the last time an individual was exposed to more than 10 lux of light. Sleep offset was later with later exposure, even when correcting for DLMO (R^2=0.64, $p < 0.001$). Higher average light intensity was associated with longer REM latency (R^2=0.18, $p < 0.05$), lower percentage of REM sleep (R^2=0.18, $p < 0.05$) and higher percentage of SWS sleep (R^2=0.39, $p < 0.05$). However, individuals with very low average light exposure also showed a larger percentage of SWS sleep than those with intermediate light exposure. REM latency was shorter with later peak light intensity exposure (R^2=0.31, $p < 0.005$). Conclusions: These results show that the relationship between light exposure and sleep is also observable in the field, and that light effects on REM sleep regulation are consistent with laboratory findings, even under field conditions.

Research Funding: NWO-STW consortium grant OnTime

S31

Constant Light During Lactation Programs Circadian and Metabolic Functions in Rat Pups

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Abstract: Epidemiological and experimental evidence support an association between constant light (LL) expossure and an increased incidence of overweight and metabolic disease in rats. The present study aimed to describe the effects constant light (LL) during lactation on the development of the suprachiasmatic nucleus (SCN), on body weight and metabolism in rat pups.

New born rats were randomly assigned to one of three groups: Control photoperiod 12:12 (LD), Constant darkness (DD) or Constant light (LL). Lighting conditions were maintained along lactation from P0 to P21. In order to specifically evidence the effects of light conditions in the pups, the nursing

mothers had a normal LD cycle. At P21 after weaning we determined daily rhythms of glucose, general activity, Vasoactive Intestinal Peptitde (VIP), Argininge Vasopresine Peptide (AVP) and the clock protein PER1 in the SCN.

General activity was assessed from P14 to P21 and we found that 100 % of the litters in LD conditions, 33.33% litters in DD conditions and 16.67% litters in LL were rhytmic [(percentage: #rhytmic litters/ total litters)X100]. In pups exposed to DD and LL rhythm was observed for the peptides AVP and VIP and the clock protein PER1 in the SCN additionally the number of positive inmunoreactive cells of VIP and AVP was decreased in DD and LL conditions. In LL-exposed animals body weight gain was significantly increased as well as fat mass, the glucose rhythm was lost in both DD and LL conditions. Body weight mainteined higher until P90 for animals who had LL conditions during lactation.

Overall our results show that expossure to LL as well as DD affects the development of the SCN leading to a decreased VIP and AVP cell number and a loss of rhythmicity. These animals also had metabolic disturbance and increased weight gain. Present point out the relevance of a normal LD cycle for the development of the SCN and urge to revise the conditions in Neonatal Intensive Care Units as well as in the house enviroment in order to avoid long lasting perturbations in the metabolic and circadian systems.

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S32

A New Standardized Method to Assess the Endogenous and Light–Response of the Retinal Clock in Mammals

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Abstract: The mammalian retina contains an endogenous pacemaker that regulates retinal physiology and tunes the temporal phase of the central clock of the suprachiasmatic nucleus (SCN) to environmental time. This entrainment process involves rods, cones and melanopsin containing retinal ganglion cells (ipRGCs). In contrast with the SCN, the role of these photoreceptors in the light response of the retinal clock is still controversial. While recent studies suggest that none of them is involved in local entrainment of the retinal clock (Buhr et al., 2014, 2015), others support a role for ipRGCs in retinal light response (Dkhissi-Benyahya et al., 2013; Zhang et al., 2008, 2012).

In order to resolve this issue, we first developed a standardized method to determine the phase of the retinal clock in vitro using retinal explants from Per2Luc mice. Retinas were dissected and cultured just before light offset (ZT12) and medium changes were always done at the projected ZT12. We analyzed the effect of medium change and mechanical movement of the culture dishes on the phase of PER2Luc activity in constant darkness. We then applied 465 nm monochromatic light stimulations of different irradiances and durations at CT 16.

We found that using the projected ZT12 as a reference to determine the biological time of the retinal clock is not a valuable marker since medium change does not uniformly reset the phase of PER2Luc. We thus defined the first complete PER2Luc oscillation after start of recording as a marker and found that the trough and the peak of the oscillation occurred respectively around CT8 and CT20. This peak time was used to determine CT16. To apply light stimulation, retinal explants were transferred in darkness

from the Lumicycle into an incubator at the same temperature, near the Lumicycle. Surprisingly, this mechanical displacement produced a random robust effect on the phase of PER2Luc activity, even in dark-control retinas. We thus developed a light stimulation device, embedded within the Lumicycle that avoids mechanical disturbances and ensures stable experimental control conditions. This approach allows us to accurately establish dose response curves that support a role of ipRGCs in the light response of the retinal clock.

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S33

Pineal Serotonin Modulates Entrainment of Central Circadian Clock by Light

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Abstract: The function of melatonin and serotonin (5-HT) produced in the pineal body on the light entrainment of the circadian rhythm has not been fully investigated. In order to delineate the roles of the two pineal hormones, we observed the effects of the two hormones on the phase shift of the circadian clock by light in melatonin-proficient CBA/N or melatonin-deficient C57BL/6 mice. At first, we examined the effect of pinealectomy on the process of light entrainment. After advance or delay of the 12h light and 12 dark (LD) cycle for 6 hours, not only pinealectomized CBA/N but also pinealectomized C57BL/6 mice showed faster resetting than sham-operated animals. Furthermore, administration of melatonin to the pinealectomized CBA/N mice did not modify the recovery from the jet lag. The findings together suggested that melatonin has little effect on the light entrainment of the circadian rhythm. In contrast, 5-HT showed apparent effects on the light resetting process. 5-HT administered to the pinealectomized CBA/N and C57BL/6 mice delayed the resetting of locomotor activity rhythm after an abrupt shift of LD cycle. In addition, in the pinealectomized mice, administration of 5-HT attenuated the pinealectomy-enhanced phase shift of the circadian locomotor activity as well as c-Fos induction in the SCN after a light pulse during the night. Further, administration of p-chlorophenylalanine (PCPA), a 5-HT synthesis inhibitor, to the sham-operated mice generated increase of the phase shift as well as increase in c-Fos induction after a light exposure during the night. The findings suggest that pineal 5-HT suppresses light entrainment by attenuating the light signal transduction to the central circadian clock in the SCN.

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S34

Cryptochrome is a Direct Neuronal Ultraviolet Light Sensor

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Abstract: Short wavelength light, including ultraviolet (UV) and blue light modulates a wide range of behaviors in Drosophila melanogaster and other insects. It has been long assumed that opsin-based

external photoreceptors are solely responsible for UV sensing in insects. In Drosophila melanogaster, a circadian protein CRYPTOCHROME (CRY), functions as a direct blue-light photoreceptor in the central brain, in an opsin-independent manner. CRY mediates behavioral and electrophysiological responses to blue light in circadian and arousal lateral ventral neurons (LNv) in Drosophila. However, spectroscopic and biochemical assays of heterologously expressed dCRY suggest that the photoreceptor may mediate functional responses to UV light as well. To determine whether CRY-based phototransduction system is able to mediate UV light physiological and behavioral responses, we tested mutants lacking CRY and mutants with disrupted opsin-based phototransduction. Here we show that CRY mediates a wide array of behavioral and electrophysiological responses to UV light, in addition to opsin-based external photoreceptors. Mutants lacking CRY show significant defects in a wide array of behavioral responses to UV light, including: circadian entrainment, acute arousal response, and phototaxis. While both dCRY and opsin-based external photoreceptor systems contribute as UV light activated sensory systems, dCRY appears to mediate behavioral responses related to executive function, consistent with its expression in central brain neurons. UV light evokes rapid depolarization and increased action potential firing rate in arousal and circadian neurons, but is significantly attenuated in cry-/- mutants. We show that CRY mediates a novel UV light-sensing mechanism that is able to function as a direct neuronal UV-light sensor. We conclude that CRY- and opsin-based phototransduction act in a coordinated fashion to mediate UV light activated sensory system and modulate many complex behaviors.

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S35

Can a Poor Sleep/Wake Cycle Contribute to Hippocampal Malfunction in a Mouse Model of Neurodevelopmental Disabilities?

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Abstract: There is increasing evidence suggesting that environmental stressors may act as triggers of neurocognitive and behavioural disturbances in genetically predisposed individuals. We have become interested in the possibility that circadian disruption can be one of these negative stimuli or second hit in neurodevelopmental disabilities. To test this hypothesis, we have been exploring the pallid mouse, which carries a mutation in the Bloc1s6 gene encoding a subunit of the dysbindin-containing complex, BLOC-1 (Biogenesis of Lysosome-related Organelles Complex-1). This complex is developmentally regulated and implicated in intracellular protein trafficking. We have shown that the lack of BLOC-1 alters the trajectory of central nervous system (CNS) development with this mouse exhibiting anatomical deficits during development which are generally recovered by adulthood. In exploring the circadian phenotype, we found that the BLOC-1-deficient mice exhibited reduced levels of activity, and that their activity rhythms were significantly more fragmented and of lower precision than in age-matched wild type. Furthermore, the pallid mutants not only slept less than the wild type, but the quality of sleep was compromised by the highly fragmented nature of their sleeping pattern. At the level of the suprachiasmatic nucleus (SCN), the neural activity rhythms appeared normal. In contrast, in the hippocampus, the rhythms in Period2 as well as tCREB were altered. Pallid mice performed poorly in a battery of cognitive measures such as the novel object recognition, elevated plus maze, and open field test. Our data indicates that the lack of BLOC-1 does not negatively influence

the central clock in the SCN. Nevertheless, the extra-SCN molecular clockwork in the hippocampus is disrupted and this may drive cognitive dysfunction in this model.

Research Funding: NIH R01 GM112942

S36

Comt Allelic Variation and Sleep Organization in Human Neonatal Opioid Withdrawal

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Abstract: Neonatal Abstinence Syndrome (NAS) is a common withdrawal complication from prenatal exposure to opioids such as methadone. NAS severity is associated with poor neonatal sleep (O'Brien & Jeffery, 2002). Preliminary work from our group (Wachman et al., 2013) has shown that NAS withdrawal severity differs based allelic variants in the COMT (catechol-o-methyl-transferase) gene. Carriers of minor allelic variants required shorter hospitalization and reduced pharmacotherapy. SNPS in the COMT gene result from a valine to methionine mutation at position 158 (Val158Met) rs4680. Val variant catabolizes dopamine approximately four times faster and more efficiently than methionine. Our study asked if allelic variants of COMT predict NAS severity, would neonatal sleep organization be affected as well. Sleep was assessed using videosomnography and actigraphy in methadone-exposed neonates (N=31) from 2400-0500 h on PND 1 or 2, and compared to genetic data harvested from saliva samples (Genotek OGR-250 kit with CS-1) in the same early withdrawal period. UsingTaqman technology, 158A>G (rs4680, dbSNP; assayC_25746809_50) within the COMT gene was analyzed using the dominant model (AA vs AG/GG). Newborn genetic status was compared to sleep-related variables (e.g. sleep state, arousal and movement parameters) using two factor GLM with maternal prenatal alcohol consumption used as a covariate.The results for gene-behavior corollaries revealed that the carriers of AG/GG genotypes associated with milder NAS had comparatively better sleep organization, more robust sleep related movements, less arousal and cry, and increased sleep frequency and duration compared to carriers of the dominant allele. COMT SNPs are associated with NAS phenotype and functionally linked to neonatal sleep organization.

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S37

Diurnal Regulation of Cocaine Self-Administration

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Abstract: We have previously reported that psychostimulant reward as measured by the conditioned place preference paradigm varies diurnally with a nadir late in the light period. Here, we use drug self-administration paradigms to examine daily rhythms in cocaine taking and in the seeking of

drug-associated cues. Male Sprague-Dawley rats were implanted with jugular vein catheters and trained to self-administer cocaine on a fixed ratio 1 schedule. Following acquisition, daily rhythms in cocaine intake (0.6 mg/kg/infusion) were assessed by giving the animals limited access to cocaine using a discrete trials procedure over consecutive or non-consecutive days. In addition, potential masking influences of test start times were assessed. Daily rhythms in cocaine intake were observed consistently with a low point near the light-to-dark transition. Next, to examine daily rhythms in drug seeking, a separate group of animals was trained to self-administer cocaine during either the late light (zeitgeber time [ZT] 9-12) or late dark period (ZT21-24), and each drug infusion was paired with the presentation of a tone. Again, cocaine self-administration was significantly lower during the late light period compared to the late dark period. In addition, active lever presses for drug-associated cues were higher at ZT23 compared to ZT11. These latter results suggest that drug seeking is increased during the late dark period, although general arousal effects can't be ruled out. Taken together, these data indicate that cocaine reward varies in a diurnal fashion with a nadir prior to the light-to-dark transition.

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S38

Aging Decreases Circadian Regulation of Alcohol Sensitivity and Increases Alcohol-Induced Tissue Injury and Mortality

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Abstract: Alcohol abuse, a growing problem in middle-aged and older individuals, has serious health and economic consequences (CDC, 2015). Although increased sensitivity to alcohol-induced motor and cognitive impairments is observed in aged rodents and humans, little research has been done to determine the endogenous factors influencing alcohol toxicity during middle and old age (Novier et al., 2015). As the circadian clock modulates alcohol sensitivity and toxicity across species, potentially age related changes in circadian function affect alcohol toxicity during aging. Given the highly conserved neurobiological and behavioral effects of alcohol, we investigated the interaction between decreased circadian function associated with aging and alcohol-induced toxicity using Drosophila as a model system. Previously, we found that the circadian clock modulates the loss of motor control, sedation and recovery following acute alcohol exposure in Drosophila, with the greatest alcohol sensitivity observed at night (Van der Linde & Lyons, 2011, De Nobrega & Lyons, 2016). In the current research, we found that as wild type flies age, alcohol sensitivity increases and circadian regulation of alcohol sensitivity weakens. The circadian rhythms in alcohol-induced loss of righting reflex, sedation and recovery remain significant at 10 days following eclosion but disappear by 20 days of age. The circadian clock also modulates alcohol-induced mortality with increased mortality occurring following alcohol exposure during the night in younger flies. Older flies exhibit significantly longer recovery times and increased mortality following alcohol exposure. Flies can be rendered arrhythmic in constant light or genetically using circadian mutants. Per01 flies or wild-type flies in constant light exhibit significantly increased alcohol sensitivity with shorter alcohol exposures needed to induce sedation, longer times needed for recovery and drastically increased mortality, similar to older flies. We hypothesize that the circadian clock phase specifically buffers the effects of alcohol exposure and that this influence on alcohol-induced behaviors is diminished with age. These studies demonstrate that Drosophila is a practical model system for studying the effects of alcohol in aging populations.

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S39

Rev-Erbα Deficiency is Associated with Mixed Affective Behaviors in Mice

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Abstract: Mood disorders often show a mixed affective episode, a condition during which features of both mania and depression/anxiety are observed at the same time. While the molecular mechanisms underlying each manic and depressive/anxious state have been intensively investigated, how these two conditions occur simultaneously remains unclear. Here we report that Rev-erbα knockout (KO) mice develop a mixed affective state with age. In the open field test, KO mice exhibit increased locomotor activity, a sign of a mania-like phenotype, and reduced exploration time in the central area, a symptom that indicates an anxiety-like state although KO mice do not show an anxiety-like behavior in the elevated plus-maze test. Importantly, a DNA microarray analysis reveals that the mRNA expression levels of genes associated with psychiatric diseases including bipolar disorder and anxiety disorder are altered in the prefrontal cortex of KO mice. Our findings suggest that Rev-erbα plays an important role in the regulation of broad classes of genes linked to mood disorders, and thus, dysfunction of the Rev-erbα gene contributes to the development of a mixed affective state.

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S40

Evaluation of Circadian Rhythms and Sleep in the APP/PS1 Mouse Model of Alzheimer's Disease

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Abstract: Alzheimer's disease (AD) is associated with disrupted circadian rhythms and sleep. An estimated 60% of community-dwelling AD patients experience insomnia or other sleep disturbances, which are highly disruptive, stressful, and a leading cause of patients requiring institutional care. Circadian and sleep disruptions may also contribute to cognitive decline and disease progression. To evaluate this hypothesis and test interventions, a suitable preclinical mouse model of AD is needed. We assessed circadian rhythms and sleep in the APP/PS1 mouse model of AD (APPswe/PSEN1dE9) using longitudinal and cross-sectional designs, and behavioural, immunocytochemical, and clock gene assays. Mice were housed in isolation chambers for continuous recording of locomotion using infrared motion sensors. Circadian rhythm endpoints included phase of entrainment to LD 12:12, precision of activity onsets, mean and peak activity level, masking and phase shifting responses to light, endogenous period in DD and LL, damping of rhythm amplitude in LL, and rate of entrainment to shifted LD and scheduled mealtime. Sleep was evaluated by cranial implants of EEG/EMG electrodes. Slow wave sleep was of special interest, as it may be essential for clearing misfolded proteins contributing to AD pathogenesis. At 5-7 months of age, Tg APP/PS1 mice, compared to non-Tg littermates, exhibited a significant phase delay of nocturnal activity onset in LD. At this early time point, group differences were not apparent in rhythm precision, mean or peak daily activity, masking or phase shift responses to light, or tau in DD and LL. A delayed phase of entrainment to LD in APP/

PS1 mice was similar to phase delayed activity and temperature rhythms reported in human AD (e.g., Harper et al., 2001), and may be an early biomarker of AD in this model. APP/PS1 mice may be a useful model for testing interventions to evaluate the role of sleep and circadian desynchrony in AD onset and progression.

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S41

Individual Differences in the Rate of Re-Entrainment to a Phase Advance Predict Anxiety and Depression-Like Behavior

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Abstract: There are considerable individual differences in circadian rhythms in mammals; some people are early risers ("larks") whereas others prefer to be active at night ("owls"). It is important to elucidate the mechanisms accounting for individual differences in circadian rhythms because it provides insight into both normal and aberrantly functioning circadian systems.

In various psychiatric conditions there are disruptions to circadian rhythms (e.g., sleep, hormone release, mood and appetite). Although there is a strong association between disrupted rhythms and various forms of mental illness (e.g., depression, PTSD, psychosis), there is limited evidence that indicates the aberrant rhythms precede the onset of the disorder.

The purpose of this research is to investigate whether individual differences in circadian locomotor parameters predict anxiety and depression-like behaviors. The study is comprised of two parts; first, the circadian locomotor phenotype of male Lewis rats was characterized by collecting wheel running data under different lighting conditions: 12:12 Light/Dark, constant dark, constant light and a six-hour phase advance. The same rats were then tested on a battery of behavioral tests: open field, restricted feeding, elevated plus maze, forced swim test, and fear conditioning.

For data analysis, locomotor parameters were regressed onto the behavioral measures to identify circadian variables that predict anxiety and depression-like responses. Within the current study, rate of re-entrainment emerged as the best circadian predictor. Animals that take longer to re-entrain spend more time struggling in the forced swim test (i.e., climbing), less time immobile (i.e., an adaptive response; see Molendijik & de Kloet, 2015) and are more distressed (measured as defecations). Slower rate of re-entrainment is also associated with more anxiety on the elevated plus maze (i.e., more time spent in the closed arms) and in the open field (more time and distance travelled in the margins, fewer center entries and less distance travelled in the center).

These results indicate that rate of re-entrainment can be used to predict anxiety and depressive-like behaviors in male Lewis rats. By demonstrating that individual differences in circadian rhythms can predict anxiety and depression-like behaviors we provide support for the theory that aberrations within the circadian system precede the onset of certain psychiatric conditions.

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S42

Global and Hepatocyte-Specific Ablation of BMAL1 Induces Hyperlipidemia and Enhances Atherosclerosis

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Abstract: Circadian rhythms controlled by clock genes affect plasma lipids, known risk factors for atherosclerosis. We observed that global ablation of a critical clock gene, Bmal1, in Apoe–/– and Ldlr–/– mice, and liver-specific ablation of Bmal1 in Apoe–/– (L-Bmal1–/–Apoe–/–) mice increases hyperlipidemia and atherosclerosis. In contrast, overexpression of BMAL1 in L-Bmal1–/–Apoe–/– mice decreases hyperlipidemia and atherosclerosis. Physiologic studies showed that Bmal1 deficiency augments hepatic lipoprotein secretion and diminishes cholesterol excretion to the bile. Molecular studies identified that Bmal1 deficiency reduces expression of Shp, a repressor, and Gata4, an activator. Reductions in Shp increase Mtp expression and lipoprotein production, whereas reductions in Gata4 diminish Abcg5/Abcg8 expression and cholesterol excretion to the bile. SHP expression normalized lipoprotein secretion with no effect on cholesterol excretion to the bile, while GATA4 expression increased cholesterol excretion to the bile and reduced plasma lipids in L-Bmal1–/–Apoe–/– mice. Taken together, our data indicate that Bmal1 modulates lipoprotein secretion to the plasma and cholesterol excretion to the bile by regulating the expression of Mtp and Abcg5/Abcg8 via Shp and Gata4, and that the loss of Bmal1 promotes hyperlipidemia and atherosclerosis.

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S43

Interdisciplinary Approaches for Identification of Circadian-Controlled Glycogen Metabolism in Neurospora Crassa

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Abstract: As other living organisms, cell-autonomous oscillations of circadian rhythms provide temporal information to other physiological processes in Neurospora crassa, which is a well-established fungal model for clock study. With a combination of mathematical modeling and experimental validations, we investigate the underlying molecular mechanisms of how circadian clock regulates glycogen metabolism, which is a major pathway for glucose homeostasis. Our data demonstrate rhythmic expression of glycogen metabolic genes, glycogen synthase (gsn) and glycogen phosphorylase (gpn), which are involved in glycogen synthesis and glycogen breakdown, respectively. As a consequence of rhythmic gene expression, glycogen abundances show circadian oscillation. These oscillations are abolished in arrhythmic mutant, frqKO. The extended data uncover that circadian oscillations of glycogen metabolism are regulated by combinatorial actions between different transcription factors. Core clock transcription factors, WC-1/WC-2, cooperate with other clock-controlled transcription factors to regulate the circadian oscillation of gsn and gpn.

Research Funding: DARPA, NIH T32

S44

Effects of Wheel Running Exercise on Feeding Patterns and Glucose Tolerance in C57BL/6J Mice

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Abstract: Increased dietary fat intake and decreased exercise have led to a widespread epidemic of diet-induced obesity and diabetes. Within animal models, exercise has been shown to mitigate the effects of high fat diet on the onset of diet-induced obesity and diabetes. It has been suggested that feeding patterns influence circadian rhythms in metabolically relevant peripheral tissues. In our experiments we tested the effects of diet and wheel running activity on the feeding patterns of male and female C57BL/6J mice and for their ability to maintain glucose homeostasis. Daylight caloric intake increased significantly in mice on a high fat diet. In male mice, food consumption during the daytime was decreased with wheel running activity. In a glucose tolerance test, male mice with wheel running activity displayed a decreased spike in blood glucose levels and had a quicker return to baseline compared to mice with a locked wheel. In addition, body weights and perigonadal fat deposits were reduced in mice with wheels. Insulin sensitivity was impaired within high fat diet treatment regardless of wheel access. In glucose tolerance tests females had greater glucose tolerance than male counterparts. Exercise did not affect body weight, perigonadal fat deposits, or glucose tolerance in female mice on either diet. These results suggest that wheel running activity may mitigate the effects of high fat diet by influencing the timing of food consumption. Future experiments will address the possibility that altered timing of food consumption may alter circadian rhythmicity in peripheral tissues to yield these effects.

Research Funding: Rider University Department of Biology

S45

Mouse Strain Differences in Response to Glucose Tolerance Test

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Abstract: High fat diet (HFD), which is increasingly prevalent in western society, has been shown to induce early signs of obesity and type 2 diabetes in both humans and mice. Effects of HFD in C57BL/6J mice are mediated in part by increased daytime feeding. The glucose tolerance test (GTT) which examines the initial spike of circulating glucose after injection followed by a gradual decline in blood glucose levels over time helps to infer in mouse models whether or not peripheral sensitivity to glucose intake has been affected by treatments such as diet or circadian disruption. Experiments in our lab have shown that BALB/cJ mice show significantly greater glucose tolerance and are resistant to the weight gain and perigonadal fat deposits resulting from HFD. Glucose tolerance is impaired in C57BL/6J mice on a HFD (indicated by a large spike in glucose concentrations as well as consistently high circulating glucose levels over time), whereas the glucose tolerance in BALB/cJ mice is unaffected by HFD, exhibiting dampened initial glucose spike and gradual decrease in circulating glucose over time. Preliminary data indicate that glucose levels rise higher and stay elevated longer in C57BL/6J than BALB/cJ mice on HFD after either intraperitoneal or intravenous

injection of glucose. The purpose of this project is to identify any effects that handling the animals or the stress of glucose administration could have on GTT and to determine if HFD alters feeding patterns in BALB/cJ mice. Preliminary results indicate that for both HFD and low-fat diets (LFD), circulating glucose levels in C57BL/6J mice rose in saline-injected control groups to nearly 25% of the levels following glucose injection. BALB/cJ data collection on handling effects in GTT and on HFD-induced changes in feeding patterns is underway.

Research Funding: Rider University Department of Biology

S46

Dosing Time-Dependent Changes in Beneficial Effects of Sesamin on High Fat-Induced Hyperlipidemia in Rats

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Abstract: Numerous previous studies have reported circadian variation in lipid metabolism, including serum cholesterol and triglyceride levels, the activities and expression of their metabolic enzymes and some molecules involved in fatty acid transport in the liver and white adipose tissue. It is also known that clock genes regulate oscillatory gene expression related to lipid metabolism and transcription factors, such as peroxisome proliferator activated receptor alpha (PPAR alpha). Sesamin, which is an abundant lignan in sesame seeds, has been demonstrated to improve hepatic fatty acid and cholesterol metabolism through the activation of the PPAR alpha pathway in rats. In this study, we investigated whether the effects of sesamin on lipid metabolism are affected by dosing time.

After feeding rats high-fat diet (60 kcal% fat) for two weeks, 100 mg/kg sesamin was administered orally for 7 days at ZT13 (early phase of dark period), ZT23 (late phase of dark period) or ZT1 (early phase of light period). The effects of sesamin on the contents of cholesterol in the liver varied with dosing time; administration at ZT23 and ZT1 showed stronger cholesterol-lowering effects than ZT13 administration. Based on gene expression analysis, CYP7A1, a metabolic enzyme that converts cholesterol to bile acid, and ABCG (ATP binding cassette subfamily G) 5 and ABCG8, transporters involved cholesterol excretion from liver, were increased more effectively at ZT23 and ZT1. These results suggest that activation of these pathways contributes to the dosing time-dependent effects of sesamin on hepatic cholesterol. Sesamin did not affect hepatic triglycerides, although the genes involved in lipid oxidation and synthesis clearly changed with administration. In the weights of visceral adipose tissue, sesamin showed dosing-time dependent effects. Only the ZT23 administration group showed significant inhibitory effects, although we could not clearly identify the reasons based on gene expression analysis.

Overall, these findings indicate that the beneficial effects of sesamin on lipid metabolism vary in a dosing time-dependent manner, particularly with regard to hepatic cholesterol contents, and that they are attributable to the activation of cholesterol metabolism and excretion.

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S47

Circadian Control of Oscillations in Mitochondrial Rate-Limiting Enzymes and Nutrient Utilization by PERIOD Proteins

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Abstract: Mitochondria are major suppliers of cellular energy through nutrients oxidation. Little is known about the mechanisms that enable mitochondria to cope with changes in nutrient supply and energy demand that naturally occur throughout the day. To address this question, we applied mass-spectrometry based quantitative proteomics on isolated mitochondria from mice sacrificed throughout the day and identified extensive oscillations in the mitochondrial proteome. Remarkably, the majority of cycling mitochondrial proteins peaked during the early light phase. We found that rate-limiting mitochondrial enzymes that process lipids and carbohydrates accumulate in a diurnal manner and dependent on the clock proteins PER1/2. In this conjuncture, we uncovered daily oscillations in mitochondrial respiration that peak during different times of the day in response to different nutrients. Notably, the diurnal regulation of mitochondrial respiration was blunted in mice lacking PER1/2 or upon high fat diet. We propose that PERIOD proteins optimize mitochondrial metabolism to daily changes in energy supply/demand and thereby serve as a rheostat for mitochondrial nutrient utilization.

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S48

Characterizing DNA Binding Activities of Mammalian Circadian Clock Protein Complexes

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Abstract: In mammals, circadian clocks are built upon a transcriptional negative feedback loop. The essential transcriptional driver is a heterodimeric complex composed of the proteins CLOCK and BMAL1. CLOCK-BMAL1 complexes are known to activate the transcription of target genes by specifically binding the E-box DNA consensus sequence. Among their many targets are genes encoding key components of the transcriptional repressor known as the PER complex (Kim et. al 2014); a protein complex including PER1-3, CRY1-2, Casein Kinase 1, and many proteins involved in modifying the epigenetic landscape. We performed mass spectrometric analysis of DNA-bound CLOCK-BMAL1 protein complexes and discovered an association with ubiquitin ligase complex components (Tamayo et. al 2015). Mono-ubiquitination of nucleosomes by CLOCK-BMAL1 associated proteins leads to an increased binding of the PER complex to E-box DNA in vivo. What are the mechanisms behind PER complex recruitment to their sites of action within the genome? To further investigate the recruitment of the PER complex to DNA via CLOCK-BMAL1, we have begun studying

its E-box DNA binding in vitro, using tissue extracts as a source of clock protein complexes. The 1.9-megaDalton nuclear PER complex is recruited to naked E-box DNA (from the Per1 promoter) in a CLOCK-BMAL1 dependent manner. By contrast, the cytoplasmic PER complex (Kwak et. al poster in this conference) cannot bind E-box DNA, presumably because it is largely devoid of CLOCK-BMAL1. Furthermore, we found that CLOCK-BMAL1 dependent E-box DNA binding of the PER complex is inhibited by Casein Kinase 1 delta (CK1d) activity. Our previously published findings have concluded that that the nuclear PER complex modifies the chromatin environment at sites of Clock-Bmal1 DNA binding. Here we report that Clock-Bmal1 mediated DNA binding of enzymatically active nuclear PER complex can be reconstituted in vitro, opening the door to a host of experiments that may provide detailed mechanistic insights about circadian clock regulation.

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S49

Prolyl Isomerases-Flipping the Circadian Switch

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Abstract: Mammalian circadian rhythms regulate metabolism, physiology and behavior in synchrony with the 24-hour rotation of the Earth. Desynchronization or decreased robustness of cellular clocks interrupts this homeostasis to increase the likelihood of diabetes, obesity, cancer and cardiovascular disease. The molecular clock is driven by a transcriptional feedback loop, largely controlled by activation and repression of the transcription factor heterodimer CLOCK:BMAL1. Our lab recently discovered a slow timescale conformational change in the C-terminal transactivation domain (TAD) of BMAL1. Locking the TAD into its trans conformation shortens clock period and improves its robustness, suggesting that isomerization may provide a kinetic barrier modulating circadian transcriptional regulation. Peptidyl-prolyl isomerases (PPIs) are enyzmes that exhibit cis-trans isomerase activity, accelerating isomerization by 4-5 orders of magnitude. PPIs of the cyclophilin family are reported to be involved in many biological processes involving signal transduction, viral infection and RNA splicing. Here, we investigate cyclophilin function on the BMAL1 TAD to identify a regulatory mechanism that is poised to control CLOCK:BMAL1 activity. Using NMR spectroscopy, we characterized the activity of nuclear cyclophilins on the TAD, showing that isomerase activity varies in vitro over four hundred-fold for different cyclophilins. Inhibition of the cyclophilin family alters circadian timing in cell-based assays to suggest that isomerases may play a role in the circadian clock. Identifying how prolyl isomerases regulate the intrinsically slow conformational switch of the BMAL1 TAD will allow us to better understand how protein dynamics contribute to circadian timing.

Research Funding: R01

S50

HITS-CLIP Reveals a Role for the RNA-Binding Protein FBP3 in the Circadian Clock

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Abstract: In mammals, circadian clocks drive rhythmic expression of thousands of RNAs, but a significant portion of these rhythmic mRNAs are not transcribed rhythmically. Post-transcriptional regulation plays a very important role in shaping the rhythms of both central clock mRNAs and output mRNAs. Here we characterize the RNA-binding protein FBP3, and show that it is a novel circadian regulator of mRNA stability. We performed high-throughput sequencing of RNA isolated by crosslinking immunoprecipitation (HITS-CLIP) to examine RNA targets of FBP3. Genome-wide mapping of FBP3-RNA interactions indicates that FBP3 binds preferentially to U-rich elements in the 3'UTR region of many genes that exhibit rhythmic mRNA profiles, including several clock genes. Altering FBP3 levels (by CRISPR-generated knockouts or siRNA knockdowns in cells) caused changes in the half-lives and abundance of a subset of target mRNAs. Knockdown of FBP3 in a Per2::luciferase fibroblast cell line caused a significantly shortened period and decreased amplitude. Our results reveal that FBP3 plays a significant role in the post-transcriptional regulation of the circadian clock and its outputs.

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S51

Regulation of Reverbα by the Spsb1-4 E3 Ligase Family

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Abstract: The time-dependent degradation of core circadian clock proteins is essential for the proper functioning of biological rhythms that drive gene expression and ultimately an organism's physiology. Dysfunctional circadian clocks, as occurs frequently in those engaging in shift work, has been shown to increase the risk of development a myriad of negative health consequences, including obesity/diabetes and several cancers. We hypothesize that identifying the ubiquitin ligases and mechanisms involved in degradation of these core clock proteins will lead to development of appropriate therapy for clock-related diseases. We focused on RevErba, an essential circadian transcriptional repressor protein that also coordinates rhythms in metabolic pathways and aimed to identify the ubiquitin ligases that induce its daily degradation and clearance. Our laboratory has developed a simple cell-based functional screening approach to quickly discover ubiquitin ligases targeting proteins like RevErba for degradation, and identified the E3 ligase Spsb4 as a potential novel regulator of RevErba degradation.

Since Spsb4 is one of four Spsb paralogs, we first asked if the other Spsb ligases (Spsb1, Spsb2 and Spsb3) were also capable of destabilizing RevErba. Our data from these experiments suggest that Spsb1 and 4, but not Spsb2 or 3, can induce rapid degradation of RevErba. We also determined if RNAi-meditated knockdown of each of these 4 E3 ligases slowed circadian clock function in U2OS

cells. We found that siRNAs targeting only Spsb1 and Spsb4 slowed clock function, whereas siRNAs targeting Spsb2 and Spsb3 had no effect. Thus, the two Spsb ligases that can cause degradation of RevErba also slow clock function, suggesting that these E3 ligases (Spsb1 and Spsb4) are important ubiquitin ligases in the regulation of RevErba stability and circadian clock function.

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S52

Circadian Clock Regulation of Translation Initiation Through eIF2α Phosphorylation

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Abstract: Roughly half of eukaryotic mRNAs accumulate with a circadian rhythm, demonstrating the profound impact of the clock gene expression. In addition, mounting evidence supports a role for the circadian clock in controlling mRNA translation, extending the influence of the clock on gene expression beyond its role in rhythmic transcription control. However, the mechanisms and extent of translational regulation by the clock are largely unknown. Previous studies in our lab revealed that the clock, through rhythmic activation of the stress-associated p38 MAPK pathway, regulates the activity of translation elongation factor eEF-2 in Neurospora crassa cells. This discovery prompted us to also examine if the clock controls translation initiation, where we first focused on determining if the activity of eIF2α, a conserved component of the translation initiation machinery, is clock-controlled. Phosphorylation of eIF2α typically blocks initation, and is carried out by kinases that are activated in response to various stress signals in eukaryotic cells, including nutrient starvation through GCN2 kinase. However, some mRNAs are actively translated by phosphorylated eIF2α, including mRNAs encoding proteins involved in adaptation to stress that have particular motifs in their 5′-UTR, including uORFS and internal ribosome entry sites (IRES). We show that phosphorylation of eIF2α is clock-controlled, and that the Neurospora eIF2α kinase CPC-3, a homolog of GCN2, is necessary for phosphorylation of eIF2α. In addition, we show that cpc-3 mRNA levels and CPC-3 protein levels accumulate rhythmically. Ribosome profiling, coupled with RNA-seq, in wild type versus Δcpc-3 cells is currently underway to determine the impact of rhythmic phospho-eIF2α levels on translation and rhythmic protein accumulation.

Research Funding: NIH grant

S53

Applications of Machine Learning in the Processing and Analysis of Large Circadian Proteomics Time-Series Datasets

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Abstract: High throughput techniques are becoming a staple of circadian analyses. Unlike RNA-seq in which all transcripts are potentially identified, proteomics analysis by tandem mass spectrometry (MS) produces measurements in which the failure to observe a peptide is partly a factor of its abundance and partly effectively random. Additionally, the large number of samples required for circadian analyses necessarily introduces between-run variation resulting in complex and arbitrary biases. We have developed methods to circumvent these inherent obstacles. A test case involves analysis of circadian proteomics data derived from time courses of Neurospora. This dataset encompasses 48 hours with a two hour resolution, three replicates and two genotypes for a total of 144 samples.

In MS only a small number of peptides are detected in all samples yet many are detected in almost all. Since most methods for analysis of rhythmicity require complete datasets the random missingness can greatly reduce the usefulness of the data; however, the number of proteins detected and the reliability of abundance estimates can be greatly improved by imputing the values of partially missing peptides. We accomplish this using the K nearest neighbors method which uses the mean of the K nearest data points. In order to remove the bias trends introduced by MS run variation we employ the SVD method, which takes a known pattern of replication within the dataset, in this case biological and circadian time replicates, and models variation not shared between those replicates as trends which can then be removed from the data. In addition to the previously described method of selecting the number of trends to remove based on bootstrapping, we have calculated an empirical false discovery rate from randomized data in each case as a more conservative guide in selecting this number.

In the case of the dataset described here, this method has been quite successful. The K Nearest Neighbors imputation has allowed us to recover 29,032 peptides, approximately doubling the number of peptides recorded from the original value of 30,681. The SVD normalization removed major bias trends associated with date of isobaric labeling and MS run order which otherwise dominated the circadian signal. In the wild type set ~ 12% of detected and ~6% of total proteins were observed to be circadian.

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S54

Roles for Period Binding Domain of Dclock in Drosophila Circadian Clock

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Abstract: In the core loop of Drosophila circadian oscillator, PERIOD (PER) physically binds to dCLOCK (dCLK) to inhibit dCLK/CYCLE (CYC) activated transcription. To better understand how PER inhibits the transcriptional activity of the dCLK/CYC complex, we sought to identify the PER interaction domain on dCLK. Mapping experiments in S2 cells identified a PER binding domain (PBD) on dCLK. Transgenic flies expressing dCLK with a PBD deletion in Clkout genetic background (herein referred to as p{dClk delta PBD};Clkout) manifested significantly decreased behavioral rhythmicity in light/dark cycles. In p{dClk delta PBD};Clkout flies, peak level and amplitude of core clock gene mRNA rhythms were lower than those in control flies; furthermore, highly abundant dCLK delta PBD proteins showed significantly decreased binding with PER, indicating a critical role for dCLK PBD in transcriptional activation and repression. Surprisingly, the levels of dCLK target gene-encoded core clock proteins were significantly lower in large and small ventral lateral neurons (LNvs) but not in LNd and DNs in light/dark cycles. Based on these results, roles for PBD on dCLK to regulate cell specific circadian transcriptional activation will be discussed.

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S55

Important Roles of the RNA Editing Enzyme in the Mammalian Circadian Clockwork

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Abstract: Physiological rhythms with about 24-hr period were controlled by thousands of rhythmically expressed genes, which are believed to be generated by circadian transcription via transcription-translation feedback loops. However, recent development in next-generation sequencing technology found an unexpected fact that approximately 70-80 % of rhythmic mRNAs are not rhythmic at de novo transcription levels. So, post-transcriptional regulation should be important for explaining the large part of circadian gene expression. Our previous study showed circadian phosphorylation of CLOCK-BMAL1 complex, and we identified its phosphorylation sites that inhibit DNA-binding ability of the complex. CLOCK-ChIP-Seq identified the genomic regions targeted by the rhythmic DNA-binding of the complex, and RNA-Seq gave us information about rhythmically expressed genes. Here, we focused on an RNA editing enzyme, which has functional E-boxes in its intronic region and rhythmically expressed in mouse liver. The rhythmic expression of the enzyme and the RNA modification rhythms mediated by the enzyme were perturbed in Bmal1-deficient mice. Importantly, not only the RNA modification rhythms but also large populations of mRNA oscillations were attenuated by deficiency of the enzyme. Such a dramatic change of rhythmic transcripts should affect many physiology as

outputs from circadian clock systems. Furthermore, the mutant mice exhibited short-period rhythms in locomotor activity and gene expression. Among the clock genes, only CRY2 protein levels were up-regulated in the mutant, and the period shortening effect was abolished when the mutant mice were backcrossed with Cry2-KO mice. Molecularly, down-regulation of several miRNAs caused the abnormal accumulation of CRY2 protein in the mutant. The present study identifies the RNA editing as a key mechanism of post-transcriptional regulation in the circadian clockwork.

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S56

A Slow Conformational Switch in the BMAL1 Transactivation Domain Modulates Circadian Cycling

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Abstract: Circadian cycling is driven by temporally specific interactions between the heterodimeric transcription factor, CLOCK:BMAL1 and its cognate transcriptional regulators. The C-terminal transactivation domain of BMAL1 is a regulatory hub where transcriptional activators CBP/p300 and the repressor CRY complete for binding. These dynamic interactions are responsible for setting the period of the molecular clock, yet mechanistic details of these interactions have not been fully elucidated. The BMAL1 transactivation domain is intrinsically disordered, allowing it to sample many conformational states at a fast timescale. Using NMR spectroscopy, we discovered the existence of two distinct conformational states that exchange on a slow timescale within this dynamic domain. Here, we report that these two states result from cis/trans isomerization about a highly conserved Trp-Pro imide bond. Both isomers interact directly with CBP/p300 and CRY. Because isomerization occ¬urs on a timescale of minutes, several orders of magnitude slower than the predicted lifetimes of the regulatory complexes, it is poised to serve a regulatory role in assembling transcriptional regulatory complexes in vivo. Genetic complementation of trans-locked mutants in Bmal1–/–PER2Luc cells results in shortened circadian periods to suggest that a proper equilibrium of isomers is key for normal circadian cycling. These data highlight the critical role of protein dynamics at the BMAL1 transactivation domain in establishing the long timescale biochemistry of the molecular circadian oscillator and pave the way for future studies to explore the mechanistic basis of circadian timekeeping.

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S57

Changes in Titin Isoform Compositino Following Inducible Knockout of BMAL1 in Skeletal Muscle

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Abstract: Recent work from our lab has shown that disruption of the endogenous molecular clock mechanism via Bmal1 knockout in skeletal muscle is sufficient to induce muscle weakness with changes in fiber type and fibrosis. To begin to discern the molecular mechanisms that link the changes in the molecular clock with changes in muscle function, we tested whether the muscle of inducible, skeletal muscle specific Bmal1 knockout (iMSBmal1-/-) mice would also exhibit changes in sarcomeric protein expression. One of the proteins we focused on was titin, the giant filamentous protein that maintains sarcomeric structure, underlies passive tension, and serves as a scaffold for many proteins and signaling molecules. In our studies, we determined that the tibialis anterior muscle of the iMSBmal1-/- mice exhibits a significant increase in the longer, more compliant isoform of titin (38% long isoform/total titin in iMSBmal1-/- mice vs. 19% in iMSBmal1+/+ mice) 5 weeks post gene recombination (17 weeks of age). While there is no change in the total amount of titin expressed, comparisons of results from a microarray data set between the two treatment groups indicate that probe sets covering the PEVK region were significantly up-regulated in the gastrocnemius muscle of these mice. Alternative splicing of this region of titin pre-mRNA is known to be regulated by the RNA binding protein RBM20. Using this logic, we noted that expression of Rbm20 mRNA is decreased approximately 24% in iMSBmal1-/- gastrocnemius muscle compared to iMSBmal1+/+ controls suggesting that the molecular clock could be acting through this protein to modify titin splicing patterns. Ongoing studies are focusing on the connection between the molecular clock and RBM20 and if altered expression of RBM20 is responsible for the change in titin alternative splicing.

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S58

Gravitational Loading at the Beginning of the Active Phase Attenuates Muscle Loss in Unloaded Mouse Hind Limb

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Abstract: Muscle mass is maintained by a balance between protein synthesis and degradation. Muscle disuse due to conditions such as bed rest, immobilization, and microgravity induces muscle atrophy. By contrast, muscle stimulation such as by resistance training and gravitational loading protects against muscle loss. However, the effective timing of muscle stimulation for muscle loss is unknown. In this study, we investigated the timing effects of intermittent gravitational loading on muscle loss in unloaded mouse hind limb.

Six-week-old male ICR mice were divided into five groups as follows: an intact group, a hind-limb unloading (unloading) group, and three unloading + intermittent gravitational reloading (reloading)

groups. Unloading was performed by using a tail suspension. Each reloading during unloading were performed at ZT12-16, ZT16-20, and ZT20-24, which we defined as morning, noon, and evening, respectively, for 4 hours per day. Unloading for 2 weeks induced hind limb muscle atrophy (gastrocnemius, soleus, and plantaris muscles). Reloading in the morning significantly prevented unloading induced-gastrocnemius muscle loss when compared with those at noon and evening. In the soleus and plantaris muscles, reloading in the morning also tended to protect against muscle loss. Activity levels in reloading in the morning and evening groups were measured with the area sensor, and their activity levels during unloading and reloading were not changed, suggesting that the preventive effects of reloading in the morning on muscle loss were independent of activity level. The levels of Hsp70 expression, which has a protective function against muscle loss, increased by reloading of the gastrocnemius muscle 3 times, and the Hsp70 gene expression level induced by reloading in the morning was higher than that induced by reloading in the evening.

Therefore, our study suggests that reloading at the beginning of the active phase, morning for humans, is effective to prevent from muscle atrophy.

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S59

Diurnal Variation in G-Protein-Coupled Inwardly Rectifying Potassium (GIRK) Channels in Hippocampus.

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Abstract: Circadian rhythms are biological processes that reoccur every 24 hours and are regulated by a central pacemaker in the suprachiasmatic nucleus (SCN) as well as secondary oscillators in other areas of the brain, such as the hippocampus. G protein-coupled inwardly rectifying potassium (GIRK) channels are important for regulating excitability and mediating cell-to-cell communication. While protein expression of GIRK2 (but not GIRK1) is rhythmically expressed in the SCN, rhythmicity of GIRK channels in other areas of the brain where they have been localized is unknown. We hypothesized that GIRK channels express a circadian rhythm within hippocampal subregions, including areas CA1, CA3 and dentate gyrus. To address this hypothesis, hippocampal subfields were dissected and rapidly frozen from wild-type C57Bl6J mice at 4-hour intervals over a 24-hour cycle. Preliminary results reveal a significant diurnal rhythm in both GIRK1 and GIRK2 expression in dentate gyrus (cosinor analysis, $p < 0.05$) with higher levels in day (ZT 5-6) and lower levels at night. Surprisingly, no significant rhythms of GIRK1 or GIRK2 expression were evident in the other hippocampal subfields. Interestingly, previous work has shown that adrenalectomy plus chronic corticosterone replacement increases GIRK2 expression in the dentate gyrus, suggesting that GIRK channel activation responds to circulating levels of this hormone that is regulated by the circadian clock in physiological conditions. In the SCN, the circadian-regulated hormone melatonin increases GIRK current amplitude. Given the importance for melatonin and corticosterone in regulating hippocampal plasticity and neuronal excitability, it will be important to determine whether day-night differences in GIRK channel expression underlie daily variation in responsivity to these critical neurotransmitters in future studies.

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S60

Circadian Transcription Factor NPAS2 and Metabolic Redox Sensor SIRT1 Interact in the Mouse Striatum to Regulate Reward-Related Behavior

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Abstract: Cocaine addiction is a substance use disorder that is widely prevalent in the United States, causing both social and economic burdens. With the lack of successful therapeutic options, it is important to understand the cellular and molecular level changes following cocaine use, and how these changes may contribute to addiction. As a mechanism of action, cocaine increases mesolimbic dopaminergic signaling via inhibition of dopamine transporter (DAT). This heightened activity is energy taxing and can cause both severe oxidative stress and altered mitochondrial function. Metabolic changes associated with cocaine use may directly regulate the circadian molecular clock and its output genes through associated metabolic redox sensors. More specifically, the core circadian transcription factors CLOCK and NPAS2 and the NAD+ dependent histone deacetylase SIRT1 have all been shown to directly respond to changes in NAD(H) levels, a mitochondrial coenzyme. Previous work in the lab has shown CLOCK and NPAS2 to differentially regulate cocaine reward; mutations in Clock increase cocaine preference and self-administration, while mutations in Npas2 yield an opposite phenotype. Moreover, our data suggest NPAS2 regulates reward through its enriched expression in the nucleus accumbens (NAc). Interestingly, SIRT1 agonists have also been shown to increase cocaine preference, and this effect can be reproduced through the NAc specifically. Given these observations, we sought to investigate the role of cellular metabolic state and the circadian molecular clock in regulating cocaine reward, and how the interaction between NPAS2 and SIRT1 in the NAc may mediate this regulation. Preliminary results suggest chronic cocaine affects the association between NPAS2 and SIRT1 across the circadian cycle, which may be driven by cocaine-induced changes in NAD(H) levels. Chronic cocaine also alters the DNA binding of NPAS2 in the NAc. Furthermore, NPAS2 and SIRT1 interactions in the NAc may be required for the increased cocaine preference seen with SIRT1 activation. Ultimately, our findings highlight a mechanism by which chronic cocaine's metabolic changes can directly alter circadian molecular clock function, and how this interaction, mediated by NPAS2 and SIRT1, can regulate reward-related behavior associated with cocaine addiction.

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S61

Alterations of the Circadian System with Chronic Administration of the Serotonin (1A) Mixed Agonist/Antagonist BMY7378.

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Abstract: Acute systemic injections of the serotonin (5-HT) mixed agonist/antagonist BMY7378 greatly potentiate phase advances to light. The effects of this drug, however, have not been observed under chronic administration protocols. With this study we aim to characterize the effects

of BMY7378 under prolonged administration. Experiments are in progress to look at the effects of chronically administered BMY7378 on circadian wheel running behavior in male hamsters – under 14:10 light:dark (LD) cycles and in response to phase advancing and phase delaying light pulses in constant darkness (DD). Animals are entrained to a 14:10 LD cycle for at least 2 weeks. Subcutaneous osmotic mini-pumps are then surgically implanted between the scapula. Following surgery animals are housed in LD for an additional 2 weeks before being placed into DD. Following a week of DD animals are exposed to 15 minute light pulses at CT 18 and CT 13 to compare phase advance and delay responses respectively. Preliminary results show that animals chronically treated with BMY7378 have significantly diminished light-induced phase advances compared to control animals ($p<0.001$). This could possibly be attributed to a change in 5-HT receptor activity that results from receptor desensitization. No significant differences were found between groups when comparing phase angle of entrainment, total activity or duration of activity ($p>0.05$). These preliminary findings hence suggest a differential effect of BMY7378 on phase shifting responses depending on the duration of administration. This has implications both therapeutically and in terms of the cellular level changes that might occur at the receptor level and possibly in the SCN itself.

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S62

Chronic Sleep Deprivation Inhibits Short and Long Term Memory in Aplysia

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Abstract: Acute and chronic sleep deprivation impair the formation of short and long term memories, although the underlying mechanisms are not clearly understood. Given the rising prevalence of cognitive impairments associated with chronic sleep restriction, there is a need for a simple animal model with established behavioral interactions between sleep and memory to understand the mechanisms underlying memory impairment due to sleep loss. The marine mollusk Aplysia californica, with its simple nervous system and well-characterized learning paradigms, is a good model to study the interactions between chronic sleep restriction and memory formation. Aplysia sleep in consolidated bouts, demonstrate rebound sleep after sleep deprivation, and exhibit longer latencies to sensory stimuli during sleep. Previously using an operant learning paradigm, learning that food is inedible (LFI), wherein the animal associates a specific netted seaweed with the inability to swallow, we found that acute sleep deprivation of 9 h impairs the induction of short-term (STM) and long-term (LTM) associative memory for at least 24 h. In the current study, we investigated the effects of chronic sleep deprivation on memory formation using two different patterns of sleep deprivation. Animals were sleep deprived manually using periodic context changes and tactile stimulation for either 6 h on two consecutive days or 4 h for three consecutive days. They were trained using the LFI paradigm following sleep deprivation. We found that two nights of 6 h sleep deprivation during ZT 12-ZT 18 or during ZT 18-ZT 24 blocked the induction of STM measured 30 minutes after training and LTM measured 24 h after training. The impairment in STM due to 6 h of chronic sleep restriction for 2 nights persisted even after 24 h of recovery. We also found that three nights of 4 h sleep deprivation during ZT 12-ZT 16 or during ZT 20-ZT 24 blocked STM. Interestingly, when the animals were sleep deprived for 3 days during ZT 20-ZT 24, it did not impair LTM with sleep deprived animals showing response times similar to control animals. The results demonstrate the differential sensitivity of STM and LTM to the detrimental effects of chronic sleep restriction. The current study provides a basis for future studies delineating the underlying mechanisms through which sleep affects memory formation.

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S63

Daily and Annual Rhythms of Activity in the Alpine Chamois Under Natural Conditions

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Abstract: Activity rhythms play an important role in the ecological relations of a species and form part of its evolutionary adaptation. We studied daily and annual activity rhythms of Alpine chamois (Rupicapra rupicapra) by analysing high-resolution data of animals monitored with GPS-collars. This first detailed field study of chamois activity showed that this species exhibited clear daily and annual activity rhythms entrained to the light-dark cycle. Actograms showed a diurnal pattern of locomotor activity affected by the changes in photoperiod naturally occurring across the year. Bouts of nocturnal activity were also present during most of the year. Interestingly, the pattern of locomotor activity showed a marked seasonal variation in both females and males; it gradually changed from unimodal (with a single peak of activity from December to May/June) to bimodal/trimodal during summer and autumn. Chamois were more active during spring-summer and less active during winter, likely in response to the variation in the availability of food resources: both sexes appeared to maximise energy intake during the season offering the highest amount of food resources to compensate for poor food supply during winter. Daily activity was influenced by the climatic factors considered: we showed a negative correlation between daily activity and adverse climatic conditions like precipitation and, during winter, snow depth. Because the activity was also strongly influenced by the interplay between temperature and wind throughout the year and by radiation and wind in winter, we hypothesized that it was critically dependent upon animals' thermal balance. In conclusion, our study highlighted that chamois is well adapted to the Alpine environment and seasonality but also raised questions about its ability to adapt to future climate change.

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S64

Clock-Modulation of Virulence in the Phytopathogenic Fungus Botrytis Cinerea and the Evolution of Clock Negative Elements in Fungi

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Abstract: Little is known about fungal circadian clocks besides the well-studied Neurospora crassa FRQ-WCC oscillator. Remarkably, despite the availability of numerous fungal genomic resources, only recently an exhaustive examination of over 450 fungal genomes has allowed the in silico identification of the clock negative element (FRQ) outside a couple of previously identified fungal clades. Not only we identified some putative FRQ-like sequences within basidiomycetes but also, our analysis revealed a FRQ-like sequence in the genome of Rhizophagus irregularis, a fungus located before the divergence of basidiomycetes and ascomycetes. Thus this phylogenetic reconstruction supports the assertion that FRQ might have been present in a more ancient ancestor than previously suggested.

To further explore the importance of clocks in other fungi and the roles served by clock proteins in fungal physiology, we have studied the circadian system of the phytopathogenic fungus Botrytis cinerea. By characterizing a functional circadian clock in this economically important pathogenic organism, we have provided a new perspective on the impact of circadian regulation on the plant-pathogen interaction. Thus, although necrotic lesions on Arabidopsis leaves are smaller when the interaction between these two organisms occurs at dawn, this result does not depend solely on the plant clock (which has been proven to modulate defense mechanisms), but instead largely relies on the pathogen circadian system. Several lines of evidence support the latter assertion: genetic disruption of the B. cinerea oscillator and overexpression of BcFRQ1 abrogate circadian regulation of fungal virulence. Importantly, by conducting experiments with out-of-phase light:dark cycles, we confirm that indeed, it is the fungal clock that plays the main role in defining the daily outcome of the Arabidopsis–Botrytis interaction. Finally, our data also indicates that the Botrytis FRQ homologue may serve extra-circadian functions, being involved in the integration of metabolic signals and developmental decisions. These results highlight the importance of studying well-defined clock proteins in "non classical" circadian models.

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S65

A Fear-Entrained Oscillator in the Mouse

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Abstract: Fear and the physiological and behavioral responses it triggers are essential to avoid threats that can otherwise diminish fitness, or even cause death. Because fear perception is crucial for survival it is not surprising that it is evolutionary conserved. Under natural conditions, fearful stimuli, such as a predator, are likely to present themselves with a 24-h periodicity. Therefore, we hypothesized that cyclic fearful stimuli presented during the active phase of the animal could lead to a shift in the temporal distribution of foraging and feeding of the animal. To test this hypothesis, we housed mice or rats in a naturalistic cage setup consisting of a nesting area and a foraging area. The animals are forced to leave the nesting area to gain access to food and water, which is restricted to the foraging area. The foraging area is rendered dangerous by applying an aversive stimulus (an electric footshock). Using this setup we delivered footshocks in the foraging area that were randomly distributed throughout the dark phase of the LD cycle, the natural foraging and feeding time for nocturnal rodents. We have previously shown that when these shocks were preceded by a cue (a dim light pulse) rats were able to learn the contingency between the conditioned stimulus and the shock; by effectively avoiding the footshock they did not alter their nocturnal pattern of foraging and feeding. In contrast, when the nocturnal footshocks were not signaled by a cue, both rats and mice avoided foraging during the night and switched the phase of their foraging and feeding behavior until most of it took place during the light phase. Interestingly, upon release into constant darkness and in the absence of footshocks, both rats and mice continued foraging and feeding with the same phase as under nocturnal fear conditions, indicating that nocturnal fear entrained a circadian oscillator. Our results indicate that the neural centers that code fear are part of the neural circuitry that constitutes the circadian system, and that the ability of cyclic fear to entrain circadian oscillators may be an evolutionary conserved trait.

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S66

Histone Demethylase JARID1a Regulates Hepatic Glucose Metabolism and Enables Rapid Transcriptional Response to Food Intake

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Abstract: The liver is a key regulatory organ of systemic energy homeostasis whose proper function is dependent on the circadian oscillator. Previously we have shown that the histone lysine demethylase JARID1a is an evolutionarily-conserved component of the circadian transcriptional machinery. To further characterize the role of JARID1a in circadian control of physiology, we have generated a liver-specific JARID1a-null mouse model. Here, we show that loss of JARID1a severely disrupts energy metabolism within the liver, rendering the animal unable to respond normally to feeding cues. Specifically, RNA-seq analysis reveals that the rapid hepatic transcriptional response to feeding is eliminated in JARID1a-deficient livers. We additionally observed decreased expression levels or circadian phase misalignments of glycolytic genes (Gck, Pfkfb3, Pklr), with simultaneous up-regulation of key gluconeogenic (G6pc, Pepck1, Pgc1a) and lipolytic (Cpt1a) genes. JARID1a occupancy at various key regulatory regions of the promoters of these genes demonstrates a circadian profile. In line with these observations, animals demonstrate increased glucose output by the liver and higher levels of hepatic ketone bodies. Furthermore, loss of hepatic JARID1a up-regulated the starvation hormone FGF21 and protected animals from fasting-induced steatosis. In all, our data show that liver ablation of clock-component JARID1a disrupts the circadian control of energy metabolism and gives rise to a pseudo-fasted state.

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S67

Circadian Rhythms in the Sea Anemone Nematostella Vectensis

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Abstract: Most research on animal circadian clocks has been conducted within the clade Bilateria, which includes organisms with brains and organs. Cnidaria (including jellyfish, sea anemones and corals) is the sister group to Bilateria, and unlike bilaterians, cnidarians do not have brains or complex multi-tissue organs. Using the model sea anemone Nematostella vectensis, we previously identified homologues of Cycle (Bmal1), Clock and cryptochromes, and showed that transcripts are expressed on a daily rhythm, and are strongly entrained to blue light. This suggests that components of the core animal clock were in place in the most recent common ancestor of cnidarian and bilaterians. While the physiological outputs of the clock are not well understood, daily cycles in behavior, respiration rate, and chaperone expression have all been observed. Recently, the oxidative state of peroxiredoxins was identified as a universal marker of circadian rhythms. To understand how redox state may interact with the core N. vectensis clock, we identified four N. vectensis peroxiredoxins and determined subfamily affiliations. While these peroxiredoxins do not exhibit diel cycles of expression, these four genes differ in abundance and developmental expression patterns, suggesting functional specialization.

Studies are underway to characterize daily cycles in cellular redox state and peroxiredoxin oxidation, as well as environmental impacts on redox homeostasis.

Research Funding: Moore Foundation

S68

The New Main Factor Influence on a Circannual Rhythm

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Abstract: The main external factors influence on biologic rhythms have cosmophysics reason in going Earth around Sun and Moon around Earth. By this mechanism many ecological factors on our planet are changing periodically. These determine periodical changes of circannual rhythm. The main factors which influence on this rhythm are photoperiod and temperature. In my research I discovered a third main factor which influence on a circannual rhythm. This is a microwave radiation of Sun. Well known that the broad range of electromagnetic radiations come from Sun to Earth, like Gamma ray, X-ray, Ultraviolet, Visible light, Infrared, Microwave, Radio waves. But the atmosphere absorbs about all of them, only ultraviolet, visible light, infrared, microwave, and radio waves can come throw the atmosphere. But ultraviolet, visible light, and infrared waves are not constant because their penetration depends on meteorological condition. The radio waves don't represent a length of day because have a significant diffraction. Unlike them, the microwaves have about the same time frame like infrared (temperature) and visible light (photoperiod) waves but don't depend on meteorological condition of atmosphere and go through shelters and bodies of living beings. For the experiments was selected insect Calliphora vicina R.-D., because it has a demonstrative effect of changing circannual rhythms as diapause in a larva stage in winter and pupation after reactivation in spring. 952 insects in 18 groups on a larva diapause stage were used in the experiments. The season time structure and the intensity of microwaves of Sun was reproduced accordingly the data of Radio Astronomy Station "Zimenki" (Russia). In the experiments was reproduced a long day structure with 18h microwaves radiation and a short day structure with 7h radiation. The experiments were isolated from external factors. Statistically significant changes in the circannual rhythms on the 7 day were observed in reactivation under the reactivation temperature and the long day microwave radiation in 57.14 ± 3.90 % cases, but under short day microwave radiation and reactivation temperature in 40.71 ± 4.62 % cases. The knowledge of microwaves influence on circannual rhythms is very important because many devices radiate these waves like 4G/LTE cell phones, Bluetooth, Wi-Fi, and etc.

Research Funding: Nizhniy Novgorod State Agricultural Academy (Russia) funded it.

S69

Red and Green Luciferases Reveal Phase-Dependent Protein Productivity During Metabolic Rhythms of Yeast

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Abstract: Luciferase is often used as a non-invasive reporter for gene regulation in rhythms studies because it does not require excitation by light (which can perturb rhythms) and its output can be recorded continuously by photodetectors. However, its use is problematic in fast growing microbes because rapidly changing cell numbers and metabolic states also influence bioluminescence. Here, we use two spectrally distinctive luciferases that share a common substrate to overcome these limitations. Green luciferase is used to report transcriptional activity of a gene of interest while a stably expressed red luciferase is used to normalize the signal for non-transcriptional changes to bioluminescence. This, in conjunction with a light-inducible promoter system, was used to test whether different phases of the yeast respiratory oscillation are more suitable for heterologous protein production than others. We show that the early reductive phase of the yeast metabolic cycle produces more luciferase than other phases. This phase corresponds to a time when yeast have recently completed biosynthesis of ribosomes, amino acids, and nucleotides and would therefore be an ideal phase for synchronized cultures to produce heterologous proteins for industry.

Research Funding: This research was supported in part by contributions from the Mary C. Dunn endowment, MTSU URECA, and MTSU FRCAC programs.

S70

Is the Zugunruhe Oscillator Related to MASCO?

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Abstract: The regulation of avian migration in songbirds is a complex phenomenon that has been associated with changes in a variety of traits, and these changes can be collectively called the "migratory syndrome." Most prominent among these traits is the appearance of intense, stereotypical nocturnal activity, commonly called Zugunruhe, in these normally diurnal birds.

The seasonal appearance of Zugunruhe and the accompanying changes in physiology that allow birds to migrate are controlled by endogenous circadian and circannual clocks, and the output of each of these clocks can be shaped by photoperiod. Previously, we had suggested that when a unique circadian oscillator is in a particular phase relationship the expression of Zugunruhe can occur, thereby permitting the bird to migrate. We sought to identify this unique circadian oscillator responsible for the appearance of Zugunruhe and focused on the Methamphetamine Sensitive Circadian Oscillator (MASCO), an SCN-independent circadian oscillator, due to similarities in their behavioral properties. White throated sparrows were provided methamphetamine in their drinking water either outside of the migratory period or during the migratory period while their locomotor activity and behavioral profiles were recorded. Our findings suggest that activation of MASCO can influence the seasonal and daily expression of Zugunruhe.

Research Funding: This work was funded by Office of Naval Research Award N00014-14-1-0703 and by the Pennsylvania State University College of Agricultural Sciences.

S71

Tissue Specific Response of Clock Genes Expression in Peripheral Oscillators in a Rat Model of Shift-Work

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Abstract: Rats are reported to become arrhythmic when forced to be active in a rotating wheel, for 8h daily during their sleeping phase. Using this model, internal desynchronization is elicited due to a combination of an inverted pattern in their food intake, and a SCN driven by the LD cycle. At a molecular level, clock genes expression in the liver is shifted to the forced activity hours for per1 and bmal1 whereas per2 is flattened. Since the shift-work model affects peripheral oscillators, we aimed to investigate a gene/tissue specific effect on clock gene expression for per2, cry1, bmal1 and clock in different tissues, and the misaligned phase relation between genes and tissues that result in internal desynchronization. We also looked at the rhythm of circadian expressed proteins in the SCN. In white adipose tissue, bmal1 was in anti-phase when compared to the control group while per2 and cry1 acrophases were advanced. Regarding the heart and skeletal muscle, per2 maintained his acrophase at the beginning of the dark phase, in contrast, cry1 was advanced and bmal1 had a delayed acrophase in both heart and skeletal muscle. Shifting food intake to the normal activity phase prevented the lost of the correct phase relationship in the expression of clock genes in all tissues. In conclusion, our results indicate a tissue dependent misaligned clock gene expression by this chrono-disruption model and the prevention of rhythmic disturbances by food intake restricted at the dark phase.

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S72

Cryptochromes Suppress PPARd and Limit Exercise Endurance

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Abstract: Circadian clocks coordinate cellular and organismal metabolism to optimize cycles of energy storage and utilization. This global coordination of metabolic rhythms involves Clock and Bmal1 driven transcriptional cycles as well as rhythmic repression of additional pathways by the negative limb of the core clock. We previously demonstrated that the circadian repressors Cry1 and Cry2 drive rhythmic repression of the glucocorticoid receptor (GR). GR is a member of the nuclear hormone receptor superfamily that coordinates the expression of transcriptional networks in response to fluctuations in a variety of hormones and dietary lipids. Among these, the peroxisome proliferator activated receptors (PPARa, d and g) are critical regulators of liver, muscle and fat tissues respectively. PPARd can be activated by AMP-activated protein kinase (AMPK), which can phosphorylate Cry1 and enhance its degradation. Prolonged activation of AMPK and/or PPARd enhances muscle metabolic flexibility and exercise endurance. We demonstrate that muscle explants and primary mouse myotubes exhibit robust circadian rhythms and their circadian period is lengthened in the presence of AMPK

activating compounds. We also show that the interaction of Cry1 and Cry2 with PPARd recapitulates the biochemical features expected of a classical nuclear hormone receptor co-repressor. Genetic deletion of Cry1 and Cry2 in primary mouse myotubes increases PPARd target gene expression and enhances fatty acid oxidation to promote metabolic flexibility. In vivo, Cry1/2-deficient mice exhibit increased expression of PPARd target genes in response to exercise and significantly enhanced exercise capacity in the absence of major changes in muscle fiber type composition or mitochondrial numbers.

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S73

The Arcuate Nucleus: Site for Time-Of-Day-Dependent Negative Feedback on Corticosterone Secretion

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Abstract: The Suprachiasmatic nucleus (SCN) influences autonomic and corticotrophin releasing hormone (CRH) neurons in the Paraventricular Nucleus of the Hypothalamus (PVN) to generate a daily rhthm in corticosterone (Cort) secretion. Although CRH is important for the release of ACTH from the pituitary, the daily variation in Cort secretion is mainly regulated by the autonomic portion of the PVN that projects to the adrenal cortex.

In the present study, we show that PVN neurons expressing glucocorticoid receptor (GR) and those projecting to the adrenal gland are separate populations. Therefore, we hypothesized that the autonomic neurons present in the PVN may require an additional system to sense Cort changes in the bloodstream. Since the arcuate nucleus of the hypothalamus (ARC) expresses GR and projects to the PVN, we investigated whether the ARC is able to detect and produce fast negative feedback of Cort in a time dependent way.

In the morning, at ZT2, under basal conditions, mineralocorticoid receptor (MR) antagonist but not GR antagonist, produced a fast and sustained increase in Cort which was not accompanied by changes in total ACTH.

At ZT10 -the moment of the daily Cort peak- GR antagonist in the ARC increased Cort levels. The importance of the ARC to produce an adequate feedback of Cort was demonstrated by the infusion of a GR agonist in the ARC, which prevents the increase of Cort after stress.

Finally, unilateral retrodialysis of GR antagonist produced a clear unilateral activation of autonomic PVN neurons. Our findings show that sensing actual levels of Cort in the ARC is essential to maintain accurate glucocorticoid levels. Consequently, within the PVN, this feedback information is integrated altogether with other inputs (e.g. the SCN) to maintain an adequate homeostasis of corticoids in a time-of-day-dependent manner. Furthermore, it shows the different role of GR and MR to sense and respond to high and low Cort levels in the ARC, respectively, and adjusting circulating Cort levels via autonomic neural pathways.

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S74

Circadian Clock Regulation of the Melatonin MTNR1B Receptor in Human Myometrial Cells

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Abstract: Circadian genes are expressed in virtually all cells and tissues, and circadian rhythms influence many bodily processes, including reproductive physiology. The expression of the human melatonin hMTNR1B receptor is suppressed during pregnancy until late in term (much like the oxytocin receptor), at which time it is upregulated to allow for the nocturnal melatonin/oxytocin synergy, which promotes strong nocturnal contractions. Little is currently known about the regulation of hMNTR1b, nor about its functional significance in the myometrium. We, therefore, aimed to elucidate some of the transcription factors that regulate hMNTR1b gene expression in the human myometrium, and to determine if hMNTR1b is under circadian control. In this study, we used immortalized and primary myometrial cells that were assessed for circadian gene expression rhythms using real-time bioluminometry and quantitative PCR. Chromatin immunoprecipitation examined the binding of the clock gene product BMAL1 to the promoter of the hMTNR1B gene. Overexpression studies tested the role of CLOCK and its partner BMAL1 in regulating hMTNR1B expression. We confirmed circadian clock gene expression in both immortalized human myometrial cells as well as in primary myometrial cell cultures. We further showed that the hBMAL1 protein binds to an E-box motif in the proximal promoter of the hMTNR1B gene. Overexpression studies demonstrated that the BMAL1/CLOCK complex activates expression of hMTNR1B leading to a circadian rhythm in phase with the E-box driven clock gene hPER2. These results indicate the presence of a functional circadian clock in the human myometrium with the hMTNR1B gene as a clock controlled target. Further investigations along these lines could open new vistas for understanding the regulation of uterine contractions and the timing of human labor.

Research Funding: This work was supported by grants from the Florida State University Council on Faculty Research & Creativity, and the Florida State University College of Medicine.

S75

Lack of Exercise Leads to Altered Activity Patterns in Wild-Type and Vip-Deficient Mice During Light-Dark Cycles

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Abstract: Background and Objectives: In mammals, locomotor activity displays daily/circadian rhythms that are controlled by the central circadian pacemaker (suprachiasmatic nucleus: SCN) in synchrony to day-night cycles. Disrupting the rhythms has many serious health consequences including sleep and metabolic disorders. Overwhelming evidence indicates that lack of exercise is detrimental to health. Here we tested how lack of exercise impacts activity rhythms in wild-type mice and mice with SCN dysfunction from deficit of vasoactive intestinal polypeptide (VIP).

Methods and Results: We studied 9 VIP-deficient (VIP-/-), 6 wild-type (VIP+/+), and 6 heterozygous (VIP+/-) mice (age: 6-35 weeks). VIP +/- mice (congenic with C57BL, courtesy of Christopher Colwell, UCLA) were bred to produce the three genotypes. To monitor locomotion, animals were housed individually under 12h:12h light-dark cycles for ≥10 days with or without access to running-wheels. Locomotor activity was monitored continuously by infrared motion detectors, and data were stored over 1-min epochs. To quantify daily/circadian rhythms, we calculated interdaily stability (IS) and intradaily variability (IV) using the motion data resampled at multiple time scales (1-180min).

As compared to VIP+/+ and VIP+/-, VIP-/- had lower IS (less stable rhythms) ($p=0.03$) but similar IV ($p > 0.05$). Depriving the exercise opportunity (running wheels) reduced IS by ~35% ($p<0.0001$), and increased IV by ~50% ($p<0.001$). Interestingly, the effects of exercise were more pronounced in IS and IV at smaller time scales (e.g., IS, $p\leq0.0001$ at 1-30min and $p=0.0006$-0.02 at 60-180min) while the genotype difference in IS was the most significant at 60-120min ($p=0.006$-0.008). Additionally, aging had an independent effect, causing a decrease in IS ($p<0.0001$).

Conclusions: Lack of exercise leads to less stable and more fragmented daily activity in both wild-type and VIP-deficient mice during light-dark cycles. The effects of exercise and of VIP deficit on activity timing can be better observed at certain but different time scales.

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S76

Differences in Circadian Light Response of Nasonia Wasps from Different Latitudes

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Abstract: Seasonal and daily environmental patterns are formed by gradual fluctuations of temperature and photoperiod, which change with latitude. Latitudinal adaptation of the circadian system is therefore expected. Previous studies demonstrated involvement of the circadian system in photoperiodic time measurement, which varies with latitudes in many insect species. Pittendrigh and Takamura hypothesized that light sensitivity of the circadian system would decrease with latitude, to compensate for the longer light exposure during summer at higher latitudes. To test this hypothesis, we investigated the circadian response to single light pulses with different durations and intensities applied at different time points. We measured the phase response of two Nasonia vitripennis populations, one from northern (Oulu, Finland 65° N) and one from southern Europe (Corsica, France 42° N).

Our results indicate that these two populations react differently to the light pulses. By plotting the results in a phase response curve, firstly, we found that the northern population showed strong resetting in response to all light pulse durations, whereas the southern population shows strong resetting exclusively to long light pulses. Secondly, the northern strain always showed strong resetting in response to all different light pulse intensities, using short light pulses, where the southern population always showed weak resetting. Further analysis revealed a latitudinal cline in light sensitivity and response, but opposite to Pittendrigh and Takamura's hypothesis: Nasonias from northern Europe are more light sensitive and have a higher response than the ones from southern Europe.

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S77

Same-Phase Circadian Rhythms of Trimethylated Lysine 4 on Histone 3 at Promoters of Diversely-Expressed Genes in the Green Alga Chlamydomonas

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Abstract: Chlamydomonas reinhardtii is a unicellular green alga often utilized as a model organism for circadian clock research. A number of genes in this alga were reported to exhibit a circadian rhythm of expression. Since different genes differ in the phase of their mRNA amount rhythm, we were interested in determining whether these differences in phase are reflected in differences in histone modifications at the promoters of these genes. We studied the promoters of two genes whose circadian mRNA amount rhythms show opposite phases and a gene with constitutive mRNA amount. We tested for differences in trimethylated lysine 4 of histone 3 (H3K4me3). This modification has been correlated with active gene transcription. Using chromatin immunoprecipitation and quantitative PCR, we investigated total histone 3 and H3K4me3 in a sequential precipitation. We expected that the amount of H3K4me3 would closely correlate with each mRNA amount pattern. However, our results suggest that H3K4me3 amounts show a circadian rhythm with identical phase at all three promoters. A similar circadian pattern was recently reported for mouse liver, but with the one difference that peak H3K4me3 amounts occur in the middle of subjective night whereas for Chlamydomonas, they occur during subjective day. In conclusion, same-phase circadian rhythms of H3K4me3 at diversely-expressed genes seem to be conserved in such distantly related species as a mammal and a green alga.

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S78

Insulin Resets the Circadian Clock via Induction of Clock Gene PER2

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Abstract: The mammalian circadian clock is an approximately 24-hour rhythm that is entrained by a number of physiological and environmental cues. Prominent among these is feeding time, which can act as a zeitgeber for peripheral tissues. When this occurs out of phase with other timing cues, such as light, the result is a disruption of internal timing — associated with an increased risk of pathologies such as type II diabetes and obesity. However, the specific mechanism by which feeding affects the phase of circadian rhythms in peripheral tissues is not well understood.

Previous work has suggested that the metabolic hormone insulin contributes to entrainment by feeding cycles in liver and adipose tissue, but have not addressed its action in other tissue types. Here, we show that administration of insulin at physiologically relevant concentrations stimulates acute expression of the circadian clock gene PER2 in a range of cell types. This insulin-induced increase in PER2 protein is sufficient to modulate key parameters of the cellular clock in a dose-dependent, phase-independent manner. These effects are independent of glucose availability, but are

reliant upon a number of components of the insulin receptor signalling pathway. Our data further suggest that this mechanism also functions to regulate rhythms in vivo. Thus insulin may constitute a primary means by which mammalian circadian rhythms entrain to feeding cycles.

Research Funding: Medical Research Council PhD Studentship

S79

Coupled Oscillators, Synchronization and (Photoperiodic) Entrainment of Circadian Clocks

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Abstract: Mutual coupling mechanisms between individual circadian oscillators have been observed at the cellular and the behavioral level. In the mammalian Suprachiasmatic Nucleus (SCN), intact cell-to-cell communication has been shown to be quintessential for a precise and robust generation of circadian rhythms. Additionally, social synchronization has been reported in several cases such as bee colonies, co-habited mice or cave bats. In general, mutual interactions between oscillators lead to emergent properties at the network level which have an impact on the performance of the circadian system as a whole. However, the estimation of the underlying coupling strength remains an elusive task. Here, we propose theoretical considerations how to determine the coupling strength between circadian oscillators based on emergent network properties such as the oscillators phase coherence or amplitudes.

Under natural conditions, entrainment by Zeitgeber signals ensures a stable phase-relation between the circadian clock and the 24h rhythms of environmental factors in our spinning world. Importantly, the internal reorganization mediated by mutual coupling also affects the sensitivity of the clock to entrainment signals. In the second part of our work, we study how such intrinsic properties of generic oscillator models influence the systems entrainment behavior under varying conditions. The phase of entrainment as well as the entrainment range is investigated for different Zeitgeber intensities and photoperiods. Finally, we probe our theoretical results with contextual molecular models and experimental data.

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S80

How Can You Tell Your Signal Is Rhythmic?

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Abstract: Spectral analysis is a widely used technique in the detection of rhythms in biochemical and behavioral studies. Multiple methods differing in resolution and computational complexity exist for

calculating power spectra (PS) of biological time series data. These methods often show peaks in the power spectra, suggesting the presence of potential rhythmic signals in the data. But, how does one know if the PS peaks represent actual rhythms and not simply noise in the data? Answering this critical question requires use of a statistical metric that can distinguish real rhythms in the data from noise at a certain confidence level. However, many commonly used methods do not readily offer such a metric and, if not used carefully, application of these methods can lead to incorrect interpretations. We use Monte Carlo approaches to generate significance metrics for four PS methods: Discrete Fourier Transform (DFT), Lomb Scargle periodogram (LS), Maximum Entropy Spectral Analysis (MESA) and Bayesian periodogram. For each method we obtain an analytical expression for confidence levels as a function of variance and length of data. We show how these expressions can be further used to estimate the significance of spectral peaks. Finally, we demonstrate utility of our method by applying it to various kinds of data. The full software package that utilizes our new significance metric will be made publicly available in user friendly format.

Research Funding: This work was supported by funds from the University of Miami.

S81

Circadian Rhythms in Wound Healing in Female Siberian Hamsters

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Abstract: A circadian pacemaker in the hypothalamic suprachiasmatic nucleus imposes temporal control over multiple aspects of immunity. To examine the functional significance of coherent circadian rhythms (CRs) in the immune system towards organismal-level aspects of immune function, we examined whether CRs in cutaneous wound healing exist in female Siberian hamsters, and tested the hypothesis that a functional circadian pacemaker drives such rhythms. Adult female Siberian hamsters were rendered behaviorally circadian arrhythmic via a series of disruptive light pulses. Behavioral arrhythmia (or retention of entrained CRs) was confirmed via periodogram analyses. Hamsters with (entrained; "ENTR") and without (arrhythmic; "ARR") behavioral circadian rhythms remained housed in a 16L:8D photoperiod and received a cutaneous wound in the early portion of either the light (ZT03) or dark phase (ZT18). Wound size was documented daily. Among ENTR hamsters, wounds delivered at ZT03 healed significantly faster than wounds delivered at ZT18, whereas in ARR hamsters, time-of-day did not affect wound healing rates. Wounds also healed significantly more slowly in circadian-arrhythmic hamsters. The data document a robust circadian rhythm in skin wound healing, and indicate that circadian organization in the immune system confers enhancements in this measure of immune function.

Research Funding: Grant AI-67406 from the National Institute of Allergy and Infectious Diseases.

S82

Dosing-Time Dependent Reproductive Toxicity of Everolimus in Male Mice

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Abstract: The circadian timing system controls a variety of biological functions in mammals such as xenobiotic metabolism and detoxification, cell cycle events and may affect pharmacokinetics, target organ toxicity and efficacy of drugs. Everolimus is an orally used selective mTOR (mammalian target of rapamycin) inhibitor which is active against renal, breast and pancreatic cancers. Toxicities associated with everolimus included lymphoid organs, lungs and reproductive organs in mammals. The aim of this study was to investigate dosing-time dependent testicular toxicity of everolimus in mice. C57BL/6J male mice were synchronized with 12 h of Light and 12 h of Dark (LD12:12, with Zeitgeber Time 0 – ZT0 – corresponding to Light onset) for 3 weeks. 5 mg/kg everolimus (n= 8) or vehicle (n= 5) were orally administered to mice at ZT1 (rest period) or ZT13 (activity period) for 4 weeks; body weight loss and changes in testes weights were recorded. Testicular toxicity was histologically investigated. The damage was scored on testis tissues and graded as follows: 0-absence, 1-very rare, 2-weak, 3-moderate and 4-severe. The organ/body indexes were calculated and the statistical significance of differences between groups were validated with ANOVA. Control tissue sections of testes exhibited well-organized histoarchitecture when compared to everolimus treated groups. Drug-induced toxicity on testes included decreased testes weights and histopathological changes such as vacuolisation of germinal epithelium, loss of germinal cell attachment and atrophy of germinal epithelium. Decreased food intake and decreased body weights of mice were observed as general toxicity. Most severe alterations on body weights ($p<0.001$) and testes weights ($p<0.001$) were found in mice treated with everolimus at ZT1 as compared to control. There were dramatic differences between ZT1 and ZT13 groups in the pathological morphology of testis which was more evident when everolimus administered at ZT1 than ZT13 ($p<0.001$) to mice. The delivery of everolimus near its time of least toxicity produced least histological alterations on testes and hereby testicular toxicity. These findings support the concept of everolimus chronotherapy for minimizing the reproductive toxicity and increasing the tolerability of drug which is effective in the clinic.

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S83

Endothelin-1 Regulates the Diurnal Variation of Sodium Excretion in Male and Female Rats

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Abstract: We have previously shown that rats lacking ETB receptors in non-neuronal tissues (ETB def) have an impaired ability to handle an acute sodium load that is time-of-day dependent. Subsequent studies suggested that the attenuated natriuretic response was more evident in male compared to

female ETB def rats. ETB receptor deficiency not only causes salt-dependent hypertension, but also results in elevated plasma ET-1 and unopposed ETA-dependent vasoconstriction that can reduce sodium excretion. Therefore, we hypothesized that ETA receptor blockade (ABT-627, 5mg/kg/day, po) would improve the attenuated natriuretic response to acute salt loading. Male and female ETB def rats and littermate controls were implanted with telemetry transmitters to monitor mean arterial pressure (MAP). After a recovery period of at least a week, baseline urine samples were collected in 12 hr light/dark intervals. A separate group of rats received ABT-627 in the drinking water throughout the experiment. Rats were then given a single 900μEq Na salt load (NaCl) in 1 mL H2O by oral gavage at the beginning of their active (7pm-7am, dark) or inactive (7am-7pm, light) period. Control rats of both sexes given ABT-627 did not show any change in the pattern of urinary sodium excretion and excreted the majority of the salt load during the first 12 hrs regardless of time of day similar to untreated rats. ETB def males treated with ABT-627 showed a significantly improved natriuretic response to a salt load given during the inactive period after the first 12 hrs (490 ± 41 treated, n=7; 80 ± 54μEq Na/12hr untreated, n=6; $P<0.05$). ETB def females treated with ABT-627 also showed an improved natriuretic response during the first 12 hrs of the inactive period as well, though this improvement was not statistically significant (512 ± 35 treated, n=5; 212 ± 34μEq Na/12hr untreated, n=10; NS). MAP was unchanged in response to salt loading although ABT-627 significantly reduced MAP throughout the entire treatment period in both male and female rats ($P<0.05$). These results show that ETA receptor activation impairs the natriuretic response to salt loading in both male and female rats but does not diminish the time–related sex difference in sodium handling.

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S84

Back to the Basics: A Simplified Model of Mammalian Circadian Rhythms

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Abstract: Enormous progress has been made in recent years in understanding mammalian circadian rhythms. In particular, great strides have been made in understanding the coupling mechanisms between circadian cells in the suprachiasmatic nucleus (SCN). Here we focus on two of these coupling agents, Vasoactive intestinal polypeptide (VIP) and Gamma-Aminobutyric acid (GABA).

In parallel with these advances the coupled oscillator community has developed a powerful dimension reduction tool for phase oscillator networks. The Ott-Antonsen reduction allows coupled phase oscillator networks to be reduced to two macroscopic variables describing the mean phase and phase coherence (collective amplitude) of the population. Remarkably, this reduction is exact in the sense that it captures all the long-term attractors of the original high-dimensional system.

We first examine the applicability of the Ott-Antonsen reduction to mammalian circadian rhythms by comparing the predicted phase distribution with experimental data. We find the experimental phase distribution is well described by the reduction when the oscillators are divided into two populations corresponding to the dorsal and ventral subpopulations. We perform the Ott-Antonsen reduction for a model circadian network with both VIP and GABA connectivity. The resulting macroscopic equations describe the circadian clock as a three-dimensional system for the phase coherence of the dorsal and ventral populations and a phase difference between the two populations.

The low-dimension of the model and direct physiological interpretations of the variables allows for mathematical analysis of the model to be compared with experimental data from the cellular, tissue

and organismal levels. Making use of experimental data on seasonal encoding and light entrainment after-effects we apply the model to study GABA interactions in the SCN. The model predicts only a single arrangement of attractive and repulsive GABA coupling within the SCN can explain the experimental results.

As our knowledge of the detailed biochemical mechanisms underlying circadian rhythms continues to increase their is a growing need for physiologically inspired simplified models to guide, explain and interpret experimental results. The Ott-Antonsen reduction provides a powerful tool for this purpose.

Research Funding: NSF Grant: NSF DMS-1412119

S85

Don't Luc Now: How Firefly Luciferase Behaves in Mammalian Cells

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Abstract: Firefly luciferase is frequently used to report circadian gene expression rhythms in mammalian cells and tissues, with the general assumption that the enzymatic substrates are in saturating excess, such that total bioluminescence is directly proportional to transcript and/or protein production. To test this assumption, we compared the enzyme kinetics of purified luciferase with its activity in different mammalian cell types. We observed significant differences between the Km of luciferase for luciferin in solution compared with the Km determined in cellular assays. We found that, consequently, extracellular luciferin concentration can be a significant determinant of (apparent) circadian amplitude and phase. We conclude by urging some caution about the robustness of amplitude and phase measurements inferred from bioluminescence data alone, and suggest that optimal luciferin concentration should be determined empirically for each luciferase reporter and cell type.

Research Funding: Medical Research Council (UK)

S86

The Adrenal Clock Limits Disruption of Circadian Glucocorticoid Rhythms by Aberrant Light Exposure

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Abstract: The glucocorticoid (GC) rhythm is entrained to light-dark (LD) cycles via a molecular clock in the suprachiasmatic nucleus (SCN) that is reset daily by photic signals and is maintained by an adrenal clock that is synchronized by SCN-dependent signals. Aberrant light exposure observed during shift work can disrupt the GC rhythm. To determine the requirement of the adrenal clock in stabilizing the circadian GC rhythm during exposure to an aberrant LD cycle, experiments were done using a novel adrenal clock KO mouse and microdialysis sampling to monitor corticosterone

rhythmicity. We generated ASCre/+ :: Bmal1fl/fl :: R26RmTom/mGFP/+ mice (adrenal clock KO) by intercrossing ASCre/+ mice (Cre recombinase inserted in the aldosterone synthase (AS) genetic locus) with the mT/mG Cre reporter and floxed Bmal1 mice. In adult male and female control (CTRL) mice, membrane bound green fluorescent protein (mGFP) expressed in adrenal cortex, but not medulla, was co-localized with nuclear BMAL1 labeling. In adult male and female KO mice, expression of mGFP was associated with loss of BMAL1 labeling. To examine whether loss of adrenal Bmal1 results in a loss of a functioning adrenal clock, we crossed ASCre/+ :: Bmal1Fl/Fl with mPER2::Luciferase (mPER2Luc) mice. Slices of adrenal cortex from CTRL mice show mPER2Luc rhythms that persist for ~1 week in vitro, whereas slices from a ASCre/+ :: Bmal1Fl/Fl::PER2Luc mouse show rapidly dampened rhythms. Female mice implanted with subcutaneous microdialysis probes were sampled continuously at 30-60 min intervals for up to 3 days under both 12:12h (tau (T) 24) LD and 3.5:3.5h (T7) LD cycles. Dialysate corticosterone was assayed by radioimmunoassay; rhythmicity was assessed using PULSAR analysis. Corticosterone rhythms remain entrained to a T24 LD cycle in CTRL and adrenal clock KO mice. Under T7 LD, circadian rhythms in corticosterone in CTRL mice are disrupted by intermittent corticosterone pulses. In contrast, circadian rhythms are lost in adrenal clock KO mice under a T7 LD cycle due to corticosterone pulses that are magnified in amplitude. These data suggest that circadian GC rhythmicity under a T24 LD cycle is resistant to loss of the adrenal clock. In contrast, under a T7 LD cycle the adrenal clock is required to stabilize the circadian GC rhythm, buffering GC responses to aberrant light.

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S87

Chronopharmacology of Everolimus by Ubiquitin Pathway in Mouse Renal Cell Carcinoma

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Abstract: Mammalians circadian pacemaker resides in the paired suprachiasmatic nuclei and influences a multitude of biological processes, including the sleep-wake rhythm. Clock genes are the genes that control the circadian rhythms in physiology, behavior and effect and/or toxicity of many drugs associated with circadian rhythm of their target enzyme, receptor and pharmacokinetics. Mammalian target of rapamycin (mTOR) is a critical regulator of proliferative signals and a member of the phosphoinositide 3-kinase related kinase family. mTOR plays an important role in cell cycle progression and cell proliferation. The inhibition of mTOR induces cell cycle arrest and apoptosis. In addition, mTOR signal activity is higher in tumor cells as compared with normal cells. Everolimus, mTOR inhibitors, has attracted attention as antitumor drugs. In the present study, the circadian rhythm of mTOR signaling and its regulatory mechanism were investigated in tumor model mice. Furthermore, whether antitumor effect of Everolimus depend on the dosing time of the drug and/or the circadian rhythm of mTOR signaling activity were investigated. A significant circadian rhythm was demonstrated for the protein levels of mTOR in the tumor cells implanted in mice, with maximum levels in the middark phase. On the other hand, no circadian oscillation was demonstrated for the mTOR mRNA levels. Fbxw7, which ubiquitinates mTOR protein, showed circadian rhythm with antiphase manner of mTOR. Dbp binding to D-site element on Fbxw7 promoter was higher in 13:00 than at 01:00 in analysis of transcriptional mechanism. The antitumor effects of Everolimus were more potent in mice injected with the drug at 19:00 as compared with those injected at 07:00. The role of molecular clock was demonstrated for chronopharmacological findings of other drugs associated

with process of protein degradation. These findings suggest that the potency of mTOR inhibitors could be improved by considering timing of administration and the novel regulatory mechanism of cell proliferation by circadian clock systems through mTOR signal pathway modulated by ubiqutination.

Research Funding: These findings indicate the significance of chronopharmacology associated with circadian clock systems through mTOR signal pathway modulated by ubiqutination.

S88

Effects of the Duper Mutation on Phase Shifts and Estrous Cycles

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Abstract: The duper mutation in Syrian hamsters shortens free running period and exaggerates phase shifting responses to 15' light pulses. Unlike tau, duper is not a change in the coding sequence of casein kinase 1ε or any other known clock gene. In a jet lag paradigm, behavioral rhythms of wild type hamsters took 11.9+2.0 and 13.8+3.2 days to re-entrain to 8h advances and delays of the 14L:10D cycle, respectively. Duper mutants achieved the new stable phase within 2.7+0.2 and 2.4+ 0.4 days respectively. In order to assess any contribution of masking, we transferred hamsters to DD on the second day after the phase shift. Both wt and duper hamsters free ran from the phase they had achieved 2d after the change of the LD cycle, showing phase shifts of 2.4+0.4 and 9.4+0.9h, respectively.

Duper hamsters violate Aschoff's rule: locomotor period shortens as the intensity of constant light increases. In order to evaluate the role of parametric responses in phase shifting, we examined the response to an 8h advance of a skeleton photoperiod (1L:12D:1L:10D). Wild type hamsters initially regarded the 10h interval as night, but upon an 8h advance they became active in the 12h interval. When the full photoperiod was restored, they took approximately 3 weeks to regain their initial phase angle. Duper hamsters became active before lights off in 14L:10D and the rapidly entrained upon its restoration, but typically showed a positive phase angle leading to a ψ jump in the skeleton photoperiod. This suggests parametric cues contribute more to stability of phase in dupers than wt.

In order to examine effects of duper on the estrous cycle, we determined timing of the preovulatory LH surge in females maintained in 14L:10D. The surge spanned 6h on proestrus, with the peak occurring 2h earlier in duper than in wt (ZT6 vs ZT8). Hamsters showed little disruption of the estrous cycle (as judged by vaginal smears) upon 8h phase advance of the LD cycle. Nevertheless, no LH surge was evident in either duper or wt hamsters sampled at 4h intervals on proestrus 3 days after an 8h phase advance. The LH surge may be compressed during the process of re-entrainment.

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S89

Aryl Hydrocarbon Receptor Deficiency Alters Circadian and Metabolic Rhythmicity

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Abstract: PAS domain containing proteins can act as environmental sensors that capture external stimuli to allow coordination of organismal physiology with the outside world. These proteins are typically promiscuous in both their ligand binding and heterodimeric partnership, allowing for varied combinations of PAS-dependent protein/protein interactions and promoting crosstalk among signaling pathways. Previous studies report crosstalk between circadian clock proteins and the aryl hydrocarbon receptor (AhR). Activated AhR forms a heterodimer with the circadian clock protein Bmal1, and thereby functionally inhibits CLOCK/Bmal1 activity. If physiological activation of AhR through naturally occurring, endogenous ligands inhibits clock function, it seems plausible to hypothesize that decreased AhR expression releases AhR-induced inhibition of circadian rhythms. Because both AhR and the clock are important regulators of glucose metabolism, it follows that decreased AhR will also alter metabolic function. To test this hypothesis, rhythms of behavior, metabolic outputs, and circadian and metabolic gene expression were measured in AhR-deficient mice. Genetic depletion of AhR enhanced behavioral responses to changes in the light/dark cycle, increased rhythmic amplitude of circadian clock genes in the liver and altered rhythms of glucose and insulin. This study provides evidence of a constant level of AhR-induced inhibition under homeostatic conditions that influences circadian rhythm strength.

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S90

PRD-1, a Component of the Circadian System of Neurospora crassa, is a Member of the Dead-Box RNA Helicase Family

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Abstract: The circadian rhythms found in almost all organisms are driven by molecular oscillators, including transcription/translation feedback loops (TTFLs). However, TTFL-independent oscillators can drive rhythms in both eukaryotes and prokaryotes. The fungus Neurospora crassa is a model organism for studying the molecular mechanism of the circadian clock. Although a circadian TTFL involving the proteins FRQ, WC-1 and WC-1 is well-characterized in N. crassa, rhythms can still be observed in the absence of this feedback loop. These rhythms are said to be driven by one or more FRQ-less oscillator(s) (FLOs). The prd-1 mutation lengthens the period in frq wild-type and was previously shown to severely disrupt FRQ-less rhythms in frq null mutants under several different conditions; therefore the prd-1 gene product is a candidate for a component of a FLO. We report here that prd-1 also disrupts free-running rhythms in wc-1 null mutants, confirming its effects on FRQ-less rhythms. We have now mapped and identified the prd-1 gene as NCU07839, a DEAD-box RNA helicase dbp-2. Complementation with the wild-type gene corrects the rhythm defects of the prd-1 mutant in the

complete circadian system (when the FRQ-based TTFL is intact) and also the free-running FRQ-less rhythm on low choline. A PRD-1-GFP fusion protein localizes to the nucleus. The prd-1 mutant has a single base pair change in the first base of an intron that results in abnormally spliced transcripts. FRQ-less rhythms on low choline, or entrained to heat pulses, were only marginally affected in strains carrying deletions of two other RNA helicases (prd-6 and msp-8). We conclude that PRD-1 is a member of an RNA helicase family that may be specifically involved in regulating rhythmicity in N. crassa in both the complete circadian system and FLO(s).

Research Funding: NSERC

S91

The Effect of Bmal1 Deletion in Gonadotropin-Releasing Hormone or Kisspeptin Neurons

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Abstract: The preovulatory gonadotropin-releasing hormone (GnRH) surge that prompts the ovulatory luteinizing hormone (LH) surge is tightly temporally regulated. Importantly, the signal for this LH surge occurs in a circadian manner, under estrogen-permissive conditions, near the onset of the dark period. This temporal control is thought to be generated by signals from molecular clock proteins such as Bmal1. GnRH and kisspeptin (Kiss) neurons express endogenous clocks, and show evidence of suprachiasmatic nucleus-independent, clock-controlled function. To test the hypotheses that endogenous clocks in GnRH or kisspeptin neurons are regulators of fertility, we crossed GnRH-Cre or Kiss-Cre mice with Bmalfx/fx mice to produce GnRH-Bmalfx/fx or Kiss-Bmalfx/fx mice, respectively. There were no differences in age at puberty onset between GnRH-Bmalfx/fx versus Bmalfx/fx controls (28.5 ± 1.0 days vs. 27.0 ± 0.58, n = 3-4) or Kiss-Bmalfx/fx mice and Bmalfx/fx controls (28.0 ± 1 days vs. 30.3 ± 1.3 days, n = 3). Daily vaginal smears over a consecutive 24 day period revealed no differences in the amount of time spent in each stage between GnRH-Bmalfx/fx and Bmalfx/fx (met/diestrus: 10.8 ± 1.2 vs 12.2 ± 1.4 days; proestrus: 5.4 ± 0.7 vs 4.6 ± 0.5 days; estrus: 7.9 ± 0.6 vs 7.2 ± 0.8, n = 5-8), nor between Kiss-Bmalfx/fx and Bmalfx/fx (met/diestrus: 15.0 ± 1.0 vs 13.3 ± 1.5 days; proestrus: 4.0 ± 0.5 vs 5.0 ± 0.6 days; estrus: 5.0 ± 0.6 vs 6.7 ± 0.9, n = 3-6). Next, we performed a kisspeptin challenge and found no difference in LH levels after 30 nmol kisspeptin in the response of GnRH-Bmalfx/fx compared to Bmalfx/fx (3.9 ± 0.3 -fold increase vs. 7.3 ± 2.0, n = 3-6) or the response of Kiss-Bmalfx/f (5.5 ± .9 -fold increase vs. 7.4 ± 1.3, n = 4-6). We then examined pituitary function by examining LH response to 1 μg/kg GnRH, and found that GnRH-Bmalfx/fx had a significantly higher response compared to Bmalfx/fx (9.17 ± 1.32 -fold increase vs. 6.13 ± 0.28, n = 4-6, significant interaction by two-way ANOVA), whereas there was no difference in the LH response following GnRH administration in Kiss-Bmalfx/fx (7.87 ± 2.14 –fold increase vs. 10.33 ± 4.29, n = 4). Both GnRH-Bmalfx/fx and Kiss-Bmalfx/fx females are able to carry litters to term. Overall, our findings suggest that endogenous clocks in either GnRH or Kiss neurons are not required for normal fertility.

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S92

A Mathematical Model of the Liver Circadian Clock Linking Feeding/Fasting Cycles to Clock Function

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Abstract: To anticipate daily changes in their environment, living organisms have evolved a circadian clock, which synchronizes to the diurnal cycle and orchestrates numerous biological functions. At the organismal level, daylight is the main signal driving the clock. In multicellular organisms, however, clocks in peripheral organs such as the liver are primarily synchronized by fasting/feeding cycles. To better understand how metabolism entrains the liver clock, we have constructed a mathematical model of the mammalian circadian clock incorporating the metabolic sensors SIRT1 and AMPK. We used this model, which reproduces accurately experimental data from mouse livers, to investigate the response of the liver clock to various temporal patterns of AMPK activation, mimicking the effects of a normal diet, of fasting and of high-fat diet feeding. Our results suggest that the severe loss of amplitude in clock gene expression and NAD+ rhythms observed when AMPK is depressed, such as reported for mice on a high-fat diet, may be pharmacologically rescued using a timed REV-ERB agonist administration, providing a strategy to pharmacologically reset the clock in obesity.

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S93

The Transcriptional Landscape Associated With Photoperiodism

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Abstract: Nasonia vitripennis is an emerging insect model, with great potential in the field of biological rhythms and seasonality. Nasonia possess a robust a response to photoperiod, in which adult females exposed to short winter-like days generate progeny that will undergo a developmental arrest (diapause). The molecular basis underlying the photoperiodic clock is unknown. We expect this response to be underpinned by changes in gene expression between long (LP) and short (SP) photoperiod exposed wasps. To identify candidate genes, we employed RNA sequencing (RNAseq) to profile the wasp transcriptome in both conditions. We extracted RNA from the heads of LP and SP-entrained wasps. We identified 66 transcripts as being significantly differentially expressed (FDR < 0.05). Among the top candidates was the orthologue of juvenile hormone acid methyltransferase (jhamt), which displayed higher expression in LP. This gene is essential in the synthesis of the diapause-regulating juvenile hormone in many insects, including in mosquitos and Drosophila. Another gene, gamma glutamyltransferase (ggt), showed a substantial increase in SP, and could be involved in signal transduction via the production of hydrogen peroxide. Utilising RNAi, jhamt and ggt were knocked down in the wasp by injecting dsRNA into pupae. The ability of the wasps to respond to photoperiod (compared to a control group injected with dsRNA against GFP) was assessed. Both sets displayed altered behaviour compared to the control. Wasps injected with jhamt dsRNA responded more rapidly to SP conditions with an elevated level of diapause in both photoperiods, ggt-injected

wasps showed no change in response in LP but displayed significantly reduced levels of diapause in SP. The results suggest a causal role for these genes in the photoperiodic response.

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S94

Beyond Body Weight: How Impaired Leptin Signaling Can Affect Sleep Disordered Breathing

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Abstract: Background: Obstructive Sleep Apnea (OSA) affects ~22 million US Americans and is associated with cardiovascular disease and a four-fold increase in mortality. While OSA is highly correlated with adiposity, mounting evidence indicates that the cause of OSA goes beyond physical weight, and implicates obesity-related physiology, including the role of leptin, in the pathogenesis of OSA. The periaqueductal gray (PAG) is a clear candidate region in the control of sleep disordered breathing due to its abundance of leptin-responsive neurons as well as its role in sleep and respiration.

Objective: To determine how leptin and altered leptin signaling within the PAG affects baseline ventilation and the ventilatory response to hypercapnia (HCVR).

Methods: To examine the overall effect of leptin, we compared leptin deficit ob/ob mice after either receiving no treatment (control), daily leptin treatment (10 ug/day), or caloric restriction (to control for leptin-related weight loss). To drive leptin receptor (LepR) neurons within the PAG, we used LepRcre/L10 mice injected with a cre-inducible DREADD hM3dq virus directly into the PAG. To ablate LepR within the PAG, we used a local injection of an adeno-associated virus that drives cre-expression in LepR floxed mice to knockout PAG leptin action.

Results: Leptin deficient mice exhibit a disordered breathing phenotype, including hypoventilation and a reduction in HCVR. Leptin improves HCVR before significant weight loss occurs. No improvement was observed in the calorically restricted mice. Driving LepR neurons within the PAG leads to tachypnea under hypercapnic conditions, as demonstrated by increased breathing frequency and shorted expiration times. Ablating LepR within the PAG also leads to tachypnea. Moreover, driving LepR neurons within the PAG leads to a significant decrease in HCVR.

Conclusions: Traditionally, it was believed that physical weight on the airway lead to respiratory restriction and OSA. However, using this preclinical model of sleep disordered breathing, these results indicate that leptin signaling is involved in ventilation and HCVR. Moreover, modulation of LepR neurons within the PAG can alter respiration and respiratory drive, suggesting a possible neuronal mechanism involved in disordered breathing independent of physical body weight.

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S95

Crosstalk Signaling Between Circadian Clock Components and Iron Metabolism

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Abstract: There is growing evidence that iron storage and regulation in the body is connected to the circadian clock, although the extent and mechanism of this potential regulation remains largely unknown. We found that the intracellular pool of readily available iron in hepatic cells (HepG2) oscillates as a result of circadian synchronization thus, we asked whether endogenous iron homeostasis including its uptake, transformation, accumulation, and elimination are cellular processes with ties to the circadian circuit. Real-time expression of core circadian genes was downregulated in HepG2 hepatocytes maintained in high iron concentrations (1mM). Accordingly, circadian synchronized HepG2 show a concentration-dependent dampening in their amplitude as a result of iron treatment without any consequences in their viability. Conversely, altering expression of CLOCK and BMAL1 resulted in altered transcription of iron regulatory genes such as IREB2, SLC40A1, and TFRC, all of which influence uptake and elimination. Genome wide ChIP-seq and RNA-seq analyses reveal binding of positive and negative circadian modulators to regulatory regions within iron metabolic genes in a rhythmic manner. In the case of TFRC, rhythmic binding precedes defined rhythm in transcription. Because of TFRC's critical role in importing transferrin bound iron to the cell, this may prove to be crucial in synchronizing an organism's intracellular iron concentration with the circadian intake of dietary iron.

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S96

Extensive Regulation of Diurnal Transcription and Metabolism by Glucocorticoids

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Abstract: Glucocorticoid hormones are important for several physiological processes such as metabolism and their release is regulated by the circadian clock. However, not much is known about how circadian clocks and glucocorticoid hormones interact with each other to mediate circadian regulation of metabolism. We examined a zebrafish model for glucocorticoid deficiency in order to determine diurnal metabolite and transcriptome patterns in vivo. Our data revealed severe temporal dysregulation of important pathways of energy metabolism. Interestingly, constant glucocorticoid hormone treatment is able to rescue half of the dysregulated diurnal transcriptome. Thus, rhythmic expression of metabolic genes does not necessarily require the circadian release of glucocorticoids. A simple combination of E-box and glucocorticoid response elements which is enriched in the rescued gene set is sufficient to drive glucocorticoid-dependent circadian reporter gene expression. Our work highlights metabolic pathways which may contribute to disease symptoms in glucocorticoid deficiency

patients, even under glucocorticoid replacement regimes. Moreover, we provide novel mechanistic insight into the cooperation of the circadian clock and glucocorticoids in the transcriptional regulation of metabolism.

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S97

Daily Magnesium Fluxes Regulate Cellular Timekeeping and Energy Expenditure

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Abstract: Circadian rhythms are a fundamental feature of most eukaryotic cells, regulating metabolism to match our planet's day/night cycle. A fundamental knowledge gap exists between cycles of clock-controlled gene expression and the biochemical mechanisms that ultimately facilitate cell-autonomous metabolic rhythms. We here report circadian rhythms of intracellular Mg2+ concentration, [Mg2+]i, in both a human cell line and a unicellular alga that diverged from metazoans more than 1 billion years ago. [Mg2+]i oscillations are not only clock-regulated, but also exhibit features of a core clock component: regulating period, phase and amplitude. Mg2+ ions are essential cofactors for all reactions that consume nucleotide triphosphates, and thus we find that [Mg2+]i oscillations contribute to the circadian regulation of energy-intensive protein synthesis. Our results suggest that [Mg2+]i oscillations, generated at the membrane, represent a conserved eukaryotic clock mechanism, linking the circadian cycle with metabolism in evolutionarily diverse cell types.

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S98

ZeitZeiger: Supervised Learning for Oscillatory Data

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Abstract: Numerous biological systems oscillate over time or space. Despite these oscillators' importance, data from oscillatory systems are problematic for standard methods of supervised learning. We developed ZeitZeiger, a computational method to predict a periodic variable (e.g., time of day) from a high-dimensional observation. ZeitZeiger learns a sparse representation of the variation associated with the periodic variable in the training observations, then uses maximum-likelihood to make a prediction for a test observation.

We first applied ZeitZeiger to a comprehensive dataset of genome-wide circadian gene expression in mice. Using the expression of 13 genes, ZeitZeiger predicted circadian time in each of 12 mouse organs

to within approximately 1 hour, resulting in a multi-organ predictor of circadian time. Compared to the state-of-the-art approach, ZeitZeiger was faster, more accurate, and used fewer genes. When applied to 20 additional datasets, the multi-organ predictor detected the progression of the clock in vitro and recognized when the clock in vivo was phase-shifted or dysfunctional. We are now using ZeitZeiger to systematically compare circadian gene expression between different species, tissues, and phenotypes.

Overall, our results suggest that ZeitZeiger not only makes accurate predictions, but also identifies major patterns and important features, and detects when the oscillator is perturbed. As our ability to collect high-dimensional data from various biological oscillators increases, ZeitZeiger should enhance efforts to convert these data to knowledge.

ZeitZeiger is available as an R package at https://github.com/jakejh/zeitzeiger. All code, data and results for our first study are available at http://dx.doi.org/10.5061/dryad.hn8gp.

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S99

Characterizing Core Clock Gene Dynamics in Mouse and Human Peripheral Blood Using Simulated Shift Work Protocols

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Abstract: Given that mice are nocturnal and humans are diurnal, aspects of their clock gene dynamics and the outputs thereof may exhibit opposite circadian phases between the two species. To investigate this, we collected peripheral blood from both C57BL/6 mice and healthy human subjects assigned to either a simulated day shift schedule or a simulated night shift schedule. We characterized the expression levels of core clock machinery, key clock-controlled genes and plasma corticosterone (mice) or cortisol (humans). N=14 healthy humans (25.8±3.2 years old, 4 females) participated in a laboratory study. Half the sample was assigned to a simulated day shift condition with 4 days of daytime wakefulness (06:00–22:00). The other half was assigned to a simulated night shift condition with 4 days of nighttime wakefulness (18:00–10:00). On the fourth day, subjects were exposed to a constant routine with 24h wake extension and blood sampling every 3h. Further, 12-week old C57BL/6 female mice were kept for 3 weeks under LD 12:12 with light from 06:00 until 18:00. Mice were then subjected to either a regular day light schedule (LD 12:12 with light at 06:00-–18:00) or an opposite night light schedule (LD 12:12 with light at 18:00–06:00). Blood samples were collected every 4h over a 24h period (n=6 per time point); sleep was not restricted. In both studies, blood was processed immediately for plasma and RNA isolation. Clock and clock-controlled gene expression and cortisol/corticosterone levels were measured. We observed differences in the circadian profiles of clock and clock-controlled gene levels between simulated day and night shifts for both humans and mice. In the day shift condition Cry1, Cry2, Per1 and Per2 were increased in the morning hours relative to the evening hours, whereas Bmal1, Nr1d1, and Dbp were decreased in the morning hours relative to the evening hours. In the circadian-misaligned night shift condition, the reverse patterns were observed. Differences in cortisol/corticosterone levels between the two conditions indicated perturbed clock mechanisms in the circadian-misaligned condition in both humans and mice. Our findings provide a foundation for translating between mouse models and humans in future studies of clock-controlled genes that regulate cellular processes such as cell proliferation and DNA repair.

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S100

Examining the Contributions of the BrLKP2 Gene Family to the Circadian Clock in Brassica Rapa

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Abstract: Light regulated protein turnover is a common feature among circadian clocks. In Arabidopsis, ZEITLUPE (ZTL)and its close relatives LOV KELCH PROTEIN2 (LKP2) and FLAVIN BINDING, KELCH REPEAT, F-BOX1 (FKF1) encode F-box proteins with an N-terminal LOV domain, and a series of Kelch repeats at the C terminus. ZTL is necessary for maintaining a normal circadian period whereas FKF1 and LKP2 play minor and primarily redundant roles in determining circadian period. In addition, FKF1 is a necessary component in the early steps of photoperiodic flowering. To define the extent to which the Arabidopsis model can be extrapolated to other species, including crops, we wished to examine the role of these F-box proteins in the agricultural crop, Brassica rapa, a close relative of Arabidopsis. Following divergence from Arabidopsis, B. rapa has undergone whole genome triplication and subsequent diploidization resulting in considerable gene loss. In B. rapa, ZTL and FKF1 have been lost, in sharp contrast to most other clock genes which have been preferentially retained in two or three copies. However, a single locus of LKP2 has been retained. Intriguingly, a tandem triplication event has resulted in three tightly linked copies of LKP2. This raises the question of whether these three copies of LKP2 in B. rapa have undergone functional specialization and now fulfill the functions of ZTL and FKF1. Accordingly, we transformed the B. rapa LKP2 genes into the Arabidopsis long period ztl mutant and the late floweringfkf1 mutant. Circadian period and flowering time analysis of these transgenic lines provides insight into the possible roles of these LKP2 genes in B. rapa.

Research Funding: The Research Program for Agricultural Science & Technology Development (Project No. PJ10025), the Rural Development Administration, Republic of Korea.

S101

Exposure to Long Photoperiods Induces Changes in Coupling Between Single Neurons of the Mouse Suprachiasmatic Nucleus

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Abstract: Anticipating seasonal changes in temperature and in food availability is important for the survival and reproductive success of many organisms. The suprachiasmatic nucleus (SCN) is considered to be the central pacemaker, and is known to respond to changes in photoperiod. Seasonal variation in day length results in changes in phase distribution of single-cell circadian oscillations in electrical activity and gene expression in the SCN. In addition, several studies have shown regional differences in Period gene expression rhythms. In this study, we investigated phase distributions for PERIOD 2 (PER2) expression in single cells after adaptation to long (LP; 16:8), or short (SP; 8:16) photoperiod along the anterior-posterior and dorso-ventral axis of the SCN.

In long photoperiod, the anterior region shows a higher phase dispersion between cells ($P < 0.001$; LP: 3.42 ± 0.19 h, n = 5, SP, n = 4: 1.42 ± 0.04 h), which is accompanied by a higher period length variation of individual cells ($P < 0.001$; LP: 1.78 ± 0.30 h, n = 5, SP: 1.28 ± 0.17 h). These results suggest that a decrease in coupling strength contribute to the wider phase dispersal.

To assess differences within slices, we employed a new community detection approach designed specifically for correlation matrices. Interestingly, we persistently identified two communities of neurons, dividing the SCN in a ventral-medial region (VM), and a central region (C). The ventral-medial region peaks earlier than the central region, and displays a higher phase distribution between the cells in long photoperiod. The central region on the other hand shows a higher single-cell period length variability, but a lower phase distribution compared to the VM region.

An important question in the field of circadian rhythm research is how phase synchrony and desynchrony are established. Phase desynchrony can either be the result of poor coupling among SCN neurons, or it can result from stable coupling in which phase differences are actively established. The finding that higher phase dispersion is accompanied by a larger cycle-to-cycle period length variation of single cells suggest that there is less coupling among SCN neurons in long photoperiod.

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S102

A Role for the Cationic Leak Channel Nalcn in Daily Rhythms of Suprachiasmatic Nuclei Activity and Locomotor Behavior

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Abstract: 24h timing is encoded in the brain by rhythmic changes in membrane properties of the circadian pacemaker neurons of the suprachiasmatic nucleus. Here, we study the ionic basis of mammalian circadian pacemaker neuron function, building on our discovery of a role for a novel cationic leak current in both fly and mammalian clock neurons. We previously found that, in flies, the cationic leak current encoded by narrow abdomen (na) functions in clock neurons to mediate circadian behavior. We also demonstrated that this cationic leak current is under clock control in both flies and mice. In a subset of Drosophila clock neurons, we also found that the molecular clock drives rhythmic expression of NLF-1 that elevates the activity of NA and daytime neuronal activity likely via channel trafficking to the plasma membrane. Although we demonstrated a rhythmic cationic leak conductance in both Drosophila clock neurons (via NA) and in mammalian SCN neurons (via NALCN), an in-vivo functional role of this conductance in mediating rhythms in mammalian clock neurons and behavior has yet to be described. Moreover, the mechanisms of clock control of the mammalian NALCN current are unknown. Using state of the art genetic tools, we are now addressing these questions to reveal the role of NALCN in mammalian circadian physiology and behavior and its mechanism of clock regulation.

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S103

Circadian Rhythms in the Expression and Function of Synaptic and Extrasynaptic Gabaa Receptors in the Suprachiasmatic Nucleus

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Abstract: GABAA Receptors (GABAARs) are pentameric ligand gated ion channels and their subunit composition determines their channel properties and location on the cell membrane. GABAARs containing the Δ subunit are tonically active, high-affinity, non-desensitizing channels found at extrasynaptic locations, whereas classical phasic inhibition is mediated by synaptic GABAARs containing the γ2 subunit. Recent studies have revealed that expression levels of Δ and γ2 subunits may regulate the balance between tonic and phasic inhibition in multiple brain regions, and we explored this phenomenon in the SCN of male Syrian hamsters using behavioral pharmacology, qRT-PCR gene expression analysis, and immunohistochemical protein expression analysis. As previously shown, we confirm that the acute effects of GABA are mediated by extrasynaptic GABAAΔ receptors during the subjective night, whereas the acute effects of GABA during the subjective day are mediated by synaptic GABAAγ2 receptors. Independent of photic input, mRNA for GABAAΔ and γ2 were expressed in an antiphase circadian rhythm, with Δ at peak and γ2 at nadir in both the day and the subjective day. Protein for both subunits was expressed in a 24h pattern in a light:dark cycle, with the peak occurring at night. However, when held in constant dark for 10 days, expression rhythms were abolished for GABAAΔ and inverted GABAAγ2, with peak γ2 protein expression during the subjective day. These data support the hypothesis that circadian rhythms in tonic and phasic GABAAR protein expression underlie circadian sensitivity to subtype-specific agonists in phase resetting. Furthermore, within the SCN there is an interaction of light and protein expression so that circadian mRNA rhythms do not predict circadian protein rhythms.

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S104

Clock Gene Expression in the SCN of Arctic Ground Squirrels

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Abstract: While environmental light/dark (LD) cycles are the most salient cue for entraining circadian rhythms, whether or how circadian rhythms persist in species that naturally experience constant light (LL) or dark (DD) including arctic species is unclear. This uncertainty is compounded in hibernating mammals that during DD enter cycles of profound changes in body and brain temperature (Tb), including prolonged (5-24 day) bouts of torpor (Tb = 0 C) interrupted by short (12-18 hr) intervals of arousal back to euthermic levels of Tb = 36-38 C. We directly assessed on a molecular level the clock function of the suprachiasmatic nucleus (SCN) in the hibernating arctic ground squirrel (Urocitellus parryii) (AGS) by measuring the expression of the proteins PER1, PER2, BMAL1 and cFOS, in baseline, euthermic conditions under12:12 LD, in DD following the first bout of torpor and during arousal intervals. In euthermic animals under LD, daily rhythms in PER1 and PER2 expression were significant in the SCN, although the amplitude of expression levels was lower (less than 2 fold) compared to

in other non-arctic diurnal or nocturnal rodents seen in our previous work. Expression of the clock-controlled Avp mRNA and peptide were also diminished in the SCN of AGS. No daily variation was detected in BMAL1 expression in the SCN in pre-hibernation ground squirrels, consistent with other species. Over 24 h of the first torpor bout there were no clear 24h rhythms in the expression of PER1, PER2 or cFOS measured at 6 h intervals. There was great variability among individuals sampled within the same time point, with a variety of expression levels comparable to pre-hibernation controls. The abolished PER1/PER2 rhythms in torpor could due to the SCN clock free-running in DD resulting in a loss of synchrony between individuals, or alternatively, the SCN might have stopped ticking in hibernating animals. During the subsequent arousal episode, the expression of PER1 and PER2 showed no time or Tb-dependent changes, although greatly enhanced cFOS expression was observed at the beginning of arousal (Tb at 20C), consistent with previous findings in hibernating squirrels. The results from the present study provide insights into the clock function of the SCN in arctic mammals.

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S105

AVP Signaling Reprograms SCN Organization

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Abstract: The suprachiasmatic nucleus (SCN) contains distinct classes of clock neurons that communicate with one another to form a coherent network. Intercellular signaling is important for maintaining SCN function, but the underlying mechanisms remain ill defined. Arginine vasopressin (AVP) is expressed by approximately 20% of SCN neurons, which are local projection neurons that innervate hypothalamic targets. In addition to being a major SCN output signal, recent work suggests that AVP may modulate the function of the SCN itself. However, the effects of AVP signaling on SCN network properties have not been examined directly. To test the influence of AVP signaling on SCN function, we performed real-time PER2::LUC imaging on SCN slices cultured with antagonists of the AVP receptors, V1a and V1b. In the absence of AVP signaling, SCN neurons displayed longer periods, later peak times, and lower PER2::LUC expression. Interestingly, the magnitude of each effect differed by SCN region, suggesting that the influence of AVP signaling varies with neuronal phenotype. These results reveal that AVP is an important neuromodulator that reprograms the functional organization of the SCN network.

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S106

Circadian Regulation in and by SCN Astrocytes

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Abstract: Astrocytes are heterogeneous glial cells that play fundamental roles in brain functions, including ion and neurotransmitter homeostasis, synaptic modulation and cerebrovascular control. Astrocytes within the mammalian master circadian clock, the Suprachiasmatic Nucleus (SCN), are among cells that are activated by a phase-shifting light pulse and show day/night differences in surface area in vivo. It is not known if these rhythms are intrinsic to the astrocytes of the SCN or if they contribute to daily behaviors. To test whether SCN astrocytes express daily rhythms in clock gene expression, we infected cultured SCN with a novel, astrocyte-specific, Cre-activated Bmal1::luc reporter virus. We imaged the cultured SCN and found that astrocytes in SCN organotypic slices have functional, synchronized circadian rhythms in Bmal1 expression with a mean period of 23.6 ± 0.2h (n = 5 SCN). This approach allows us to investigate how rhythms in SCN astrocytes are modulated by SCN neurons. Next, we tested whether circadian rhythms in SCN astrocytes play a role in daily rhythms in the SCN and behavior. By stereotactic injection of single guide RNA-carrying (sgRNA) AAV (Adeno-Associated Virus) into SCN of an astrocyte-specific Cas9 mouse line (Aldh1L1::Cre/+; LSL-Cas9-eGFP), we specifically disrupted Bmal1 expression only in astrocytes within the SCN. Wheel-running in mice with astrocyte-specific Bmal1 deletion had a significantly longer circadian period than littermate controls (24.7 ± 0.2 vs 23.8 ± 0.1, n = at least 3 mice per treatment, $p < 0.01$). These preliminary results indicate that Bmal1 in SCN astrocytes is critical for circadian rhythms in behavior.

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S107

Spatial Segregation of PER1 and PER2 Expression in the Mouse SCN

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Abstract: Background: The SCN, locus of the master circadian clock, is comprised of ~20,000 neurons. At the cellular-molecular level, periodicity is orchestrated by feedback loops involving clock genes and their protein products, including those of Period1 and Period2. There is evidence supporting distinct functional roles of PER1 and PER2 proteins. For example, PER2 acts as a positive regulator of BMal1 transcription, while PER1 appears to act post-transcriptionally to regulate cellular clocks, possibly through protein-protein interactions. A question raised by such findings is how the SCN network organization contributes to these different functions. While the standard model assumes that PER1/2 oscillate together and occur jointly in each clock cell, it is possible that the two proteins have unique expression patterns within individual neurons.

Goal: To understand the basis and the nature of divergent functional roles for PER1/2, we explored spatial and temporal changes in these proteins in cells throughout the volume of the SCN at CT 0, 8, 12 and 18.

Method: Sagittal sections of the SCN were triple-labeled for PER1, PER2, and GRP. We designed automated programs to evaluate the intensity and location of immunofluorescent signal for each protein. Our programs successfully distinguished SCN from the background, assessed amplitude of PER1/2 expression within a slice, and identified the core SCN (using GRP as a marker), allowing for identification of distinct SCN regions.

Results: We found that SCN neurons expressing high amplitude PER1 and PER2 did not always overlap. Neurons highly expressing PER2 were concentrated in the rostral, dorsal and extreme caudal borders of the SCN, while those expressing PER1 were concentrated in a broad central area, though not within the GRP-containing cells. Confocal microscopy confirmed these findings. We also observed high PER1 and PER2 expression in neurons just beyond the area delineated by AVP-containing cells, previously characterized as outside the SCN proper.

Conclusion: The results indicate that peak expression of PER1 and PER2 in individual SCN cells is a function of their spatial location in the nucleus. Our data indicate the potential for anatomical contributions to differential functional roles for PER1 and PER2 in SCN networks.

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S108

Role of GRK2 in Circadian Behavior and Molecular Rhythms

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Abstract: Various G-protein coupled receptor (GPCR) signaling pathways are known to regulate the central molecular clock in the suprachiasmatic nucleus (SCN), a hypothalamic region that coordinates circadian rhythms in mammals. GPCR kinases (GRKs) are a Ser/Thr kinase family which canonical role involves down-regulating GPCR signaling by receptor phosphorylation and internalization. We examined the role of one of these kinases, GRK2, using two different murine models: one conventional heterozygous strain, and a GABAergic conditional knockout (cKO). We found that loss of one copy of grk2 lengthens the period of behavioral rhythms under constant darkness, enhances acute phase delays after an early night light pulse, and slows the rate of re-entrainment in a jetlag paradigm. Additionally, ablation of grk2 in vesicular GABA transporter (Vgat)-expressing cells enhanced the period-lengthening effects of dim constant light. Also, grk2 cKO animals showed attenuated phase advancing in response to a late night light pulse compared to control mice. To further examine the role of grk2 in the circadian clock, we used immunohistochemistry to characterize the rhythmic expression of PERIOD proteins as well as the light-induced phosphorylation of extracellular signal-regulated kinase (ERK) in the SCN. grk2-deficient animals showed higher peaks in PER1/2 expression at the single-cell level, as well as higher levels of ERK1/2 phosphorylation after a light pulse administered either at the early or late subjective night. When examining Period1 transactivation in grk2 mutant mice, we found that light-induced mPer1 expression was enhanced. Finally, we aimed to assess the effects of grk2 loss in the molecular clock using an ex-vivo setting, by monitoring SCN PER2::LUC bioluminescence. Compared to control SCN, intrinsic grk2 cKO rhythms showed a higher amplitude and surprisingly, a shorter period. Collectively, these findings point at an important role of grk2 in fine-tuning the pace of the circadian clock, as well as in down-regulating the activation of the ERK pathway and mPer1 expression in response to acute photic stimulation. The intriguing discrepancy

between the period of behavioral and ex-vivo rhythms opens questions about the determinants of the pace of locomotor rhythms, and possible feedback from extra-SCN oscillators.

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S109

Examination of the Suprachiasmatic Nucleus Expression in Forebrain Bmal1 Knockout Mice

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Abstract: The suprachiasmatic nucleus (SCN), located in the anterior hypothalamus of the brain, contains the central pacemaker that regulates daily behavior and physiological programs in mammals. Each neuron in the SCN is a cellular oscillator that produces a unique set of neuropeptides and neurotransmitters. The ensemble of these heterogeneous SCN neurons controls output behavior. However, until recently, it has remained unclear as to which neurons are responsible for driving coherent behavioral rhythms (but see Lee et al. 2015).

Previously, we generated forebrain knockout (BKO) mice by crossing floxed Bmal1 to a CamKIIa-iCre driver. The BKO mice displayed complete arrhythmic behavior in constant conditions. Based on in situ hybridization, we estimated that >90% of SCN cells were devoid of Bmal1 expression in these mice. Upon detailed analysis by immunohistochemistry, we unexpectedly found that a number of intact neurons still expressing BMAL1 were localized in the peripheral regions of the SCN. In other words, the majority of the neurons that were affected by the CamKIIa-iCre line were in the central portion of the SCN. While the behavior is arrhythmic, some SCN slices showed molecular rhythms in a circadian range either after 1 week in culture or after medium change probably due to these residual neurons, albeit damping significantly. We investigated this aspect, and will discuss a possibility that the functional oscillators in the central SCN, which encompass the majority of the ventral and dorsal regions, are important for robust and stable rhythms both in molecular expression and circadian behavior.

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S110

Dim Light at Night Disturbs the Daily Sleep-Wake Cycle and Sleep Architecture in Rats

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Abstract: Study Objectives: Exposure to artificial light during the dark phase of the circadian cycle is common in present society. Light provides the main input to the suprachiasmatic nucleus (SCN) which regulates sleep-wake behavior and energy metabolism. In humans, exposure to light at night has been correlated to insomnia and obesity. Here we aimed to develop a rat model to study the effects of dim light at night on sleep-wake behavior and energy metabolism.

Methods: Male Wistar rats were exposed to either a 12-h light (150-200lux):12-h dark (LD) schedule or a 12-h light (150 lux):12-h dim white light (5 lux) (LDim) schedule. Sleep-wake rhythms were assessed with EEG/EMG recordings, homecage locomotor activity, and in situ hybridizations of clock genes in the SCN. Energy metabolism was assessed with calorimetric measurements and intravenous glucose tolerance tests.

Results: LDim decreased the amplitude of the daily rhythms in waking, NREM sleep and REM sleep. Within NREM sleep, LDim induced a pronounced loss of rhythm in slow wave activity (0.75-4 Hz) and the circadian (16-19 Hz) frequency domain. LDim induced a free running rhythm in locomotor activity with a period of 25.1±0.0 h that interfered with the remaining 24 h rhythm. In the SCN, LDim reduced the amplitude in the daily rhythm of Per1 and Arntl (Bmal1) expression. LDim also decreased the amplitude of the daily rhythms in food intake and energy expenditure, but despite this it did not affect body weight, adiposity or glucose tolerance.

Conclusion: In the Wistar rat, LDim disturbs the daily rhythm in sleep-wake behavior by a direct sleep promoting effect of dim light, as well as by introducing an endogenous free running rhythm with a period of ~25-h that reduces SCN output amplitude.

Significance: We introduce the first rodent model showing decreased sleep-wake rhythms due to dim light at night. Dim light disturbances in sleep/wake rhythms were caused by the induction of a second rhythmic components in locomotor activity with a period of ~25 hours. Our data show for the first time that behavioral desynchronization can be induced despite adherence of the main L/D Zeitgeber to a 12h:12h cycle. This rat model may help to identify the causal mechanism underlying the associations between light at night and insomnia in humans. Future studies with transgenic clock-gene luciferase rats are needed to elucidate the anatomical representation of the endogenous free running rhythm.

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S111

Deficits in Temporal Processing in a Mouse Model of Autism

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Abstract: Temporal processing in the seconds-to-minutes range, known as interval timing, is a crucial cognitive function that requires activation of cortico-striatal circuits via dopaminergic-glutamatergic pathways. Interval timing is altered in disorders associated with pathological dopaminergic function, including schizophrenia, Parkinson's disease, and Huntington's disease. It has been reported that children and adults with autism spectrum disorders (ASD) are impaired in their ability to accurately perceive time. Indeed, circadian alterations – including sleep disorders and low melatonin levels – are frequently observed in ASD.

The objective of the present work was to study interval timing in a mouse model of autism, generated by prenatal exposure to valproic acid (VPA) at gestational day 12.5. Animals were evaluated for its ability to acquire timing responses in simultaneously trained 15-s and 45-s peak-interval (PI) procedures. In the PI procedure, subjects are first trained on a fixed-interval (FI) schedule of reinforcement and then transitioned to the PI protocol in which unreinforced probe trials are introduced. With repeated experience on probe trials, subjects learn to respond at a time closer to the expected time of reinforcement, producing a Gaussian-shaped response function.

Our results indicate that both male and female VPA-mice showed significant impairments in timing accuracy and precision compared to control (saline) groups. Moreover, these impairments were reversed after peer-rescue of autism-related behavior by early social stimulation in male mice. Furthermore, preliminary results indicate significant differences in striatal dopaminergic and serotonergic system in VPA male mice, consistent with previously identified alterations in dopamine and serotonin metabolism in humans with ASD. We are currently studying circadian rhythms in VPA mice.

These deficits in temporal processing in a mouse model of autism complement previous results in humans, and provide a useful tool for further behavioral and pharmacological studies.

Research Funding: ANPCyT, Argentina, Universidad Nacional de Quilmes

S112

Nitrergic Neural Communication for the Synchronization of the Mammalian Circadian Clock: A Putative Redox-Regulation

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Abstract: Background: The hypothalamic suprachiasmatic nuclei (SCN) orchestrate most mammalian circadian rhythms, which are mainly synchronized by light-generated phase changes. Both light-induced phase advance (PA) and delay (PD) pathways involve the activation of nitric oxide synthase (NOS). Nevertheless, it is still unclear how the same NOS enzyme can activate one or the other

pathway in SCN neurons. Recent reports indicate that the redox state can oscillate in a circadian fashion in the SCN, exhibiting a relatively more reduced state during the sleeping phase, and a more oxidized state when the animal is awake. We hypothesized that the NOS enzyme would produce two different messengers depending on the redox state of neurons: NO (at CT18), and S-nitrosylated gluthathione (GSNO, at CT14), respectively. NOS gets auto-S-nitrosylated in its tetra-thiolate domain, a glutathione (GSH) docking site, generating GSNO through a trans-S-nitrosylation mechanism. Since oxidized glutathione (GSSG) cannot be S-nitrosylated, NOS would not be able to carry on this trans-nitrosylation at CT18, thus producing mainly NO that would act both through GC activation and as a gaseous transmitter. Under a reduced state (CT14), GSH would be the putative substrate for NOS, and the resulting S-nitrosothiol GSNO could be working as messenger downstream NOS activity through RyR-II opening.

Results: We have used different types of NO donors in order to modulate circadian light-induced phase changes. Only when an S-nitrosothiol type of NO donor was used, both phase advance and delay pathways were potentiated. How different type of NO donors generate this enhancement is under study. In addition, a single intracerebroventricular administration of L-N-acetyl-cysteine, a well-known antioxidant agent, generated PD at CT14, but failed to generate PA at CT18. Conclusion: Our data suggests that changing the redox state of SCN neurons is enough to induce phase changes, and that the photically-induced second messengers produced by the NOS could be dependent on the redox state of the cells.

Research Funding: ANPCyT, Argentina, CONICET, Universidad Nacional de Quilmes

S113

Chronic Sleep Restriction Increases the Change in Systolic Blood Pressure Between Circadian Night and Day

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Abstract: Shiftwork and sleep restriction are risk factors for adverse cardiovascular health. How cardiovascular outcomes are affected during chronic sleep restriction (CSR) at different circadian phases is not well understood. Systolic and diastolic blood pressure (SBP and DBP, respectively) and heart rate (HR) were collected during CSR (1:3.3 sleep:wake ratio) and Control (1:2 sleep:wake ratio) conditions during all circadian phases in 16 healthy and normotensive participants free of medication use (9 female) aged 20-34y. After 3 weeks of maintaining a consistent 10h sleep schedule at habitual timing, participants began a 32-day inpatient study that included 24 cycles of a 20h forced desynchrony (FD) protocol. Participants were randomized to CSR (4.67h sleep, equivalent to 5.6h on a 24h cycle, n=8) or Control (6.67h sleep, equivalent to 8h on a 24h cycle, n=8) FD conditions. At each scheduled awakening, the participant's bed was elevated to ~45° and constant posture was maintained for ~1h while participants completed neurobehavioral testing. Upon completion of testing, BP and HR were measured. Circadian phase and period were calculated using Non-Orthogonal Spectral Analysis of core body temperature (CBT) during the FD portion of the protocol: fitted CBT minimum was defined as 0°. Repeated measures ANOVA found significant differences in SBP and HR, but not DBP in CSR vs Control; circadian phase; and their interaction ($p<0.05$). There were no changes across the 24 cycles of FD. Data were compared using both paired and independent t-tests for measurements occurring during the circadian day (120-240°) and night (300-60°). SBP and HR were higher during circadian day than during circadian night in both CSR and Control groups ($p<0.05$); there were no significant

circadian day-night differences in DBP. The magnitude of change in SBP from the circadian night to day was significantly higher in the CSR group as compared to controls (4.8 vs 1.9 mmHg; $p<0.05$), with no differences in change in DBP or HR ($p>0.05$). These findings suggest that chronic sleep deficiency can lead to an increased magnitude in the difference between waking day and night SBP. Further work is needed to examine the physiological mechanisms and the implications for cardiovascular health in night shift workers, who are known to be at higher risk for cardiovascular disease.

Research Funding: NIH R01HL114088, R01GM105018, P01AG009975, K24HL105664 (EBK),T32HL007901 (AWM), R01HL118601 (FAJLS), UL1TR001102, NSBRI HFP02802 and NKF Young Investigator Award (CMM)

8:30 pm - 10:30 pm

Poster Session II

M1

Correlation of between Circadian Rest Activity Rhythm and Nucleic Acid Turnover in Patients with Metastatic Colorectal Cancer

Sandrine Dulong[1], Alain Pruvost[2], Pasquale Innominato[3], René Adam[1], Mohamed Bouchahda[4], Mazen Haydar[4], Ayhan Ulusakarya[4], Christophe Junot[2], Francis Lévi[3]

[1]*INSERM*, [2]*CEA*, [3]*Cancer Chronotherapy Unit, Warwick Medical School,* [4]*APHP*

Abstract: Background: Colorectal cancer is the second cause of cancer-related deaths. The circadian rest-activity rhythm is an independent prognostic factor of survival in patients with metastatic colorectal cancer. High urinary excretion of modified nucleosides could reflect tumor burden, and be rhythmically regulated in some patients.

Purpose: To determine the relation between the robustness of the rest-activity rhythm, as assessed with dichotomy index I<O, and the urinary excretion of modified nucleosides, a measure of DNA and RNA breakdown. Their modification around the clock were evaluated and correlated to rest-activity rhythms.

Methods: Pseudouridine (PS), 1-methylguanosine (MG), N2-N2-dimethylguanosine (DMG), 1-methyladenosine (MA), 4-acetylcytidine (AC), 1-methylinosine (MI), adenosine(AD), cytidine (CY), were determined with LC-MS/MS spectrometry and normalized against creatinine in the urines of patients with metastatic colorectal cancer. Their circadian rest activity rhythms were recorded using Actigraph from Ambulatory Monitoring for 5 days, with urine samples being collected at 7:00, 11:00, 15:00, 19:00 and 23:00 on the last 2 days of actigraphy. The relative amount of activity In-bed that is less than the median activity Out-of-bed (I<O) was computed over the 5-day span, while circadian rhythms in urinary nucleosides excretion were analyzed with ANOVA and Cosinor.

Results: 30 patients (12 females and 18 males) aged 23-76 years participated and provided a total of 290 urines. Mean nucleoside excretion (AU) ranged from 74 for PS, to 9 (MG), 8 (DMG), 6.5 (MA), 3.7 (AC), 2.6 (MI), 0.75 (AD), and 0.7 (CY). No circadian variation was statistically validated with Cosinor in the group of 30 patients. However, circadian changes in at least one nucleoside excretion were demonstrated in 7 individual patients. 19 patients with an I<O exceeding the mean value of 96.5 were considered as having non-disrupted circadian rest-activity rhythm, while circadian disruption was obvious in the 11 other patients. We found that the better the rest-activity rhythm, as indicated with a high I<O value, the higher the 24-h urinary excretion of MA (p= 0.022), AD (p=0.025).

Conclusion: These data suggest some link between the circadian timing system and specific nucleosides breakdown.

Research Funding: Supported by the ANR (CANCERSENSOR ANR TECSAN 2011-2015)

M2

The MYC Oncogene Disrupts Circadian Rhythm and Metabolism in Cancer through Modulation of REV-ERB and BMAL1

Brian Altman[1], Annie Hsieh[1], Arjun Sengupta[1], Saikumari Krishnanaiah[1], Sharon Diskin[2], John Maris[2], Mitchell Lazar[1], John Hogenesch[3], Aalim Weljie[1], Chi Dang[1]

[1]*University of Pennsylvania Perelman School of Medicine*, [2]*Children's Hospital of Philadelphia*, [3]*University of Cincinnati*

Abstract: MYC is an oncogenic transcription factor that is amplified or upregulated in many human cancers and has been shown to be critical for growth and metabolic rewiring. Given that MYC binds the genome at E-box sites identical to those bound by the CLOCK-BMAL1 master transcriptional regulators of circadian rhythm, we hypothesized that oncogenic MYC could inappropriately regulate clock components and disrupt circadian rhythm to provide an advantage to MYC-driven cells.

To study the role of MYC in circadian rhythm, we utilized inducible oncogenic MYC or the related protein N-MYC (which is frequently amplified in aggressive neuroblastoma) in cell models of osteosarcoma, hepatocellular carcinoma, and neuroblastoma. In each of these cell lines we both studied static circadian gene expression and circadian gene oscillation in the presence or absence of MYC. We found that MYC or N-MYC led to a profound suppression of BMAL1 expression and subsequent disruption of circadian gene oscillation. Using siRNA, we determined that MYC disrupted BMAL1 expression and circadian oscillation through induction of REV-ERBα, as knockdown of REV-ERB could rescue this disruption of oscillation. Examination of clinical data revealed that elevated REV-ERBα and low BMAL1 both correlated with poor patient outcome in neuroblastoma, and expression of BMAL1 suppressed neuroblastoma cell clonogenicity, suggesting that BMAL1 is a potential tumor suppressor. Finally, we used NMR (nuclear magnetic resonance) to study oscillation of selected metabolites over time. We found that several metabolites including glucose oscillated, and intriguingly, ectopic MYC profoundly disrupted the oscillation of glucose metabolism and strongly altered glutaminolysis.

We conclude that oncogenic transformation driven by MYC leads to circadian and metabolic dysrhythmia, providing a potential competitive advantage to growing cancer cells.

Research Funding: Partially supported by the NCI of the NIH, The Leukemia and Lymphoma Society, & the Abramson Family Cancer Research Institute. J.M.M was supported by RC1MD004418.

M3

Active-phase Restricted Feeding Restores the Blood Pressure Circadian Rhythm in Type 2 Diabetic db/db Mice

Tianfei Hou[1], Wen Su[1], Ming Gong[1], Shreyas Joshi[1], Bruce Ohara[1], Zhenheng Guo[1]

[1]*University of Kentucky*

Abstract: Objectives Blood pressure (BP) exhibits a 24-hour rhythm. Loss of BP oscillation has been found in up to 75% of diabetic patients and is associated with increased risks of target organ injuries. We and others have shown that type 2 diabetic db/db mice have hypertension and disrupted BP circadian rhythm. The objective of this study is to investigate whether the active-phase restricted feeding (TRF) is able to recover the BP circadian rhythm in db/db mice.

Methods and Results Group I: 4-week-old male db/db and control mice (db/+) were subjected to either TRF (chow diet was only available at nighttime from ZT13 to ZT21) or ad libitum feeding. BP was recorded for 3 consecutive days using radio telemetry after 10 weeks TRF. The results showed that TRF prevented the disruption in BP circadian rhythm. We then tested whether TRF can recover the already disrupted BP circadian rhythm in Group II mice: 14-week-old male db/db mice, which already showed disruption in BP oscillations, were treated with TRF for 9 days. Baseline and after treatment BP was recorded for 3 days. The results showed that the disrupted BP circadian rhythm is corrected. In order to study the mechanism underling the improvement in BP circadian rhythm with TRF, we tested metabolic parameters, including respiratory exchange ratio (RER), energy expenditure, and locomotor activity were for 3 consecutive days in both group I and II mice. The results showed that TRF significantly improved oscillations in metabolic parameters in both groups of mice. In addition, sleep-week states, which affect both BP and metabolism, were measured in group II mice using PiezoSleep system at baseline and at the first and third week of TRF. The results showed that by the third week of TRF, the sleep time during light and dark phase were recovered to control mice level in db/db mice. In addition, light phase sleep bout length was significantly increased and breath rate during sleep was decreased in db/db mice with TRF.

Conclusions Our studies illustrate that TRF is able to prevent and recover db/db mice BP circadian rhythm, suggesting that eating only at right time may serve as a novel therapeutic strategy to prevent the disruption and recover the normal BP circadian rhythm in patients with diabetes.

Research Funding: This work is supported by National Institutes of Health grants RO1 HL106843.

M4

Clock Genes Regulate Circadian Gating of Parturition and Gestation Length

Carmel Martin-Fairey[1], Ronald McCarthy[1], Xiaofang Ma[1], Sarah England[1], Erik Herzog[1]

[1]*Washington University*

Abstract: Circadian rhythms are endogenous rhythms with periods close to 24 hours. Many physiological and behavioral processes show circadian rhythms that are synchronized to environmental stimuli. Alteration of environmental stimuli or mutations in circadian genes can perturb, or chronodisrupt, circadian rhythms. Although chronodisruption has been linked to poor reproductive and pregnancy outcomes such as preterm birth, the role of circadian oscillators in the time of day of delivery and gestation length is not fully understood. Here, we tested the hypothesis that mutations in circadian clock genes increase the risk of preterm birth.

We compared pregnancy outcomes of pregnant mice of three different circadian genotypes: C57BL/6NJ (wild type [WT]) mice have a 23.7hr period, hPer2SG mice have a 20.7hr period, and Per1-/-Per2-/- mice that have no circadian rhythm. All mice were maintained on a 12h:12h light:dark cycle. Mice were mated for two hours and then singly housed after a post copulatory plug was observed. We used infrared cameras to measure the time (+/- 1 min) from the appearance of a copulatory plug until the appearance of the first pup. We compared gestation duration and the time of day of delivery among dams for their first two parities.

We found that first parity hPer2SG dams had a shorter gestation length than WT and Per1-/-Per2-/- dams (18.02 +/- 0.62 days, n=7, vs. 19.48 +/- 0.89 days, n=14; P=0.0001 and 19.65 +/- 0.74 days n=19; P=0.0001). Gestation length of Per1-/-Per2-/- dams was similar to that of WT (19.65 +/- 0.74 days; P=0.6). The time of day of delivery during the first parity was significantly clustered near dawn for all genotypes. In the second parity, gestation length of both hPer2SG and Per1-/-Per2-/- dams was shorter than that of WT (17.94 +/- 0.31days, n=7 ; P=0.001 and 18.94 +/- 0.61 days, n=10; P=0.0424, vs.

20.3 +/- 0.2390 days, n=11). Notably, a third of the Per1-/-Per2-/- dams gave birth 72–96 hrs earlier than WT. Per1-/-Per2-/- dams also varied more in the time of day of delivery in their second parity than WT (r=0.99, P=0.0001).

These data reveal that mutations that either shorten circadian period or abolish circadian rhythms shorten gestation length and alter the time of day of delivery. We conclude that the circadian clock plays a role in both the length of gestation and the gating of the time of day of delivery.

Research Funding: Supported by the March of Dimes.

M5

Magnetic Field Effects in Drosophila Melanogaster

Giorgio Fedele[1], Celia Hansen[1], Ezio Rosato[1], Charalambos Kyriacou[1]

[1]*University of Leicester*

Abstract: Many higher animals have evolved the ability to use the Earth's magnetic field, particularly for orientation and navigation. However, the biophysical mechanism by which magnetoreception is achieved, remains elusive. One theoretical model (the radical pair mechanism - RPM) proposes that the geomagnetic field is perceived by chemical reactions involving the circadian blue-light photoreceptor Cryptochrome (CRY).

Previously, we have confirmed that exposure to electromagnetic fields (EMF) affects the circadian behaviour of the fruit fly (i.e. it shortens the period under constant dim blue light in wild type flies), supporting a mechanistic link between the circadian clock and magnetoreception. Here we have attempted a preliminary genetic dissection aiming to identify responsible neuronal clusters. Interestingly, we have found that in addition to some CRY positive canonical clock neurons (LNDs), the eyes seem to be necessary for mediating the EMF-mediated response, thus providing a putative input pathway. These findings have also been confirmed using a non-circadian paradigm and suggest alternative models by which EMFs can mediate behaviour.

Research Funding: EMF Biological Research Trust 2 years PostDoc grant

M6

Integration of Light Intensity Information into the Clock Neuron Network of Drosophila Melanogaster

Matthias Schlichting[1], Fang Guo[1], Pamela Menegazzi[2], Charlotte Helfrich-Förster[3], Michael Rosbash[1]

[1]*Brandeis University*, [2]*Neurobiology and Genetics, Biocenter, University of Wuerzburg*, [3]*University Wuerzburg*

Abstract: A general feature of the circadian clock is its ability to entrain to day and night. To do so, it can use several external cues (Zeitgebers), with light being the most important for synchronizing fly behavior. In light-dark regimes, flies exhibit a bimodal activity pattern with a morning (M) peak and an evening (E) peak of activity, which are clearly separated by a siesta, during which flies tend to sleep. Here we show that high light intensities (HI) significantly decrease activity and increase sleep during the siesta, also resulting in a delayed onset of E activity. Immunostaining against PER

and TIM reveal no differences in TIM cycling. However, PER appears more stable during the first half of the day at HI in both, s- and l-LNvs, suggesting that light information is integrated into the clock network via the lateral neurons. To learn more about this phenomenon we tested the behavior of photoreceptor mutants and show that the HB-eyelet, a photoreceptor comprised of four receptor cells per hemisphere, is essential for prolonging the siesta at HI. GRASP experiments reveal that this photoreceptor is connected to the lateral neurons. In addition, a recently developed Ca2+ sensor shows that the neuronal firing rate of the s-LNvs increases at HI, highlighting an excitatory input from the visual system. HI and the resulting change in neuronal activity also influences the s-LNv projections in the dorsal brain and causes them to adopt an open conformation. This suggests that the s-LNv modulate the downstream DN1s differently at HI, which we confirmed by illustrating that DN1 Ca2+ levels are altered in HI. Taken together we suggest that light information from the HB-eyelet activates and delays the lateral neurons, which modulate in turn the DN1s. They contain sleep-promoting neurons, which enhance the siesta and delay the E activity peak at HI.

Research Funding: This project was funded by the Howard Hughes Medical Institute (HHMI), the German Research Foundation (DFG, SFB1047) and the Hanns-Seidel-Foundation (Funded by the BMBF).

M7

An RNAi Screen for RNA Binding Proteins Controlling Drosophila Circadian Behavior

Lauren Foley[1], Jinli Ling[1], Patrick Emery[1]

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Abstract: The Drosophila circadian pacemaker consists of transcriptional-translational feedback loops subjected to both post-transcriptional and post-translational regulation. While post-translational regulatory mechanisms have been studied in detail, much less is known about circadian post-transcriptional control. RNA binding proteins mediate post-transcriptional regulation at every level, including splicing, nuclear export, stability, localization and translation. Thus, by knocking down these proteins, we can uncover potential regulatory events that might occur during any of the different stages in the life of circadian mRNAs. We have completed an RNAi screen of approximately 350 RNA binding and RNA-associated proteins. We have identified candidate hits with period lengthening or shortening exceeding two standard deviations from the mean. These hits represent about 50 genes, and include regulators of pre-mRNA splicing, mRNA polyadenylation and localization. We will describe preliminary data on promising candidate clock-regulating genes from this collection.

Research Funding: NIH

M8

Genome-Wide Characterization of the Molecular Response of the Circadian Clockwork to Temperature in Drosophila

Naveh Evantal[1], Osnat Bartok[1], Reut Ashwal-Fluss[1], Ron Weiss[1], Avigayel Rabin[1], Eran Meshorer[1], Sebastian Kadener[1]

[1]*Hebrew University of Jerusalem*

Abstract: Temperature is an environmental variable that affects the rate of chemical and enzymatic reactions. Hence, it has great impact on animal physiology. As a result, animals have developed sophisticated systems to mediate/compensate for external temperature changes. The importance of these mechanisms is even more prominent in ectotherm organisms such as Drosophila melanogaster, whose body temperature, even on an intracellular level, is subjected to drastic environmental changes. Until now, little has been known about how drosophila deals with this perturbation, and how molecular events such as transcription, RNA turnover, protein synthesis and protein turnover are affected by temperature changes. Compensatory mechanisms must exist for biological systems, such as circadian rhythms, which rely heavily on transcription and that are known to run at the same speed at different temperatures. In this work, we systematically determined the effect of temperature on transcription and RNA metabolism in Drosophila. For that purpose, we generated tools for genome-wide assessment of transcription rate, co-transcriptional and post-transcriptional processing and mRNA stability using high throughput RNA-seq of nascent RNA, total RNA, Ago-1- bound RNA and miRNAs, and 5'- and 3'-end mapping. We coupled this methodology with new library construction and computational pipelines, which we recently developed. Using these tools, we determined how gene expression is globally affected by temperature in drosophila.

We found many specific RNAs that change their transcription, stability and splicing patterns due to temperature changes. Of particular interest was the regulation of circadian clock genes. Our results show that the central circadian component Timeless (Tim) is strongly regulated by temperature at the post-transcriptional level. Although Tim mRNA expression is not affected, TIM protein levels are lower at 18C. This may be due to the generation of a short isoform of Tim and to a 3' UTR variant generated by alternative splicing that is strongly downregulated by miRNAs. In addition, we found that an additional isoform of Tim, likely with different regulation at the localization levels is highly expressed at 29C. In summary, in this work we comprehensively assessed how temperature shapes the drosophila gene expression map.

Research Funding: European Research Council (ERC)

M9

Light Induced Bursts in Drosophila Locomotion

Stanislav Lazopulo[1], Jeffrey McMahan[1], Sheyum Syed[1]

[1]*University of Miami*

Abstract: Although sudden bursts are thought to be common in animal behavior, their random occurrence and short temporal life often make bursts difficult to study. We have recently shown that the temporal patterns in fly locomotion, generally characterized by the so-called morning (M) and evening (E) peaks in activity, are punctuated by even stronger bouts of activity that are reminiscent

of bursts. The bursts are more than ten standard deviations larger than basal activity and appear in two bands, on average around 2 hours after the M peak and 2 hours before the E peak. In particular, bursts in the morning hours are highly dependent on light. Adult Drosophila senses light through the eyes, the ocelli, Hofbauer-Buchner eyelets and structural changes in the protein Cryptochrome. These photoreceptors each have distinct activation spectra. To identify the light input pathways that trigger these bursts, we have measured fly locomotion patterns under different wavelengths of light. To complement these studies, we have also measured locomotion of mutant fly strains with impaired light inputs. Together, these data help us better understand an overlooked feature in Drosophila behavior.

Research Funding: This work was supported by funds from the University of Miami.

M10

Circadian Translational Profiling of the Drosophila Head Fat Body Reveals Potential Novel Roles for a Peripheral Oscillator

Amy Yu[1], Yanmei Huang[1], F. Rob Jackson[1]

[1] *Tufts Medical School*

Abstract: Circadian oscillators are expressed in a wide variety of cell types. Presumably, these oscillators orchestrate cell type specific programs of gene expression. However, genome-level study of cell type specific oscillator function has been hampered by the difficulty of isolating RNA from a single clock cell type. Dissection studies have provided tantalizing hints that oscillators do indeed have cell type specific functions. However, approaches based on physical separation of the cell type of interest are inherently limited. Our lab has therefore adapted translating ribosome affinity purification (TRAP) to selectively isolate ribosome-associated RNAs from selected clock cell types of interest. In TRAP, a GFP-tagged ribosome is selectively expressed in the target cell type, allowing immunoprecipitation of associated mRNA. Previously, our lab employed a tim-GAL4 driver with TRAP to express the GFP-tagged ribosome in all clock cells of the Drosophila head. This permitted detection of novel cycling mRNAs that are also expressed at high levels in non-clock cells, and had thus been missed in studies using bulk tissue. We are now using fat body specific GAL4 drivers to profile circadian rhythms in mRNA-ribosome association in the Drosophila head fat body, with the goal of identifying fat body specific cycling genes and elucidating their circadian functions. The Drosophila head fat body is a complex organ which shares functions with mammalian adipose tissue and liver; it secretes a wide variety of signaling molecules. Using TRAP and bioinformatic analysis, we have identified approximately 200 cycling genes that are expressed primarily in the fat body. Many of these genes cluster in functional groups related to immunity, wound healing, energy metabolism, and sex-specific physiology. The mRNAs expressed from certain of these genes have not been previously shown to cycle in abundance. Studies are currently underway to determine whether the cycling of these mRNAs depends primarily on the fat body clock, or if output from other clock cell types is also required.

Research Funding: K12GM074869 from the National Institute of Health's IRACDA program to Claire Moore and NS065900 from the National Institute of Health to F. Rob Jackson

M11

Diapause in Drosophila melanogaster

Ane Martin Anduaga[1], Lewis Collins[1], Rodolfo Costa[2], Charalambos Kyriacou[1]

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Abstract: Diapause (or overwintering) in Drosophila, and in several other insects, has circadian input. Most diapause experiments on D. melanogaster are performed by growing the flies at 25 °C in LD 12:12, placing the newly hatched females at 12 °C and scoring ovary development after 12-28 days. However, this is an artificial protocol so for my experiments I developed a new and more natural protocol, which consists on raising the flies at 15 °C or 18 °C from late embryonic-early larval stages under short (LD 8:16) or long (LD 16:8) photoperiods before placing them at 12 °C Diapause, resistance to acute stress (exposure to -20 °C) and levels of several metabolites (total levels of glucose, glycogen and trehalose) were assessed in Northern and Southern natural fly populations from Europe.

The results show that the temperature in which D. melanogaster larvae are reared significantly affects the three phenotypes, diapause, stress responses and metabolites.These results are discussed in the context of geographical variation. In addition, we discover that the temperature sensitive splicing of the period 3′ UTR, which generates seasonal circadian locomotor phenotypes, also plays a dramatic role in diapause induction.

Research Funding: INsecTIME ITN (European community).

M12

Cold-Induced Period Transcription Links Environmental Temperature to the Drosophila Molecular Clock

Akanksha Bafna[1], Tadahiro Goda[2], Brandi Sharp[2], Herman Wijnen[2]

[1]*University of Southampton*, [2]*Centre for Biological Sciences and Institute of Life Sciences, University of Southampton*

Abstract: Many organisms synchronize their daily timekeeping systems as well as associated physiological and behavioural rhythms to environmental temperature cycles. However, the molecular mechanisms underlying circadian temperature entrainment remain relatively poorly understood. In a study of temperature-mediated resetting of molecular circadian oscillations in the fruit fly, Drosophila melanogaster, we noticed that the core-clock gene period (per) exhibited an early and prominent temperature-driven response. Here, we have used the instrumental model organism, Drosophila melanogaster, to study the mechanisms underlying temperature entrainment at the level of the molecular circadian oscillator. In particular, we aim to pin-point the cis-regulatory elements responsible for temperature-driven transcription of the Drosophila per gene and elucidate the transcription factors mediating this response.

First, qRT-PCR analyses of transgenic arrhythmic flies carrying a series of per-luciferase reporter constructs revealed that per is subject to cold-induced transcription via cis- regulatory elements residing between the transcriptional and translational start site. Next, we investigated cold-mediated induction of per across various Drosophila species and found that this response is well conserved at both mRNA and pre-mRNA level. An in silico approach was adopted to predict candidate transcription factors that might bind the per gene between its transcription and translation start sites. Transgenic

knock-down and/or over-expression of the 23 identified putative transcription factors was then tested for temperature-driven per regulation in clock-bearing cells with the help of in-vivo luciferase assay.

We demonstrated that per transcription is stimulated in response to a drop in environmental temperature. This phenomenon is well-conserved across Drosophila species and is separable from temperature-dependent post-transcriptional regulation of per. The cis-elements responsible for cold-induced per transcription map between the transcription and translation start sites. Ongoing efforts to identify the responsible transcriptional regulators will be discussed. So far, we have been able to exclude a requirement for HSF as well as the circadian regulators CLK/CYC and CWO.

Research Funding: BBSRC Responsive Mode Grant L023067

M13

The Dopamine Transporter is Not Required for Entraining Circadian Rhythms to Scheduled Feeding

Jennifer Enriquez[1], Andrew Steele[1]

[1]*California State Polytechnic University, Pomona*

Abstract: The dopamine transporter (DAT) is a transmembrane protein responsible for recycling dopamine back into dopamine neurons. This transporter is vital for the termination of dopamine signaling and many common drugs of abuse, such as cocaine, inhibit the activity of DAT. Changes in DAT expression level and regulation are also implicated in a number of human diseases, including attention deficit disorder, schizophrenia, depression, anxiety disorders, and in susceptibility substance abuse. Recent studies have described a DAT-dependent, dopamine ultradian oscillator in mice. Our laboratory recently implicated dopamine signaling to D1R neurons as crucial to entraining circadian rhythms to scheduled feeding. We sought to test whether DAT knockout mice, which lack dopamine ultradian oscillations, would show altered circadian entrainment to feeding. When mice are placed on a calorie restricted (CR) diet (60% of their daily calorie intake) they shift their activity and many physiological rhythms to predict scheduled food availability. Much to our surprise, we observed that DAT knockout mice had normal, or even enhanced, food anticipatory activity, showing that they entrained their behavioral rhythms to scheduled feeding.

Research Funding: California State Polytechnic University, Pomona, Huntley Fellowship, NIH MRBS RISE

M14

Mapping dopaminergic-D1R Circuitry that Mediate Circadian Entrainment to Feeding

Andrew Steele[1]

[1]*California State Polytechnic University, Pomona*

Abstract: Daily rhythms of food anticipatory activity (FAA) are regulated independently of the suprachiasmatic nucleus, which mediates entrainment of rhythms to light, but the neural circuits

that establish FAA remain elusive. We have described that dopamine D1 receptor (D1R) knockout mice show greatly reduced FAA, whereas mice lacking the dopamine D2 receptor have normal FAA. To determine where dopamine exerts its effect, we limited expression of dopamine signaling to the dorsal striatum of dopamine-deficient mice; these mice developed FAA. In addition, pharmacological activation of D1R at the same time daily was sufficient to establish anticipatory activity in wild-type mice. Recently, we have created conditional deletions of D1R to further refine the population of D1R neurons responsible for mediating food entrainment. We have also determined that Pitx3- and dopamine transporter (DAT)-expressing dopamine neurons are unlikely to be the mediators of FAA. Thus, our working model is that Pitx3-negative, DAT-negative dopamine neurons mediate circadian entrainment to scheduled feeding.

Research Funding: CSUPERB new investigator award

M15

Time-Restricted Feeding of a High-Fat Diet Attenuates its Deleterious Effects on Middle-Aged Mice

Marilyn Duncan[1], Julio Narbaiza[1], Farhana Mueez[1], Liza Bustle[1], Sadia Qureshi[1], Joshua Smith[1], Sandra Legan[1]

[1]*University of Kentucky Medical School*

Abstract: High-fat diet (HFD) induces obesity and glucose intolerance, risk factors for heart disease. In young adult mice time-restricted feeding (TRF) of HFD lessens these negative effects. Because obesity is prevalent in the middle-aged population, we hypothesized that TRF would ameliorate HFD effects in male, middle-aged (12 mos) C57BL6 mice. Groups of mice (n=15 ea) of similar weights were fed: 1) HFD (60% fat) ad-libitum (HFD-AL), 2) low-fat diet (LFD, 10% fat) ad-libitum (LFD-AL), or 3) HFD-TRF, i.e., HFD access from ZT13-21 (ZT12 = lights off). All diets were fed for 21 or 25 weeks during exposure to a 12L:12D photoperiod. Body weight gain differed among the groups ($p<0.0001$): HFD-TRF gained less weight than HFD-AL (~20% vs 55%, respectively). Caloric intake only differed between HFD-TRF and HFD-AL mice at weeks 3 & 5 and thus intriguingly did not account for their body weight differences. Average daily cage activity assessed with motion detectors varied among the groups ($P=0.042$) and was lower in the HFD-AL than the LFD-AL group ($P<0.05$) while the HFD-TRF mice did not differ from either group. After 16 weeks, a glucose tolerance test was conducted after an overnight fast (ZT 21-ZT13) by injecting glucose (2 g/kg, i.p.) at ZT 13 and collecting tail vein blood samples at 0, 15, 30, 60, and 120 min after injection. Glucose tolerance, assessed as incremental area under the curve (iAUC), was not different between HFD-TRF and LFD-AL, but was lower in HFD-AL than in HFD-TRF ($p<0.02$). After euthanasia, livers but not fat pads (retroperitoneal and epididymal) from HFD-TRF mice weighed more than those from HFD-AL mice ($p<0.0001$), but did not differ from those of LFD-AL mice. In conclusion, similar to its effects in young mice, TRF of a HFD improved body weight, liver weight, and glucose tolerance in middle-aged mice. Because TRF does not require altering caloric intake, it may constitute a valuable alternate strategy for ameliorating the health risks of human obesity.

Research Funding: Funds from the University of Kentucky College of Medicine, the Department of Anatomy and Neurobiology, and the Department of Physiology.

M16

Role of Gonadal Hormones in Food Anticipatory Activity in Response to Timed Restricted Feeding

Jessica Krizo[1], Eric Mintz[1]

[1]*Kent State University*

Abstract: Temporal restriction of food access has a major effect on daily patterns of locomotor activity. If given access to food only for a limited period during the light phase of a light-dark cycle, mice demonstrate food anticipatory activity (FAA) – an increase in activity for approximately three hours prior to food availability. Female mice exhibit significantly less FAA than males, and we sought to investigate the mechanism for this difference. Females showed no daily changes in FAA that might correspond to the estrous cycle, suggesting that variation in estrogen levels are not responsible for the difference between males and females. Therefore, we hypothesized that testosterone might be responsible for sex differences in FAA. Male and female mice were randomly selected for gonadectomy (GDX) and sham procedures. Following surgical procedures mice were individually housed in 12:12 LD and placed on 4-hr daily restricted feeding. In males, orchiectomy significantly decreased overall wheel-running activity compared to both intact and sham groups but did not affect levels of FAA. In females, ovariectomy (OVX) decreased overall wheel-running activity compared to both intact and sham groups but FAA was increased in both sham and OVX groups. We then added groups in which testosterone or estradiol were replaced in GDX mice. Following surgical procedures mice were individually housed in 12:12 LD and placed on 4-hr daily restricted feeding. Testosterone replacement in males significantly increased activity at baseline but did not alter the activity levels during restricted feeding or influence levels of FAA. Estrogen replacement did not significantly alter activity levels at any point. Taken together, these data suggest that the sex difference in female locomotor response to restricted feeding is not a function of circulating gonadal hormones. However, our data do not rule out a developmental role for steroid hormones in establishing this sex difference.

Research Funding: Research is funded by the Department of Biological Sciences, Kent State University.

M17

Decreased Food Anticipatory Activity of Obese (Neotomodon alstoni) Mice Relates to Changes in Hypothalamic Fos Expression

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Abstract: Nearly 50% of Neotomodon alstoni mice become obese when raised on a standard commercial laboratory diet, whereas the rest remain lean. Blood profiles of obese mice indicate high tryacilglycerides, insulin and leptin. The mechanisms underlying this interindividual susceptibility to develop obesity remain unknown. Previously we reported that during a daily Food Restriction Protocol (FRP), obese N. alstoni mice present a reduction in amplitude and duration of the so called Food Anticipatory Activity (FAA). Because hypothalamus plays a major role in the regulation of body weight and the expression of FAA, the present study aimed to find differences in hypothalamic

regions involved in circadian and food intake regulation between lean and obese mice during a FRP, using immunoreactive detection of Fos as a marker of neuronal activation. Our results showed that obese mice have an overall reduction of Fos positive neurons in the Suprachiasmatic Nucleus (SCN), regardless of the feeding conditions. During FRP, an increase in Fos positive neurons was noted in the Arcuate Nucleus, 5 fold and 4 fold for the lean and obese mice respectively. The number of fos positive cells in the Dorsomedial Hypothalamic Nucleus (DMH) of obese mice was increased during ad libitum feeding and reduced during the FRP, compared to the corresponding group of lean mice. These results are consistent with the locomotor activity recordings where obese displayed a reduction of FAA. Furthermore, the Fos increase observed in cortico-limbic areas in the lean mice during the FRP are not shown in obese mice. The daily food intake of obese mice was severely reduced during the FRP, compared to the ad libitum condition, whereas lean mice intake shows no differences by the seventh day of FRP. The current data suggests that decrease of neuronal activity in the SCN and the increase in key autonomic regulatory centers may contribute to the excessive fat storage and the inability of obese N. alstoni mice to anticipate daily meals.

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M18

Time Perception Relates to Cognitive Performance, Anxiety and Subjective Reports of Well-Being

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Abstract: Time perception reflects some known basic and current human psychics qualities. The purpose was to reveal the correlations of time perception parameters to cognitive performance, anxiety and perceived well-being.

Methods: 62 volunteers were tested by the computer based tests: time perception (2-5 s intervals), short-term memory for figures, attention concentration (Landolt's rings) and switching (red and black figures), efficiency of the information flow processing under time pressure, anxiety (by Spielberger-Khanin), self-reports on alertness, workability and fatigue. Pearson correlation was used at $p<0,05$.

Results: Mean overall group individual time was 92,75±1,21 % from the presented one and mean deviation was 17,62±0,75 %. In this, the longer was the presented interval the smaller was the individual playback time (for 2s: 97,16±2,03 %; for 5s: 87,44±1,37 %) while the deviation manifested no significant differences for different time intervals.

An increase in the deviation of the overall time perception was accompanied with the deterioration in some parameters of the attention concentration and the efficiency of the information flow processing. An increase the deviation of the 2-s intervals perception was accompanied with the similar changes of the same cognitive tasks (due to lesser number of parameters), of 3-s intervals – with the decrease in short-term memory volume, of 4-s intervals – with the deterioration in the attention concentration, of 5-s intervals – with the deterioration in the attention concentration, social workability and alertness.

An increase in the mean playback time of 2-s intervals was accompanied with the min time elongation of the correct information flow processing, of 5-s intervals – with the increase in anxiety, attention switching quality and attention concentration productivity.

No correlation was found to the perceived physical or mental well-being indices.

Conclusion: Deviation in the playback time is more sensitive to cognitive performance and other psychological parameters compared to its mean scores. Parameters of playback time of 4-5-s intervals are sensitive also to the perceived current psychics state parameters (anxiety, alertness, social workability). Time perception is found to be related mainly to cognitive and social oriented human properties.

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M19

Objectively Measured Late-Morning Physical Activity Predicts Mortality in the NHANES 2003-2006 Cohorts

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Abstract: Background: Objectively measured physical activity using accelerometers has emerged as a novel health risk factor that is independent of other known risk factors. Many studies have shown that self-reported activity and objectively measured sedentary time predict mortality; however, no studies have investigated the relative importance of the time-of-day intensity of activity.

Objective: The objective of our study was to examine the association between objectively measured activity expressed as counts and the relative importance of the time-of-day activity intensity on all-cause mortality in the nationally representative sample of NHANES.

Methods: Accelerometry data for N=6579 adults aged 18 to 84 from the U.S. nationally representative National Health and Nutrition Examination Survey (NHANES) 2003–2004 (N1 =3233) and 2005-2006 (N2=3346) were analyzed. Objective physical activity was measured as average activity counts in 8 two-hour daytime intervals between 8am-12am.

Results: Over an average of 6.8 years (7.8 years in the 2003-2004 cohort and 5.9 years in the 2005-2006 cohort), there were 518 deaths (324 in the 2003-2004 cohort and 194 in the 2005-2006 cohort). After adjusting for potential confounders, including age, sex, race/ethnicity, education, body mass index, and comorbidities, we found significant association between objectively measured physical activity in the 10am-12pm period (hazard ratio (HR) for one unit change: 0.71; 95%CI: 0.66-0.76). This corresponds to hazard ratio of 0.65 (95%CI: 0.60-0.71) when comparing the first versus the third quartile of activity intensity. Once adjusted for activity intensity during this time interval, the activity intensity in the other time intervals was not found to be statistically significant.Conclusions: Our study suggests that objectively measured activity intensity using accelerometers between 10am and 12pm is an independent risk factor for mortality. Our results may also be indicative of age-related disturbances in circadian rhythms as independent predictors of mortality. Consequently, our results appear to show that it might be possible to extend live duration either by normalizing circadian rhythm through the use of chronobiotics such as melatonin or using light treatment interventions.

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M20

MEQ Predicts Optimal Performance Time in Addition to Morning-Evening Preference

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Abstract: A major criticism that has been made about the Horne-Ostberg Morningness-Eveningness Questionnaire (MEQ) assessment of chronotype is that the instrument returns only a relative preference score for morning versus evening times for activities. We have developed an alternative approach for assessing chronotype (UTIME) which asks each subject how they would perform at different times of day in a set of 20 common situations. The 20 individual preference scores produced a large range of correlations with MEQ score; however, the highest were found in scenarios that placed high demands on executive function and cognitive performance. Importantly, the UTIME instrument not only acquires a temporal profile (from which preference is derived), but also peak times that each subject reports they would perform best. We have found that for the 5 UTIME items showing the highest correlations with MEQ, the relationships between peak (optimal) time and preference score are highly significant ($r > .75$; $p < .00001$). Therefore (1) MEQ score is determined predominantly from the perceived cognitive demand of a situation, and (2) MEQ scores reflect the optimal time of day for performing activities with high cognitive performance requirements. Supported by a grant from the Natural Sciences and Engineering Research Council of Canada to MRR.

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M21

Trends in Self-Reported Hourly Lighting and Sleep in a Global Dataset of Travelers

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Abstract: In 2014, we released a mobile app for iOS and Android, ENTRAIN, which relays optimal schedules for overcoming jet lag to travelers. After installing, users are given the option of anonymously submitting their data back to our servers. This data includes their age, sex, home timezone, "typical" lighting, and normal sleep habits. It also includes their self-reported hourly lighting and sleep during travel, as well as their responses to a survey about their experiences of jet lag (the Columbia Jet Lag Scale).

Here we revisit trends in global sleep occurring in our dataset of normal sleep habits. These include observations that women schedule more sleep than men, that individuals reporting typical light as "outdoor" sleep earlier and longer than those reporting "indoor" light, and that the variability of a population's sleep habits decreases as the population ages.

Further, we discuss new data from the hourly light and sleep histories of users in our dataset as they traveled, with a focus on differences with age and sex. We compare these sleep and lighting histories to scores on the Columbia Jet Lag Scale, and using these scores develop a simple model for predicting jet lag severity from recent lighting, sleep, and user demographic information.

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M22

Circadian Profiles of Light, Activity, and Body Temperature for Non-Invasive Physiology Prediction in Humans

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Abstract: Circadian rhythms play a central role shaping behavior and physiology. Modern infrastructure such as light exposure at night and work or school schedules cause a loss of synchrony across the body's clocks, resulting in increased risk of diseases. Advances in sensor design and data processing make research into the manifestation of internal desynchrony possible across large and diverse populations. Here we share design information of an open-source wrist-band device configured to gather activity, light exposure, and body temperature. Gathered at high temporal resolution, we show that these data could be useful for predicting internal hormonal state - covering infradian, circadian, and ultradian time scales. These data also appear to allow detection of internal circadian synchrony or desynchrony within individuals. This device is being miniaturized to a cubic millimeter as part of a collaboration with the distributed sensor Swarm Lab at UC Berkeley, and we include plans for dispersed networks of these devices in human and animal research.

Research Funding: Engineering work was supported by a grant from the American Society for Engineering Education.

M23

Molecular Basis for Chronotype and Time-Of-Day Effects on Decision-Making

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Abstract: Recent reports have highlighted time-of-day effects on human behavior, including on ethical decision-making. 'Morning-morality' and similar time-of-day effects can be moderated by an individual's chronotype—a self-reported preference for activity early in the day or late in the evening; morning-type 'larks' cheat more in the evening and evening-type 'owls' cheat more in the morning. Here we show that time of day effects in decision-making between larks and owls can be explained by phase differences in oscillating circadian clock genes. Our results provide evidence that endogenous variation in the molecular circadian clockwork may influence inter-individual differences in decision-making behavior.

Research Funding: Picker Interdisciplinary Science Institute, Colgate University

M24

A Prospective Study of Rotating Night Shift Work and Incident Depression in the Nurses' Health Study 2

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Abstract: Evidence linking shift work to mental health, and more specifically to depression and depressive symptoms, has been mixed, and largely based on cross-sectional studies, with limited information on potential confounding factors. We, therefore, plan to assess the association between rotating night shift work and incident depression (assessed as self-reported physician-diagnosis and anti-depressive medication intake) over 10 years of follow-up (2001-2011) in the Nurses' Health Study 2 cohort (N=33,992), a prospective cohort study with detailed information on a number of important confounders. We hypothesize that longer duration of rotating nightshift work is associated with an increased risk of incident depression. We will also examine whether this association is modified by chronotype - a proxy for individual phase of entrainment. It has been suggested that later chronotypes, while their baseline risk of developing depression might be higher than that of morning chronotypes, are less impacted by shift work, including rotating night shift work. We will use Cox proportional hazard models to estimate age- and multivariable-adjusted hazard ratios (HRs) and 95% CIs of developing depression across exposure categories of lifetime history of rotating night shift work (none (ref), <5, 5-10, 10-15, ≥15yrs). Covariates will include marital status, pack-years of smoking, physical activity, alcohol consumption, body mass index, social support, sleep duration and quality, household income (census level), health limitations, as well as self-reported history of diabetes, cancer, coronary heart disease and hypertension. Our results will help elucidate the association between occupationally-induced strain to the circadian system and depression.

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M25

Clock Regulation of Circadian Rhythms in the Human Neocortex

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Abstract: For the past 20 years, the molecular mechanisms underlying circadian rhythm and its relevance to human health have been the subject of intense research. The transcription factor CLOCK is a key driver of circadian cycling. Although genome-wide transcriptomic profiling studies have focused on cyclical gene expression in the liver and the suprachiasmatic nucleus, little is known about the function of CLOCK in other areas of the central nervous system. We have previously shown that CLOCK is more highly expressed in human neocortex compared to non-human primates, raising questions concerning the role of CLOCK in human brain evolution and its relevance to human mental health. We hypothesize that neocortical expression of CLOCK in the human brain is critical for regulating novel transcriptional networks. To test this hypothesis, we are identifying the transcriptional network regulated by CLOCK through ChIP-seq in human neocortex and RNA-seq following CLOCK knockdown in human neurons. Additionally, we will assess the role of neocortical

CLOCK expression by creating a humanized mouse, which will further serve as an improved model for circadian functioning and neocortical-associated disease.

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M26

TNF-alpha and Ccl2 Mediate the Immune-Circadian Interaction in Inflammation and Cancer Animal Models

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Abstract: The mammalian SCN clock controls most physiological circadian rhythms, including variations in endocrine and immune variables. Bidirectional interactions between immune and circadian systems have been under intensive study in recent years. In addition, desynchronization affects immune rhythmicity and can alter animal models of tumor progression.

In a murine model of melanoma, we observed that tumors grew faster in animals under circadian desynchronization schedules than in LD conditions. Moreover, proinflammatory cytokines were induced during the day in tumors of animals living in LD conditions, but not under desynchronizated schedules. In addition, animals expressing tumors showed a decrease in the percentage of nocturnal activity and in the strength of their locomotor activity rhythms in LD conditions. In these animals, the SCN levels of TNF-α (Tumor Necrosis Factor-alpha) and the chemokine Ccl2 (Monocyte Chemotactic Protein-1) increased at night as compared to controls.

Acute inflammation by inoculation of systemic low doses of the endotoxin LPS (lipopolysaccharide) resulted in phase-delays of locomotor activity rhythms. Mice lacking TNF-α receptor 1 (TNFR1) did not show LPS-induced phase-delays, and the same effect was observed by the intracerebroventricular administration of a Ccl2 synthesis inhibitor. Daily TNFR1 and Ccl2 receptor (CCR2) expression in the SCN of WT mice exhibited increased levels at night, when LPS has a circadian effect. Finally, LPS induced an increase in TNF-α, Ccl2 and Interleukin-6 levels in the SCN both at day and at night.

In conclusion, here we show in different murine models of inflammation that the immune-circadian interaction can be mediated by TNF-α and Ccl2. We have also confirmed that circadian desynchronization can affect the development of tumors and that the inflammatory molecules in the tumor are under circadian control.

Research Funding: ANPCyT, Argentina, CONICET, Universidad Nacional de Quilmes

M27

Circadian Rhythmicity in Bone Marrow-Derived Macrophages

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Abstract: Immune responses show clear circadian time-dependency, and macrophages lie at the forefront of the innate immune response. Macrophages can be isolated from a variety of sources, and not all have yet been exploited for circadian studies. Primary resident macrophages, for instance peritoneal macrophages, are strongly rhythmic. However, cell yields are often low for the labor involved, and this can be inconvenient for functional assays. As an alternative, we have explored isolation of stem cells from bone marrow and their subsequent differentiation using appropriate growth factors derived from fibroblast L929 conditioned media (LCM). We describe isolation and differentiation of macrophages from the bone marrow of Per2-Luc mice, validating their phenotype by flow cytometry, and testing their molecular rhythmicity through luminometry. Specifically, cells harvested from the femur and tibia of Per2-luc mice were cultured (DMEM containing 10% FBS, 20% LCM, penicillin-streptomycin, and supplemented with L-glutamine) for six days with one medium change. Analysis by flow cytometry using anti-F4/80 and anti-CD11b established that the cultures were predominantly fully differentiated macrophages. The clocks in these cells can subsequently be synchronized through serum shock with 50% horse serum. While large numbers of cells can be produced, unknown variables appear to contribute to lack of complete phase coherence in otherwise identical cultures.

Research Funding: R01 GM34985 27-30, R01 GM083336-23 - 27

M28

Identifying CK1e/d Activity as a Potential Link between Circadian Rhythm Disruption and CXCL-1 Mediated Neuroinflammation

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Abstract: Emerging evidence associates circadian disruption to a number of psychiatric diseases whose pathologies have been linked to neuroinflammation. The inhibition of casein kinase 1 epsilon and delta (CK1e/d) stabilizes circadian rhythms indicating therapeutic potential for mood disorders. Therefore, the current study was designed to determine if a selective CK1e/d inhibitor alters the pro-inflammatory cytokine release in an in vitro model of neuroinflammation.

The selective CK1e/d inhibitor PF-5006739 was added to fibroblasts expressing BMAL::Luciferase and resulting luminescence was measured. For the neuroinflammation model, PF-5006739 was added to the medium of isolated mouse microglia followed one hour later by addition of lipopolysaccharide (LPS). Media was collected and 37 analytes were profiled using a multiplex assay. ELISAs for specific analytes were used to confirm cytokines/chemokines from the multiplex assay.

Addition of PF-5006739 to the BMAL::Luc fibroblasts resulted in a robust shift in BMAL oscillation at the highest doses tested. While PF-5006739 alone did not alter the release of the analytes studied in the neuroinflammation model, the addition of LPS to the microglia resulted in an increase in

a number of pro-inflammatory cytokines/chemokines and decrease in anti-inflammatory markers. Pre-treatment of the LPS treated microglia with PF-5006739 attenuated the release of the pro-inflammatory mediators (CXCL-1 and MIP-2) and increased the anti-inflammatory cytokine, IL-5. CXCL-1 was confirmed as a chemokine that is regulated by CK1e/d activity in LPS treated microglia. Further confirmations are currently on-going.

The current study identified the pro-inflammatory chemokine CXCL-1 as being under the control of CK1e/d activity following LPS treatment of mouse microglia. Elevated CXCL-1 concentration has been associated with a number of neurological and psychiatric diseases including Alzheimer's and addiction. Therefore, these results demonstrate the therapeutic potential of CK1e/d inhibitors for circadian rhythm disruption and neuroinflammation mediated by CXCL-1. Future in vitro and in vivo studies are designed to further investigate the link between CK1e/d activity, circadian rhythms, and CXCL-1 signaling.

Research Funding: Research is funded by Janssen Pharmaceutical Company of Johnson and Johnson.

M29

How Outer Retinal Photoreception and Melanopsin Phototransdcution Control Non-Image Forming Visual Functions

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Abstract: Rods, cones and ipRGCs are the only mammalian photoreceptors that influence non-image forming (NIF) visual functions. These NIF functions include the pupillary light reflex, circadian photoentrainment, sleep and direct light effects on activity and body temperature. ipRGCs are both sufficient and necessary for light to regulate these NIF functions. In the absence of melanopsin phototransduction, however, rods/cones are capable of signaling light input through ipRGCs and are able to, at least partially, compensate for the absence of the melanopsin-based phototransduction pathway in driving several NIF functions. in fact, in vitro electrophysiological recordings from ipRGCs have found little deficits in the ipRGC light response if melanopsin is removed while rod/cone input is maintained. This leads to the following conundrum: why is melanopsin conserved across evolution if rods/cones are able to signal light input through ipRGCs? Here we provide quantitative behavioral data using an array of mouse mutant animals to show how rods/cones interact with melanopsin phototransduction to influence an array of NIF functions. We particularly uncover an essential role of melanopsin phototransduction in circadian photoentrainment, sleep and direct light effects on body temperature. We provide a model for how the three photoreceptors interact with one another to optimally regulate distinct NIF visual functions, and highlight the evolutionary evidence for the conservation of melanopsin across vertebrates.

Research Funding: This research is funded by NIH-GMS.

M30

Characterization of Non-Visual Responses to Light Using Spectral, Temporal and Spatial Properties of Rods/Cones and ipRGCs in Humans

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Abstract: Intrinsically photosensitive retinal ganglion cells (ipRGCs) subtend non-visual (NV) effects of light, together with rods and/or cones. Whether all NV functions share the same photoreceptors (PR) contribution and threshold sensitivity is unknown. Our study investigated this aspect in humans, using the spectral, spatial and dynamics properties of cones and ipRGCs.

In a within-subject design, 28 subjects were exposed to 4 consecutive 50-min light stimuli (B1, B2, R1, R2), from 7-11pm. Stimuli consisted of a full field multi-LED white light (300 lux, 40-120 mlux) enriched with different levels of blue (B1, B2), or red (R1, R2), and a central white LED light (CWL, 7000 lux) centered on the fovea (20°), turned on after 1 min in order to evaluate responses dynamics. EEG was recorded at 256 Hz and submitted to FFT analysis (Fz reported here). Pupil diameter was recorded at 30 Hz and analysed for phasic and tonic constrictions. Visual and cognitive performance were tested.

All EEG bands were affected during the 1st min of LE, but there was no differential effect of light spectrum. The CWL decreased theta band (5%, $p < 0.05$) in B2 vs R. Delta, beta and gamma bands showed an effect of light, irrespective of condition, at 42min ($p<0.05$). The results do not allow to extract PR contribution in the EEG response.

Phasic constriction was similar for all light conditions (~24%) during the 1st min of LE, increased when CWL was turned on (+~11%, $p<0.0001$), irrespective of the spectra. Tonic constriction was significantly higher in the blue than in the red during the 1st min (B = B2 (22%) > R2 (19%) > R (17%), ($p<0.001$), further increased with the CWL (+ ~13%) in all conditions ($p<0.0001$) and did not differ between spectra from 2 to 50 min. These results suggest to main cone contribution in the phasic part of the PLR, a dual contribution of cones and ipRGCs in tonic constriction, with an initial (min 1) stronger cone input, replaced over time by a stronger ipRGCs input.

Visual and cognitive performance (additions, PVT, 2-back) were at same levels in all conditions.

These results show a polychromatic light stimulus can activate NV functions within 1 min of exposure to a moderate intensity (300 lux). They also show that responses involve either cones, melanopsin, or a combination of both inputs, with contribution and dynamics differing across NV functions.

Research Funding: Funded by contracts from the industry and the National Research Agency (ANR, France).

M31

Red Light at Night Does Not Suppress Melatonin in the Horse

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Abstract: 'Smart lighting' systems are widely used in animal housing within the Agri-Food sector but have yet to gain acceptance within the equine industry. The nature of equine breeding and performance management is such that it is frequently necessary to interact with the animals during the nighttime hours. As knowledge of the importance of optimal photoperiod regimes for equine circadian and seasonal rhythm maintenance and manipulation grows, it is important that lighting systems are developed that do not negatively impact these important rhythms. Six horses were used in a crossover design to evaluate the impact of an LED lighting fixture that emitted low intensity (5 lux) red light (peak wavelength approx. 625 nm) during the hours of ambient darkness and polychromatic white light (>200 lux) during daytime hours. The horses were maintained for 48 h under the Light: Red (LR) photo-schedule where the transition from white to red light coincided with dusk in the external environment and the transition from red to white coincided with dawn, and 48 h under a Light: Dark (LD) (<1 lux) regime with lights on and lights off again mimicking dawn and dusk. This experiment was conducted around the time of the Autumnal equinox in mid-September. Blood samples were collected at 2-h intervals throughout from indwelling jugular catheters followed by melatonin analysis by ELISA. Two-way Repeated Measures ANOVA revealed a significant effect of time ($p<0.0001$) with melatonin rising to similar levels at night under both LR and LD conditions but no time x treatment interaction and no effect of treatment on serum melatonin. We conclude that low intensity red light at night does not suppress melatonin secretion in the horse and is therefore unlikely to impact circadian or seasonal regulation of physiology. These findings support the development of lighting regimes that use red light at night for horse management.

Research Funding: This research is being funded by an Irish Research Council Enterprise Partnership Scheme grant award between University College Dublin and Equilume, Ltd.

M32

Photoreceptor Weighted Light Intensities and Their Dose-Response Relationships for Non-Visual Effects of Light

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Abstract: Background: Our understanding how to assess and quantify light conditions for their ability to elicit non-visual effects is still incomplete. Behavioral and biological effects of light are known to be influenced by distinct photoreceptors in the eye, melanopsin-containing retinal ganglion cells, next to conventional rods and cones. A new strategy to quantify light allows to individually characterize all these different photoreceptive inputs, and expresses light intensity in terms of five different kinds of α-opic irradiances: melanopic, rhodopic, cyanopic, chloropic and erythropic.

Methods: For each of the five α-opic irradiances, dose-response relationships are established from a meta-analysis of the scientific literature for specific non-visual effects of light in man, such as alertness induction and nocturnal suppression of the sleep-supporting hormone melatonin.

Results: Both alertness induction and melatonin suppression display a strong and significant correlation with the melanopic-irradiance, but less so with the other α-opic irradiances. These results indicate that a melanopsin weighted light intensity is highly predictive for the alerting and melatonin suppressing effects of light. Previously a "melanopic-lux" quantity has been proposed to evaluate melanopsin weighted light intensities. This quantity is easily and directly translatable into a melanopic irradiance or a melanopic "daylight equivalent" illuminance, thus ensuring compatibility with existing SI units.

Conclusions: Quantifying light exposures in terms of the different α-opic irradiances and α-opic daylight equivalent illuminances helps light designers decide what lighting conditions to use in order to promote, or avoid, certain non-visual responses to light. Moreover, it allows to define further experiments to establish what photoreceptor inputs and photoreceptor interactions are predictive for particular non-visual responses to light.

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M33

Chronotype Differences in the Distribution of Excitatory and Inhibitory Cell Populations in ipRGC Target Areas

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Abstract: Masking responses are completely reversed in diurnal and nocturnal species, with light increasing arousal in the former and suppressing it in the latter. The neural mechanisms promoting these effects are not well understood. In nocturnal rodents, masking is mediated through a subset of retinal ganglion cells that are intrinsically photosensitive (termed ipRGCs). The projections of ipRGCs are similar in diurnal and nocturnal rodents. Thus, differences in the circuitry within ipRGC targets, such as the distribution of inhibitory and excitatory cells, may lead to differences in how diurnal and nocturnal species respond to light. Here we examined this hypothesis by characterizing glutamatergic and GABAergic neuronal populations in multiple ipRGC target areas in diurnal Nile grass rats (Arvicanthis niloticus) and nocturnal Norway rats (Rattus norvegicus). We found that while many ipRGC targets were very similar in these two species, there were striking differences in the ventral lateral geniculate nucleus (vLGN) where there was a higher density of glutamate cells in Norway rats than grass rats and in the lateral habenula (LHb) where GABAergic cells were present in grass rats and virtually absent in Norway rats. These patterns suggest that the vLGN and/or LHb may contribute to differences between diurnal and nocturnal species with respect to masking and/or circadian regulation of rhythms by altering the valence of signals coming to these regions, directly or indirectly, from ipRGCs and/or the suprachiasmatic nucleus. The species difference in the LHb is particularly interesting, since this area is highly integrated with brain regions that modulate circadian rhythms and a variety of other behavioral processes (sleep, reward, pain, cognition). Future work is needed to determine what role these cells may play in modulation of behavioral state in diurnal species.

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M34

The Multifunctional Nature of Cryptochromes in the Mammalian Retina

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Abstract: Cryptochromes (Crys) can be critical for both maintenance and entrainment of circadian clocks, but their specific roles are species-dependent. Whereas mCry1 and mCry2 are core negative transcriptional regulators of the mammalian clock, dCry and dpCry1 act as blue-light photoreceptors that synchronise the clocks of Drosophila and Monarch Butterfly, respectively, to photic cues. In addition to these well characterised roles, there is evidence that insect Crys may function as light-dependent magneto-sensors (Gegear et al. 2008, Nature). It is unclear whether such a function for Crys exists in mammals, nor is the location of any magnetoreceptive organ in mammals known. Previously we showed that the mammalian master pacemaker, the suprachiasmatic nucleus (SCN), is not intrinsically magneto-sensitive (Fedele et al. 2014, PLoS Genetics). Given that the SCN does not normally receive direct light stimulation, we sought instead to explore putative magneto-sensory functions of Crys in a light-sensitive tissue: the mammalian retina. We thus asked the following questions: first, can we confirm a role for mCrys in the retinal clock; second can Cry proteins originating from Monarch Butterfly function within the mammalian circadian clock and finally, can they exhibit their intrinsic magneto-sensory functionality within the mammalian retina? Using whole retina explants, expressing the Per2::Luc reporter, we characterised the roles of mCry1 and mCry2 in the retina. Retina from both Cry1- and Cry2-null mice exhibited robust bioluminescence rhythms that had periods of ca. 22h or 26h respectively, thus phenocopying the circadian period of the SCN in vitro and overt behaviour in vivo. Furthermore, Cry1/Cry2-null retinas were arrhythmic, indicating that Crys are needed to maintain circadian rhythmicity in the retina in vitro. An AAV-dpCry2 was generated for in vivo expression in the retina of Cry-null animals by intraocular injection. Retinas from transduced animals could then be prepared in culture and the recovery of clock function and/or magneto-sensation assessed. Custom-made electromagnetic field (EMF)-generating incubators were used to assess the responses of retina explants to EMF exposure, in the presence or absence of light. This combined approach of both in vivo expression and in vitro measurements will allow us to gain greater insight the multifunctional nature of Cry proteins.

Research Funding: Supported by The EMF Biological Research Trust.

M35

Blue Light Therapy Improves Circadian Dysfunction in Two Mouse Models of Huntington's Disease

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Abstract: Patients with Huntington's disease (HD) exhibit movement disorders, psychiatric disturbance and cognitive impairments as the disease progresses. Abnormal sleep/wake cycles are common among HD patients. In addition to the reports of delayed sleep onset and greater sleepiness during the waking phase, the changed circadian pattern of melatonin suggests dysfunction in the circadian timing system. Moreover, previous studies in mouse models of HD have demonstrated that the

circadian rhythm system in HD is disrupted. Importantly, circadian dysfunction manifests early in disease, even before the classic motor symptoms, in both patients and mouse models. Therefore, we hypothesize that circadian dysfunction may interact with the disease and exacerbate the HD symptoms. Moreover, early intervention may benefit patients and delay disease progression. One test of this hypothesis is to determine whether light therapy designed to strengthen this intrinsic timing system can delay the disease process in mouse models of HD. Light is a strong environmental regulator of circadian timing with blue wavelength light having the strongest impact. In addition, the blue-enriched light therapy has potential benefits over current light therapy, including shorter therapy sessions, more comfortable light intensity, and energy savings. Therefore, this study applied blue-enriched light during the first 6h of light phase during the pre-manifest stage of two HD mouse models: the BACHD (3mo) and Q175 heterozygous (6mo) mouse models. After 3 months of treatment, both genotypes showed improvements in their locomotor activity rhythm and sleep/wake cycle. Moreover, the motor performance was significantly improved in Q175 mutants. Our results suggested the possibility that novel environmental intervention can delay the progression of HD in pre-clinical models.

Research Funding: Cure Huntington's Disease Initiative (CHDI) Foundation

M36

Time–of-Day Disruption of GSK3β Phosphorylation and Cognitive Impairment in the Tg-Swdi Mouse Model of Alzheimer's Disease

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Abstract: Alzheimer's disease (AD) is one of the most prevalent causes of dementia in elderly patients worldwide. In addition to cognitive decline, patients with AD often experience circadian rhythm disruptions in both early and late stages of the disease. Glycogen synthase kinase 3 β (GSK3β) is involved in both β-Amyloid and Tau pathways and is regulated by components of the molecular clock. In AD, GSK3β is overactive, leading to hyper-phosphorylation of Tau resulting in the formation of neurofibrillary tangles, a prominent pathological feature of AD. We hypothesized that in the Tg-SwDI mouse model of AD (which expresses human amyloid precursor protein with the familial Swedish (K670N/M671L), Dutch (E693Q), Iowa (D694N) mutations), day-night differences in phosphorylation (i.e., inhibition) of GSK3β are lost, leading to reduced cognition in early and late stages of the disease. We found that phosphorylated (S9)/total GSK3β ratios showed diurnal variation in whole hippocampus from 12-month mice, with greater levels at Zeitgeber Time (ZT) 2 and 8 and lower levels at ZT 14 and 20, where ZT 12 refers to lights off ($n = 6$-9/group, $p < 0.05$). Importantly, day-night differences were greatly reduced in AD mice. Because GSK3β activation is important for spatial memory, we next examined whether day-night differences in memory were impaired in AD mice. Spatial working memory can be assessed by spontaneous alternation (SA) in a T-maze, and prior work in hamsters reveals enhanced performance at night. Our results show that mice also exhibit enhanced SA performance at night (ZT 15) compared to during the day (ZT 3) (n =13-14/group, $p < 0.05$). Moreover, a separate cohort of 4-month-old male and female mice (AD and WT) revealed a significant reduction in spatial working memory in female AD mice at four months, compared to female WT mice ($n = 8$-9/group, $p < 0.05$). In 12-month old males, alternation of AD mice was significantly reduced at night compared to WT controls ($n = 8$-13/group, $p < 0.05$). Mice with constitutive GSK3 activation (i.e., phosphorylation-resistant) showed a loss of day-night differences

in SA performance, similar to the results found in AD mice (n = 5-9/group, $p < 0.05$). Thus, these results show that diurnal rhythmicity of GSK3β activation is compromised in AD and may underlie time-of-day dependent regulation of spatial working memory.

Research Funding: This work was supported by NIH 1R01NS082413 to KLG, and P30 NS47466 to TVG and T32HL105349.

M37

The Effects of Circadian Misalignment During Adolescence on Mood and Alcohol Sensitivity

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Abstract: During adolescence, circadian phase and sleep/wake times are delayed. When given the choice on the weekend, teens prefer to stay up late and sleep in. However, on weekdays adolescents must wake up early for school. The repeated weekend-weekday shifts, termed "social jet lag", cause the internal clock to become desynchronized from the environment. Social jet lag has been linked with mood disturbances and increased substance use in adolescents. Specifically, studies by Dr. Hasler and colleagues found that weekend-weekday advances in midsleep time during adolescence were associated with decreased medial prefrontal cortex (mPFC) and ventral striatum reactivity to monetary reward. In another study by this group, an evening chronotype in 20-year-old males was associated with decreased mPFC reactivity to monetary reward and greater alcohol consumption. To investigate the mechanisms underlying adolescent vulnerability to circadian misalignment effects on alcohol use and mood, we are developing a model of adolescent social jet lag in rats. Rats have been shown to exhibit an evening chronotype during adolescence similar to humans. In our paradigm, adolescent rats (P31-P44) experience weekend-weekday activity advances by forced treadmill walking. On weekdays rats are placed on the treadmills from ZT6-14, and on the weekends rats are on the treadmills from ZT12-20. Control rats remain on the treadmills from ZT12-20 each day. Following two weeks of timed treadmill walking, mood-related behaviors were assessed by open field, elevated plus maze, and forced swim tests. Then we examined alcohol sensitivity by assessing locomotor activity and striatal c-fos levels after an alcohol (2 g/kg, i.p.) challenge. Our preliminary data indicates that our social jet lag paradigm may increase risk taking behavior, produce hyperactivity, and increase alcohol sensitivity. Taken together, this data suggests that we can successfully model social jet lag and its effects on addiction-like behavior in adolescent rodents.

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M38

Treating Circadian Dysfunction Delays Disease Progression in Mouse Models of Huntington's Disease

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Abstract: Sleep and circadian disruption are a common complaint in patients with Huntington's disease (HD) and other neurodegenerative disorders. Even early in HD progression, patients have difficulty falling asleep at night and suffer from excessive daytime sleepiness. These sleep/wake disruptions are indicative of a dysfunctional circadian timing system. In recent work on mouse models of HD, we found significant disruption in the circadian rhythms of activity and sleep/wake behavior, along with impaired function of the circadian pacemaker in the brain, suggesting that circadian and sleep disruption is integral to HD. A disrupted circadian system not only leads to poor mood and memory, but also has a negative impact on important bodily functions like cardiovascular function and metabolism. We sought to test the hypothesis that improvement of daily rhythms in activity and sleep could improve or delay disease symptoms in two mouse models of HD: the BACHD transgenic insertion line, and the Q175 knock-in line. Both models recapitulate aspects of disease progression of HD, including disrupted circadian rhythms and progressive decline in motor coordination. We treated the pre-symptomatic mutants with 3 months of scheduled feeding aligned to their active phase, which resulted in improved entrainment to the light-dark cycle compared to HD mutants under ad libitum feeding. By limiting food consumption to the active phase, we reduced daytime activity and improved performance on motor coordination tasks at the early symptomatic stage. There may thus be significant value in therapeutic interventions to address the circadian rhythm disruption during the early stages of HD.

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M39

Constant Darkness and Constant Light Suppress Voluntary Alcohol Intake in Mice

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Abstract: Seasonal variations in photoperiod are associated with changes in physiology and behavior in both human populations and experimental animals. For example, alcohol consumption varies with both season and latitude, while exposure to short photoperiods and/or constant darkness (DD) has been reported to increase ethanol intake in rats and hamsters. Surprisingly, however, Goodwin et al. (1999) found reduced ethanol intake in rats under both DD and constant light (LL), relative to standard light-dark (LD) 12:12 conditions. The aim of the present research was to examine the effects of DD and LL on voluntary ethanol intake in inbred (C57BL/6 and C3H/He) and genetically heterogeneous (WSC2) mice. Mice were maintained in running-wheel cages with continuous free-choice access to both 10% v/v ethanol solution and plain water, and exposed to a series of lighting regimens for several weeks each. Circadian activity rhythms entrained under LD and showed typical

free-running rhythms under DD and LL. Ethanol intake was consistently reduced under both DD and LL in all lines. While these results confirm an important effect of environmental lighting on ethanol consumption, it is difficult to account for the similarity of the effects seen under both DD and LL. Thus, these effects are unlikely to be mediated by a photoperiodic mechanism, changes in melatonin secretion, circadian disruption, or "direct" behavioral effects of light and darkness. We hypothesize that drinking may be reduced due to the lack of entrainment to a 24-hour cycle in both DD and LL. Alternatively, the apparently similar effects of DD and LL on alcohol drinking may be mediated by dissimilar underlying mechanisms.

Research Funding: Departmental and University funding

M40

Clock-HIF Interaction Establishes Rhythmic Skeletal Muscle Exercise Tolerance and the Hypoxic Response

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Abstract: The core clock transcriptional activators BMAL1/2, CLOCK, NPAS2, and repressors PERIOD1/2/3 are members of the basic helix-loop-helix-PER-ARNT-SIM (bHLH-PAS) domain-containing family homologous to bHLH-PAS hypoxia-inducible factor (HIF) proteins HIF1/2α which activate gene pathways in response to low oxygen. We tested the impact of BMAL1-HIF transcription factor heterodimerization on the coordination of circadian and hypoxic transcription and exercise tolerance. Here, we demonstrate (i) that circadian transcription factors regulate hypoxic HIF1α activation, with opposite effects of Bmal1 and Cry1/2 deletion; (ii) hypoxia reciprocally regulates clock transcriptional activity and period length in myotubes ex vivo; (iii) circadian and hypoxic transcription factors co-localize within the E-box elements of the Per and Cry repressors, and (iv) the circadian clock establishes time-of-day dependent exercise tolerance and hypoxic response. Collectively, our data reveal coupling of the hypoxia-inducible factor and circadian pathway producing rhythmic adaptation to hypoxic stress.

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M41

Disruption of Daily Rhythms by High-Fat Diet is Reversible

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Abstract: In mammals a network of circadian clocks coordinates behavior and physiology with 24-h environmental cycles. Consumption of high-fat diet disrupts this temporal coordination by advancing the phase of the liver molecular clock and altering daily rhythms of eating behavior and locomotor

activity. In this study we sought to determine whether these effects of high-fat diet on circadian rhythms were reversible. We chronically fed mice high-fat diet and then returned them to low-fat chow diet. We found that the phase of the liver PERIOD2::LUCIFERASE rhythm was advanced (by 4h) and the daily rhythms of eating behavior and locomotor activity were altered for the duration of chronic high-fat diet feeding. Upon diet reversal, the eating behavior rhythm was rapidly reversed (within 2 days) and the phase of the liver clock was restored by 7 days of diet reversal. In contrast, the daily pattern of locomotor activity was not restored even after 2 weeks of diet reversal. Thus, while the circadian system is sensitive to changes in the macronutrient composition of food, the eating behavior rhythm and liver circadian clock are specifically tuned to respond to changes in diet.

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M42

Short-Term Effect of Nocturnal Transportation Noise on Glucose Metabolism

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Abstract: Background: Traffic noise from roads and railways is a growing cause of sleep disturbances and in the long run, seems to be associated with a higher risk of type 2 diabetes. However, the relationship between traffic noise, sleep disturbances and cardio-metabolic diseases remains to be investigated.

Aim: Evaluation of short-term effects of nocturnal traffic noise on morning glucose tolerance after night-sleep episodes.

Methods: So far 8 lean young volunteers (BMI: 18.5-25; age: 19-32 y) participated in a six-day laboratory study starting with a noise-free baseline night (BL) followed by 4 nights with night-time noise scenarios (railway or road traffic noise with an hourly Leq of 45 dB(A) at the ear of the sleeper, NN2-NN5), and ending with a final noise-free recovery night (RC). Carbohydrate metabolism was evaluated during a 120 min oral glucose tolerance test (OGTT) scheduled in the mornings of BL, NN5 and RC.

Results: Post-charge glucose and insulin levels increased after four nights of nocturnal traffic noise compared to the baseline night ($p=0.03$ and $p=0.0012$, respectively). Mean sleep efficiency was at least 94% and slow wave sleep at least 16% and did not significantly differ between the 6 nights.

Conclusion: Four nights of nocturnal traffic noise decreased glucose tolerance in lean young volunteers - an effect, which is most likely not related to changes in sleep efficiency or slow wave sleep. These results could explain the first step in the development of metabolic syndromes as seen in traffic dense regions.

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M43

The Liver Circadian Clock Modulates Blood Glucose Lowering Efficacy of Metformin

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Abstract: Circadian clocks in peripheral organs coordinate metabolic functions to optimize energy storage and utilization with predictable daily cycles in fasting, feeding and activity. Thousands of transcripts exhibit diurnal patterns of expression in hepatocytes, and metabolic pathways are enriched among the genes that exhibit such oscillatory patterns. The liver circadian clock plays an important role in glucose regulation as evidenced by a daily period of fasting hypoglycemia observed in mice lacking Bmal1 expression specifically in hepatocytes. Metformin is widely used in the treatment of type 2 diabetes; it inhibits the activity of mitochondrial complex I, resulting in inefficient ATP production and secondary activation of AMP-activated protein kinase (AMPK). Ultimately, these and other biochemical effects of metformin result in decreased hepatic glucose production, and amelioration of elevated blood glucose in diabetic patients. Though metformin is a relatively safe and effective drug, it can cause dangerous acute hypoglycemia and lactic acidosis. Thus, additional understanding of metformin pharmacokinetics could provide important information regarding therapeutic usage of this widely prescribed drug. We observed a significantly different acute blood glucose lowering response to metformin depending on the time of day of administration in healthy mice. Furthermore, we established that the kinetics of metformin transport into hepatocytes and of metformin-induced AMPK activation depend on circadian time. These effects were abolished in mice lacking circadian clocks in hepatocytes due to liver-specific ablation of Bmal1 expression, suggesting that the liver circadian clock contributes to circadian modulation of metformin transport and efficacy.

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M44

Effects of Photoperiod on Locomotor Activity and Glucose Regulation in C57BL/6J Male Mice

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Abstract: Currently, published literature suggests a correlation between circadian disruption and the effects of high fat diet. This in turn is also important in addressing negative health effects such as lowered glucose intolerance, which may lead to diabetes and an increase in fat deposition. Previous experiments by our group focused on the effects of wheel running activity or nighttime food restriction and high fat diet, to gain more insight on how diet and circadian disruption may affect eating behaviors and calorie consumption. Preliminary studies focused on circadian disruption and high fat diet have shown effects on glucose intolerance and increased weight gain. Mice eat primarily during the nighttime, thus manipulating the photoperiod by elongating the night or elongating the day in different treatment groups on high fat or low fat diet may allow us to gain more insight

into the eating patterns, calorie consumption and glucose tolerance in mice. Moreover, chronic circadian disruption appears to influence timing or pattern of feeding, which can influence glucose metabolism. In this experiment, young male C57BL/6J mice are housed on 16:8 LD or 8:16 LD cycles on high fat (45% kcal) or low fat diet (10% kcal). addition, half the mice in each group are subjected to a chronically advancing light-dark cycle (4 hours advanced every 3 days), or maintained on a 24-hour LD cycle, with locomotor rhythms monitored by infrared motion detector. Preliminary data show that the pattern of daily locomotor activity is altered by 4 weeks of long or short photoperiod. Mice on 8:16 LD are not able to maintain normal entrainment as well as mice on 16:8 LD. High fat diet adversely affects glucose tolerance and body weight. Data collection on glucose measures under chronic circadian challenge is currently underway.

Research Funding: Rider University Department of Biology

M45

Glucose Tolerance in Nocturnal Animals Experiencing Light-Dark Stimulus Patterns Mirroring Patterns Measured from Dayshift and Rotating Shift Workers

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Abstract: Introduction: Light-induced circadian disruption experienced by rotating shift workers has been associated with increased risk for Type II diabetes.

Methods: Using light-dark exposures data collected from nurses, we investigated how glucose tolerance in mice was affected by exposure to patterns simulating dayshift (12L:12D) and two rotating-shift workers working one night per week (RSS1) and three nights per week (RSS3). An oral glucose tolerance test was applied 3 h before the start of the dark phase on the same day of the third week of each session. Circadian disruption was quantified with phasor magnitudes based upon mouse-specific 24-h light-dark patterns and 24-h activity-rest wheel-running patterns.

Results: Glucose levels were significantly lower 30 min after glucose administration than 15 min after glucose administration when animals had experienced the 12L:12D pattern ($t10 = 5.0$; $p = 0.001$), but not when they had experienced the RSS1 or the RSS3 lighting patterns ($t10 = 0.3$; $p = 0.8$ and $t10 = 0.6$; $p = 0.6$). Glucose area-under-the-curve after animals experienced the 12L:12D pattern was significantly less than after animals experienced the RSS1 and the RSS3 lighting patterns ($t10 = 13.8$; $p < 0.0001$ and $t10 = 11.8$; $p < 0.0001$, respectively). Phasor magnitudes measured when animals experienced the 12L:12D pattern was significantly greater than after animals experienced the RSS1 and the RSS3 lighting patterns ($t11 = 20.6$; $p < 0.0001$, $t11 = 24.7$; $p < 0.0001$, respectively). Glucose intolerance in mice was inversely related to phasor magnitude.

Conclusions: The present study showed that even one night of shift work increases glucose intolerance. Human circadian disruption, defined in terms of phasor magnitudes, experienced by dayshift and rotating-shift humans, was directly linked to glucose intolerance in mice, a nocturnal animal model for onset of Type II diabetes.

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M46

Circadian Regulation of Xenobiotic Metabolism

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Abstract: Circadian clocks sustain rhythmic 24 hour patterns in behavior and physiology on a systemic as well as a tissue level. The molecular mechanism underlying mammalian circadian rhythms is based on a transcriptional feedback loop featuring the transcription factors CLOCK, BMAL1, cryptochrome (CRY), and period (PER). Forming a heterodimer, CLOCK and BMAL1 drive transcription of a large number of target genes, including those encoding the negative regulator period (PER1, PER2, PER3) and cryptochrome (CRY1, CRY2). PER and CRY dimerize and in turn act to repress CLOCK and BMAL1 transcriptional activity allowing the cycle to repeat. This core clock directly or indirectly drives oscillating transcription of a large number of target genes. Approximately 43 % of all protein coding genes are transcribed rhythmically in at least one organ. Correspondingly, many aspects of mammalian physiology are robustly rhythmic, including the detoxification of foreign, potentially harmful compounds: xenobiotic metabolism. The duration of sleep of rats under anesthesia depends on the time of day the animal was exposed to the drug. Studies in humans have shown dosing time-dependent fluctuations in drug metabolism and half life, e.g. half life of CYP3A substrates, is shortest in the afternoon. Both the circadian transcriptome and the circadian proteome are enriched for components of xenobiotic detoxification pathways. However, the underlying mechanism(s) that confer rhythmicity on these mRNAs and proteins are not well understood. Expression of the proteins involved in phase I-III is in large part facilitated by ligand activated transcription factors, the nuclear receptors pregnane X receptor (PXR) and constitutive androstane receptor (CAR). Upon binding of a xenobiotic ligand, PXR and CAR control the expression of proteins required for the detoxifying process. Interestingly, we observe that the liver xenobiotic receptors PXR and CAR show a robust interaction with CRYs. Hypothesizing that these interactions alter PXR/CAR function we characterized the interaction between CRYs and PXR/CAR biochemically and we examined the cis-regulatory elements necessary to drive strong rhythmic xenobiotic gene expression at the promoter level. We hope this new information may help to optimize drug administration routines.

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M47

24 Hour Metabolic Profiling in Obesity and Type 2 Diabetes (T2DM)

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Abstract: Obesity and type 2 diabetes (T2DM) have been associated with altered concentrations of circulating metabolites. Previous studies have mostly compared the effect of weight and/or T2DM on the concentrations at a single time point or the daily rhythmicity of metabolites in animals and healthy individuals. We thus tested the hypothesis that weight and/or T2DM affects the concentration and rhythmicity of circulating metabolites in humans across 24h.

In an environmentally controlled laboratory study (meals, lighting and body posture), 2 hourly plasma samples were measured from 8 lean (L), 10 overweight (OW) and 7 OW with T2DM men, aged 45-65 and analysed by quantitative targeted LC/MS metabolomics. A total of 130 metabolites were quantified.

Using the fit to a cosine curve as a measure of diurnal rhythmicity 50 (38%) metabolites showed a significant daily rhythm ($p < 0.05$) in at least one study group. Of which 14 displayed a daily rhythm in all 3 groups which peaked within hr of each other, nor was there a trend regarding change in relative amplitude.

Most metabolites (91) showed significant differences in 24 h mean concentrations between L and OW groups (2-way ANOVA, time and group as factors). Twenty metabolites significantly increased in OW compared to L, the largest increases being glutamate, acylcarnitines octadecenoylcarnitine (C18:1) and valerylcarnitine (C5), kynurenine and all the branched chain amino acids (BCAA). Of the 71 metabolites with significantly lower concentrations in OW, 59 (83%) were glycerophospholipids. This finding supports previous negative associations of circulating lysophoshophatydylcholines with BMI and plasma insulin concentrations.

Compared to OW, the T2DM group had significant increases in the lipid classes as well as carnitine (C0), propionylcarnitine (C3), amino acids (alanine, glutamate, phenylalanine, proline, and tyrosine), alpha-AAA, sarcosine and t4-OH-proline. Of these, propionylcarnitine, proline and sarcosine also displayed significant 24 h rhythms ($p < 0.05$) in all 3 groups. The T2DM group had significantly lower levels of histidine, ornithine, glutamine, serine and phospholipids compared to the OW group. These T2DM "hits" concord with previous studies proposing novel biomarkers of T2DM and emphasise the potential of targeted metabolomics for disease diagnosis.

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M48

Composition and Structure of Cytoplasmic PERIOD Complexes in Relation to the Nuclear PERIOD Complex

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Abstract: The mammalian circadian clock is built on a molecular feedback loop in which the transcription factor CLOCK-BMAL1 drives the expression of three PERIOD (PER) and two CRYPTOCHROME (CRY) proteins. PERs and CRYs accumulate, enter the nucleus, and form a large complex that includes chromatin-modifying machinery. This complex binds to DNA-bound CLOCK-BMAL1, repressing transcription of Per, Cry and other target genes.

While it is clear that interactions of cytoplasmic PERs and CRYs with each other and with other proteins, particularly Casein Kinase-1 (CK1), are important for timing PER and CRY nuclear entry and for clock function, there is as yet no coherent picture of the cytoplasmic part of the cycle.

What PER-CRY complexes are there in the cytoplasm, and what is their composition? We characterized, purified, and analyzed cytoplasmic PER complexes from mouse liver. We found that there are two distinct PER complexes in the cytoplasm, with masses of ~0.9 (lower complex) and ~1.1 MDa (upper complex). We affinity-purified cytoplasmic complexes that contained PER2 and size-separated upper and lower complexes by glycerol gradient sedimentation. We found that the lower complex consisted

of PER1, PER2, CRY1, CRY2, and CK1Δ (but not CK1ε). In agreement with its greater mass, the upper complex in addition contained the proteins PER3 and GAPVD1. Neither cytoplasmic complex contained CLOCK, BMAL1, or any of the chromatin modifiers of the nuclear PER complex.

How do the morphologies of the two cytoplasmic PER complexes differ from one another and compare to the physical organization of the larger nuclear PER complex? We visualized purified cytoplasmic and nuclear PER complexes isolated from mouse liver at the single particle level using negative-stain electron microscopy. These experiments provided the first physical images of intact PER complexes. Lower cytoplasmic PER complex particles are ~25-nm structures and consist of four globular modules tethered by flexible connectors. The upper cytoplasmic complex possessed additional globular modules, in agreement with its larger mass, and it appeared more rigidly organized. In comparison, the nuclear PER complex is ~40-nm in diameter, roughly the expected size of a complex of ~2 MDa, appearing as a central core surrounded by a large number of smaller globular domains.

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M49

Determination of poly(A)-Tail Lengths and 3′-End Modifications of mRNAs by Tail-Seq in Circadian Systems

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Abstract: The poly(A)-tail lengths of mRNAs have been shown to be important for the regulation of mRNA translation and stability in diverse biological processes. Recently, a new technique, Tail-seq, has been developed to measure poly(A)-tail lengths and 3′-end modifications of mRNAs. Interestingly, uridylation of 3′-very-ends of mRNAs is enriched in short A-tail mRNAs (less than 25nt) and the uridylation frequencies of mRNAs are anti-correlated with mRNA half-lives. To examine rhythmic A-tail length regulation and 3′-end modifications of mRNAs, we performed Tail-seq in fly heads and mouse liver at 6 time points under light-dark cycles. Generally, RNAs from fly heads have shorter A-tail lengths and lower uridylation frequencies than those from mouse liver. We identified transcripts with rhythmic poly(A) tail lengths in both fly heads and mouse liver. The mouse liver data largely agree with those of Green and colleagues (Kojima, Genes Dev., 2012). Surprisingly, we found that the uridylation frequencies, but not guanylation or cytidylation frequencies, are rhythmic in mouse liver. Consistent with these results, the major terminal uridylyl transferases, TUTase 4 and TUTase 7, have rhythmic mRNA and protein expression in mouse liver, implying that rhythmic expression of the TUTases may contribute to the cycling patterns of uridylation.

Research Funding: Howard Hughes Medical Institute

M50

4C-Seq in Mouse Liver Reveals Clock-Dependent Rhythmic Chromatin Contacts

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Abstract: The topological organization of chromatin plays an important role for nuclear functions such as transcription replication and DNA repair. Since the circadian oscillator provides a unique model for dynamic gene expression, we explored the topology of chromatin surrounding promoters of core clock and rhythmic output genes in mouse tissues of WT and BMAL1 KO animals, during the circadian cycle using 4C-sequencing (4C-seq). We found chromatin interaction patterns that are gene specific and localized predominantly within one or two megabases near the baits. Though the overall 4C-seq profiles were conserved between biological replicates, across tissues and genotypes, we identified time-varying chromatin reorganization and DNA loops that depended on a functional molecular clock. Here we report two oppositely phased genes, Cry1 and Gys2, displaying rhythmic contacts between promoters and nearby intragenic genomic regions containing enhancer chromatin signatures. We observed the strongest chromatin interactions concomitantly with peaks of Cry1 and Gys2 transcription in the liver of WT animals. These time varying genomic contacts were lost in BMAL1 KO animals. Moreover, 4C-seq experiments performed in kidney of WT animals supported that DNA looping was strongly correlated with the transcription state. Finally, preliminary findings with genome editing using CRISPR-cas9 suggested that the identified distal regulatory regions increased robustness of circadian gene expression. Altogether, our data revealed time varying and clock-dependent chromatin structure implicated in the regulation of circadian gene expression.

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M51

Molecular Description of the Poised CRY:CLOCK:BMAL1 Repressive Complex

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Abstract: The bHLH-PAS transcription factor CLOCK:BMAL1 sits at the core of circadian timekeeping in mammals. As the positive arm of the transcription-translation feedback loop, CLOCK:BMAL1 regulates the expression of at least 40% of the genome on a daily basis to control physiology. Precise control of CLOCK:BMAL1 activity by transcriptional activators and repressors establishes the 24 hour periodicity of peak gene expression. PER and CRY form the negative arm of the transcriptional feedback loop; however, how they act together and separately to regulate CLOCK:BMAL1 activity is still not well understood. Recent studies confirm the presence of at least three distinct circadian regulatory complexes throughout the day: a transcriptionally active state comprising the CLOCK:BMAL1 heterodimer with its coactivator CBP/p300, an early repressive state of CLOCK:BMAL1 with PER:CRY complexes, and a late repressive state marked by a transcriptionally poised but repressed CRY1:CLOCK:BMAL1 complex. Here we provide a molecular description of the CRY1:CLOCK:BMAL1 late repressive complex. We previously showed that the PAS-B domain of CLOCK and transactivation domain (TAD) of BMAL1 are both required for repression by CRY1. We show here how CRY1 binds

to the PAS domain core of the CLOCK:BMAL1 heterodimer and demonstrate that this complex is compatible with binding of the BMAL1 TAD, which sequesters it from the coactivator CBP/p300. Data-driven modeling of the CRY1:CLOCK PAS-B complex unambiguously positions CLOCK PAS-B in a secondary pocket on CRY1 opposite the FAD binding pocket, supported by mutational analysis of both CRY1 and CLOCK. Small angle light scattering (SAXS) analysis of CRY1 bound to the bHLH-PAS-A/PAS-B CLOCK:BMAL1 heterodimer in solution situates CRY1 atop the PAS domains to provide the first low resolution picture of a CRY1:CLOCK:BMAL1 ternary complex. This structural and biochemical analysis of the ternary complex describes how CRY1 can repress CLOCK:BMAL1 independently of PER. Further structural and biochemical investigations of the core clock proteins will aid in our understanding of how remodeling of clock protein complexes each evening sustains circadian rhythms in mammals.

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M52

Regulation of the Mammalian Circadian Clock Transcriptional Output by CLOCK:BMAL1

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Abstract: The mammalian molecular circadian clock relies on the transcription factors CLOCK:BMAL1 to coordinate the rhythmic expression of 10-15% of the transcriptome and promote the daily regulation of biological functions. The recent characterization of genome-wide CLOCK:BMAL1 DNA binding sites revealed surprisingly that the majority of their target genes are not rhythmically expressed. This suggests that CLOCK:BMAL1 rhythmic DNA binding alone does not directly activate transcription and that other factors contribute to transcriptional output. These may include transcription activation hallmarks such as: modifications of chromatin landscape, recruitment of transcription factors and RNA polymerase II (Pol II) to DNA, and regulation of transcription initiation. To tease out the mechanisms that enable CLOCK:BMAL1 to drive rhythmic transcription, we characterized CLOCK:BMAL1 DNA binding sites based on its transcriptional output (target genes that are transcribed rhythmically in-phase, rhythmically out-of-phase, constitutively, and not expressed). Our results indicate that CLOCK and BMAL1 DNA binding features such as strength, phase, and location, are not predictive of the transcriptional output. In addition, CLOCK:BMAL1 rhythmic DNA binding promotes rhythmic nucleosome signal and histone modifications on all target genes, independently of the transcriptional outcome. Recruitment of other DNA-dependent transcription factors at CLOCK:BMAL1 binding sites is also similar for all CLOCK:BMAL1 target genes. Our data thus strongly suggest that CLOCK:BMAL1 rhythmically primes all DNA binding sites for transcription, and that additional mechanisms contribute to transcription activation on only some selected CLOCK:BMAL1 target genes.

Research Funding: Texas A&M Startup Funds

M53

CRY Acts as a Cofactor for the SCF-FBXL3 Mediated Degradation of Novel Substrates

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Abstract: The circadian clock drives internal rhythms, which allow organisms to predict future environmental conditions and food availability. The core clock contains a negative feedback loop in which the transcriptional repressors CRY1 and CRY2 heterodimerize with PER proteins and bind the transcription factors CLOCK and BMAL1 to repress their own transcription. Irregular rhythms have been shown to increase the incidence of obesity, cancer, and diabetes. Mammalian cryptochromes are degraded in the nucleus following ubiquitination by the E3 ligase SCF-FBXL3 and this process is stimulated by AMPK-mediated phosphorylation of CRY1. Currently, cryptochromes are the only published substrates for SCF-FBXL3 ubiquitination; we have found that SCF-FBXL3 promotes the degradation of additional substrates, with cryptochromes acting as cofactors. This establishes a previously unknown mechanism for cryptochrome-mediated regulation of protein stability, and likely contributes to rhythmicity of some cycling proteins that are not associated with rhythmic transcription or translation. We have performed a screen using mass spectrometry to identify additional novel substrates of a complex between CRY1 or CRY2 and SCF-FBXL3. From our screen, we identified several proteins important in cell cycle regulation and progression. We will use this information in combination with additional biochemical approaches to define the biochemical determinants of interactions with CRY1 or CRY2 associated with SCF-FBXL3.

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M54

Epigenetic Regulation of the Drosophila Circadian Clock Involves the Interaction of a SWI/SNF Chromatin-Remodeler with Histone Deacetylases to Repress Transcription.

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Abstract: Temporal changes in levels of key clock mRNA and protein expression over the circadian cycle are critical for the normal progression of the clock. In Drosophila melanogaster, key transcriptional activators CLOCK (CLK) and CYCLE (CYC) drive cyclical gene expression by participating in an auto-inhibitory feedback loop that involves stimulating the expression of the main negative regulators, period (per) and timeless (tim). PER and TIM proteins do not peak until the evening due to the instability of PER without TIM, which is light-sensitive. As PER and TIM proteins accumulate and enter the nucleus, they dimerize and repress the circadian transcriptome, including their own transcription, by binding and inhibiting the activity of CLK and CYC. This repression is relieved upon sunrise due to degradation of PER and TIM, thus initiating another round of CLK-mediated transcription. Overlaid

on this negative feedback interaction, daily rhythms in clock gene expression are also regulated by changes in chromatin organization, RNA polymerase II (RNAPII) recruitment and elongation, and post-transcriptional mechanisms. Although previous studies have shown that clock-controlled genes exhibit rhythmic chromatin modifications, less is known about the functions performed by chromatin remodelers in animal clockwork. We have previously characterized the role of the evolutionarily conserved SWI/SNF BRAHMA (BRM) chromatin-remodeling complex in regulating the circadian clock through catalytic activities to increase nucleosome density, as well as non-catalytic roles to recruit repressive factors to limit transcriptional output during the active phase of circadian transcription. Here we uncover the interaction of histone deacetylases (HDACS) with BRM to remove active histone marks at the per promoter. In addition, we will present our investigation on the roles of PER, TIM, and CLK in modulating the activities and recruitment of BRM.

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M55

Clockwork Orange Enhances per Mediated Circadian Transcriptional Repression by Competing with Clk-Cyc for E-Box Binding

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Abstract: The Drosophila circadian oscillator controls daily rhythms in physiology, metabolism and behavior via transcriptional feedback loops. CLOCK-CYCLE (CLK-CYC) heterodimers initiate feedback loop function by binding E-box elements to activate per and tim transcription. PER-TIM heterodimers then accumulate, bind CLK-CYC to inhibit transcription, and are ultimately degraded to enable the next round of transcription. The timing of transcriptional events in this feedback loop coincide with, and are controlled by, rhythms in CLK-CYC binding to E-boxes. PER rhythmically binds CLK-CYC to initiate transcriptional repression, and subsequently promotes the removal of CLK-CYC from E-boxes. However, little is known about the mechanism by which CLK-CYC is removed from DNA. Previous studies demonstrated that the transcription repressor CLOCKWORK ORANGE (CWO) also contributes to core feedback loop function by repressing per and tim transcription in cultured S2 cells and in flies. Here we show that CWO and CLK bind E-boxes upstream of the tim gene in a reciprocal manner across the circadian cycle, thereby reinforcing repression at times when PER is bound to CLK-CYC. The ability of CWO to compete with CLK-CYC for DNA binding is PER dependent; CWO shows little or no tim E-box binding in per01 mutant flies that are unable to remove CLK-CYC from E-boxes, and CWO shows high levels of tim E-box binding in Clkout mutant flies that lack CLK-CYC binding to E-boxes. These results suggest a model in which CWO co-represses CLK-CYC transcriptional activity in conjunction with PER by competing for E-box binding once CLK-CYC-PER complexes have formed. Given that CWO orthologs DEC1 and DEC2 also target E-boxes bound by CLOCK-BMAL1, a similar mechanism may operate in the mammalian clock.

Research Funding: Texas A&M University

M56

Cry Drives Cyclic CK2-mediated BMAL1 Phosphorylation to Control the Mammalian Circadian Clock

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Abstract: Intracellular circadian clocks, composed of clock genes that act in transcription-translation feedback loops, drive global rhythmic expression of the mammalian transcriptome and allow an organism to anticipate to the momentum of the day. At the heart of this molecular oscillator are the BMAL1–CLOCK transcription factors that drive expression of Cry and Period genes, which in turn encode inhibitors of BMAL1–CLOCK–driven transcription. Phosphorylation and other posttranslational modifications also specify the activity and stability of clock proteins. Here, we unveiled the key mechanism underlying circadian rhythmic phosphorylation of BMAL1 at Ser90 by Casein Kinase-2 alpha (CK2α). Performing live monitoring of protein–protein interactions, we show that CRY proteins facilitate cyclic BMAL1–CK2β binding and subsequent cyclic inactivation of CK2α-mediated BMAL1-S90 phosphorylation; lack of this cyclic event abolishes circadian rhythmicity. We propose a dual negative-feedback model in which CRY proteins not only act as inhibitors of BMAL1–CLOCK transcription but also of BMAL1-S90 phosphorylation as the new role.

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M57

Selective Knockout of Bmal1 in Skeletal Muscle and Not the Brain Regulates Circadian Rhythms of Wheel Running and Sleep

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Abstract: The effects of whole-body Bmal1 knockout on circadian and sleep processes are well characterized. However, whole-body Bmal1 knockout also has pleiotropic effects on inflammatory and metabolic processes that can, in turn, impact rhythms of wheel running and sleep. To this end, we focused our attention on selective knockout of Bmal1 expression in brain and a peripheral tissue that accounts for ~40% of total body mass: skeletal muscle. These experiments utilized an inducible Cre-recombinase mouse driven by a Nestin (brain) or α-skeletal actin (muscle) promoter. In experiment 1, wheel running rhythms were measured across 2 wk of a 12:12 light-dark cycle (LD) and constant darkness (DD). Under LD, there were no differences in nighttime activity onset and duration (alpha) between each flox/cre strain and respective flox/wt mice ($p>0.05$, all). Under DD, flox/cre-muscle mice had a significantly shorter free-running rhythm (23.2+0.1 h) compared to flox/wt mice (23.7+0.2; $p<0.001$; n=8/strain). The free-running rhythm of flox/cre-brain mice was comparable to flox/wt mice (23.8+0.2 h vs. 23.6+0.2 h, respectively; $p>0.05$; n=8/strain). Flox/cre-muscle mice were awake for ~ 1 h less than flox/wts during the dark-phase of LD ($p=0.02$; n=6/strain). Daily amounts

of wake showed no differences between flox/cre-brain versus flox/wt mice but further analyses are required (p=0.05; n=4). To conclude, this study demonstrates robust tissue-specific effects of Bmal1 knockout on circadian processes and modest effects for sleep processes. Most notably, molecular manipulation in the periphery and not the brain produces the most significant changes in rhythms of wheel running and sleep.

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M58

Simulated Light Therapy Enhances Recognition Memory and Alters Daily Rhythms in Hippocampal Gene Expression

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Abstract: Light therapy improves cognitive function in humans, but the neurobiological basis of this effect is not well understood. One obstacle to gaining insight into this process is that nearly all rodent models used to date have employed lighting conditions that cause cognitive deficits rather than improvements. Here we have developed a mouse model where light improves cognitive function, which provides insight into the mechanisms underlying the effects of light therapy. First, we find that recognition memory is enhanced in mice receiving simulated light therapy. In the hippocampus of mice receiving simulated light therapy, we see pronounced suppression of core clock gene transcription, which indicates that the molecular circadian clock of the hippocampus is markedly altered. Moreover, simulated light therapy specifically elevated daily transcription of a growth factor (Insulin-like growth factor II, IGF-II) that is necessary and sufficient for memory consolidation. Up-regulation of IGF-II occurs in tandem with suppression of its transcriptional repressor (Wilm's tumor 1, WT1), which suggests that WT1 is a first order clock-controlled gene important for regulating daily rhythms in learning and memory. These findings provide important insight into how the molecular circadian clock regulates learning by controlling cellular processes required for memory consolidation. Furthermore, this study highlights novel neurobiological and molecular mechanisms underlying the effects of bright light therapy.

Research Funding: Marquette University Research Fellowship

M59

Integrated Multimodal Analysis of Cell- And Circuit-Specific Processes in Circadian Hippocampal Functions

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Abstract: Spatially-defined cellular heterogeneity in the brain is essential in learning, forming memories, and generating behaviors. The ability to analyze complexities that underlie cellular heterogeneities in structure, chemistry, and functional dynamics has been limited. To enable integrated understanding of the spatial, chemical, and temporal nature of the brain, new tools and technologies are being developed under the BRAIN Initiative, including methods to obtain cell-type and chemical information from individual cells and their connections throughout the day. This project creates a chemical information-rich approach that advances the emerging technique of stimulated Raman scattering microscopy (SRSM) and single-cell chemical profiles in the context of circadian function. SRSM provides vibrational spectral data from every location of a living brain slice so that dynamic chemical changes can be followed. Chemical information encoded in the molecular vibrational bands of Raman spectra will be compared to the chemical contents of individual cells analyzed by mass spectrometry (MS). Single-cell MS provides detail on hundreds of components in each cell, effectively mapping each cell's peptidome and metabolome. MS data include unique information on the metabolic state of these cells and allow us to define known and unknown cell types. Computational models will be used to correlate the SRSM data to MS-derived chemical content as well as develop strategies to examine dynamic changes and heterogeneity in brain tissue. These technologies are focused on the neurons and glia of rat dentate gyrus, their involvement in memory formation, and issues related to astrocyte morphology changes. By performing electrophysiological measurements and detailed MS-based metabolomics profiling on cells of the dentate gyrus, the SRSM—single-cell MS technology platform—investigates this complex, functionally remarkable brain region containing many cell types, heterogeneous morphologies, and chemical characteristics. These technologies will provide unmatched detail on the chemical content and dynamics within this defined brain region, answer long intractable questions related to cellular heterogeneity, and relate this information to organization and functional processes such as LTP across the circadian cycle.

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M60

The Circadian Transcription Factor CLOCK Represses the Expression of the Dopamine Rate-Limiting Enzyme Tyrosine Hydroxylase via Recruitment of the Metabolic Sensor SIRT1

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Abstract: Many studies strongly implicate alterations or disruptions to circadian rhythms as contributors to the pathophysiology of mood and addiction disorders. We have shown previously that Clock mutant mice (ClockΔ19) display a behavioral repertoire similar to human bipolar mania with a particular sensitivity to rewarding stimuli. ClockΔ19 displayed enhanced cocaine conditioned place preference (CPP), along with increased dopamine cell firing in the VTA. mRNA and protein levels of tyrosine hydroxylase (TH), the rate-limiting enzyme in dopamine synthesis, was also increased in the VTA of ClockΔ19 mice, suggesting TH is a direct target of CLOCK. We investigated how CLOCK represses TH expression in the VTA, and whether these mechanisms are involved in the hyperhedonic phenotype. We focused on two particular proteins that dynamically interact with CLOCK across the light-dark cycle, phosphoactive CRE-element binding protein (P-CREB) and the histone deacetylase sirtuin 1 (SIRT1), a sensor of intracellular changes in metabolism.

CLOCK typically drives circadian rhythms in gene transcription. However, we found that CLOCK is a transcriptional repressor of TH in the VTA through interactions with P-CREB and SIRT1 at particular diurnal phases. CLOCK and P-CREB bind the TH promoter in antiphase. SIRT1 interacts with CLOCK to inhibit CLOCK-mediated transcription of TH. P-CREB binding and TH expression were constitutively elevated in the VTA of Clock mutants, while SIRT1 protein levels were significantly reduced. Both mCREB and SIRT1-OX in the VTA of Clock mutants reduced TH expression and attenuated cocaine CPP, suggesting CREB-inactivation and restoring SIRT1 levels in mutant mice reversed the hyperhedonic phenotype. Excess NAD and NAM blocked the ability of CLOCK to suppress TH expression. These studies demonstrate a link between metabolic and circadian pathways, and how disruption to these pathways are important for behavioral phenotypes relevant to addiction.

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M61

Per1::Venus Arcuate Neurons Exhibit Robust Rhythms in Excitability

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Abstract: In mammals, the master circadian clock in the brain's suprachiasmatic nuclei (SCN) organises daily rhythms in physiology and behaviour. The intracellular transcriptional-translational feedback loop (TTFL) enables individual SCN neurons to function as cell autonomous clocks components of the TTFL such as Period1 (Per1) and Cryptochrome1/2 (Cry1/2) are also rhythmically expressed in other brain regions, but it is unclear if cells in these extra-SCN sites also show circadian changes in neuronal activity. The arcuate nuclei of the hypothalamus (ARC) are one such region. The ARC play a vital role

in energy balance, integrating metabolic cues of a central and peripheral origin, to control appetitive and ingestive behaviours. To determine whether ARC neurons show daily variation in electrical activity, we used a mouse in which Per1 promoter activity is reported by a yellow fluorescent Venus protein (Per1::Venus animals) and made targeted whole-cell electrophysiological recordings from Per1::Venus +ve neurons in acute adult brain slices. Importantly, these neurons were significantly more active at night, firing APs at ~4Hz, with lower AP discharge during the day (~2.5Hz). Resting membrane potential and input resistance, measures of excitability often associated with changes in spontaneous firing rate, remained unchanged across the day-night cycle. Leptin (10nM) supressed while orexin (100nM) both excited and decreased the electrical activity of Per1::Venus +ve neurons, demonstrating that these cells can sense signals associated with systemic energy level. To establish whether the day-night change in spontaneous AP firing rate is dependent on an intact circadian molecular clock, we made whole-cell recordings from ARC slices prepared from mice lacking Cry1/2 (Cry1-/-Cry2-/-) and found such daily variation to be absent. Collectively, these results suggest that the TTFL in the ARC drives daily changes in the electrical activity. This raises the possibility that local timekeeping processes in the ARC shape daily changes in the neuronal integration of energy status.

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M62

Local Adaptation by Losing Circadian Control of Asexual Development in Neurospora Discreta

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Abstract: The circadian clock has been attributed as a fitness trait in multiple organisms. However, the mechanism of how circadian clock variation influences organismal survival is still not well understood. Here we show that the strength of clock regulation of asexual development is shaped by local habitat and plays a role in organismal fitness. We found that habitat-specific-clock-variation is involved in local adaptation in N. discreta, a species that is adapted to two different habitats, under or above tree bark. African N. discreta strains, whose habitat is above the tree bark, have higher fitness under a light/dark cycling condition relative to a constant ambient light condition. North American strains, whose habitat is under the tree bark, gained fitness in comparison to that of African strains, regardless of light conditions, but lost their clock regulation of asexual development. Our results demonstrate a mechanism by which local adaptation involving circadian regulation influences fitness.

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M63

Regressive Evolution in the Somalian Cavefish Phreatichthys Andruzzii: Loss of Selective Constraint on Circadian Opsin Genes

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Abstract: Phreatichthys andruzzii is a Somalian cavefish that evolved in complete isolation and absence of light beneath the desert for about two million years. Constant darkness caused extreme degenerative phenotypes, such as complete depigmentation, reduced metabolic rate and complete eye degeneration. The circadian clock is also partially degenerated in this cavefish, and mutations in several photoreceptors and clock-related genes play a role in its lack of response to light. However, a detailed description of the molecular mechanisms underlying the regression of this important mechanism is still missing. Here we investigate the molecular evolution of the non-visual photoreceptor melanopsin Opn4m2, whose premature stop codon accounts for the inability of the peripheral clock to respond to light. To test the hypothesis that other light-related mechanisms are undergoing degeneration, we studied the molecular evolution of the visual pigment rhodopsin, expressed in the brain of P. andruzzii and probably involved in its photophobic behavior. The same genes were studied in another blind cavefish, Garra barremiae from Oman, a close relative to P. andruzzii that independently colonized subterranean waters and evolved troglomorphic traits. Our results based on within and between species analyses, show that both genes lost signature of selective constraints in P. andruzzii, but not in G. barreimiae. Our observations indicate that the long and extreme isolation of P. andruzzii in darkness led to a general relaxation of natural selection on light-responsive physiological mechanisms. Based on this change in selective regime, we estimate that the functional constraint on cavefish Opn4m2 was relaxed about 5.2 MYA. This predicts a long subterranean history, about half in complete isolation from the surface.

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M64

Investigating Neural Correlates of Rhythm Deterioration in Seasonal Adaptive Behavior in Aging Mice

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Abstract: Aging is often accompanied by fragmentation or loss of circadian rhythmicity of behavior in mice, which is suggested to be a consequence of reduced synchronization of rhythms in electrical activity in neurons of the suprachiasmatic nucleus (SCN). The SCN is essential for coordinating circadian rhythms in physiology and behavior, and can also respond to day length. Exposure to

long-day photoperiod results in a wider phase distribution of single-cell oscillations in electrical activity and clock-gene expression when compared to short-day photoperiod. It is assumed that this contributes to the adaptation to seasonal changes in the environment. In this study we investigated the effect of aging on the behavioral response to different photoperiods and if this is reflected in gene expression rhythms in the SCN.

To study this, we measured the behavioral and gene expression rhythms in two-year old mice, with a genetically modified clock gene (PERIOD 2; PER2::LUC). Behavior was recorded with passive infrared sensors, and gene expression using bioluminescence imaging. Prior to starting in vitro recordings of PER2 gene expression in the SCN, mice were exposed to either a long (Light:Dark; LD 16h:8h), or short (LD 8h:16h) photoperiod.

We found that old mice are less capable of adjusting their circadian behavior to photoperiodic changes compared to young mice. Peak times of single cell PER2 clock gene expression in aged mice are more dispersed in long photoperiod (n = 4; average number of cells per slice 157) when compared to short photoperiod (n = 4; average number of cells per slice 170). The peak time distribution – represented by the standard deviation of the first peak – was significantly larger in long days (SD = 2.53h) than in short days (SD = 1.35h, $P < 0.005$). These findings do correspond with what we have shown previously for young mice (long day: SD = 2.72h, short days: SD = 1.72h, $P < 0.05$). The data suggest that aging related deficits in adaptation to different photoperiods are downstream from the molecular clock.

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M65

Does the Id2 Null Mouse have a Disturbed Circadian Profile in Core Body Temperature?

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Abstract: Inhibitor of DNA binding 2 (ID2) is a helix-loop-helix transcriptional repressor rhythmically expressed in many adult tissues. Our earlier studies have demonstrated a role for ID2 in the input pathway, core clock function and output pathways of the mouse circadian system. Our previous studies have demonstrated that Id2-/- mice have sex-specific elevated glucose uptake in brown adipose tissue (BAT) suggesting a role in core body temperature regulation. Furthermore, Id2-/- mice express changes in their circadian/diurnal profiles of locomotor and feeding rhythms (Mathew et al, 2013 PLoS ONE 8: e73064). Here we further explored the role of Id2 in the regulation of core body temperature over the diurnal cycle and the impact of Id2 deficiency on genes involved in insulin signaling and adipogenesis in BAT. Inhibitor of DNA binding 2 null and wild type littermate control mice were implanted with temperature transponders, and body temperature measured every 3 hrs under both normal room (21°C) and thermoneutral (30°C) conditions. We discovered a reduced core body temperature in Id2-/- mice over the 24 hr diel cycle, but no distinct alteration in the normal core body temperature rhythm. Body temperature was found reduced in Id2-/- mice under both ambient and thermoneutral temperature conditions. Moreover, in Id2-/- BAT, 30 genes including Irs1, PPARs and PGC-1s were identified as differentially expressed in a sex-specific pattern. These data provide valuable insights into the impact of Id2 deficiency on energy homeostasis of mice in a sex-specific manner.

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M66

Functional Segmentation of the Clock by Lim-Type

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Abstract: We found that Lim1, a LIM-homeodomain transcription factor, is required in Drosophila DN1 neurons to control morning anticipation. Lim1 forms a positive transcriptional feedback loop with Clk, and its levels change in response to changes in light and temperature. We hypothesize that Lim1 is required to adjust the levels of morning anticipation to different environmental conditions. We found that two other Lim-type proteins are expressed in non-overlapping sets of clock neurons, and that all clock neurons can be classified based on which Lim-type protein they express. These proteins antagonize each other's levels by competing for a common cofactor needed for their stability. Under certain environmental conditions, the balance between them can change and this might provide the clock with increased flexibility. Lim1 is highly conserved and its mammalian orthologue LHX1 is expressed in the suprachiasmatic nucleus.

Research Funding: Whitehall Foundation Research grant, Rogulja (PI); Klingenstein-Simons Fellowship Award in the Neurosciences, Rogulja (PI)

M67

Evaluation of Novel Methods to Non-Invasively Monitor Core Body Temperature Rhythms in the Horse

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Abstract: A robust CBT rhythm has been well documented in the horse using rectal thermometer measurements. An interest exists in identifying less invasive, more convenient and more continuous methods for assessing equine CBT. In a pilot study we investigated CBT changes over a 48 h period at 5 external body sites (on cornea, face (2), and tail (2)) using IRT Imaging or remote temperature sensing technology, respectively. For this we used a SHIMMER Wearable Sensor System, (Shimmer Sensing, Dublin, Ireland). Bland Altman bioequivalency testing and simple linear regression was used to compare each external location with rectal CBT. Simple linear regression of skin vs rectal temperature means yielded significant results ($p<0.001$, $F=23.27$ (1, 23)). BT measurements from the hairless underside of the tail head showed the best correlation of all tested sites following a paired t-test ($p<0.0001$, $r=0.71$, $t=9.22$, $df=24$). Subsequently, 4 horses were maintained in individual stalls in a barn environment under ambient lighting and temperature conditions for 25 h. Temperature sensing skin probes were adhered to the hairless underside of the tail head using 'hydrogel' and the SHIMMER Wearable Sensor System fixed to the outer tail head. Readings were collected automatically. Rectal CBT was measured every 2 h by digital thermometer (Cotran Corp. Sharptemp-V). 24-h cosine

fits were computed for both sets of temperature measurements (Action4, Ambulatory Monitoring). Paired t-tests showed no statistical differences for the cosine parameters of acrophase (p=0.884, r=0.5787, t=0.1513, df=7) and mesor (p=0.1872, r=0.007284, t=1.462, df=7) between skin and rectal measurements along with significantly increased variance in skin temperature mesor values. Group and individual time series data suggest that the 24-h patterns of skin temperature under the tail head and rectal CBT share similarities but also show differences. This study identifies a potentially suitable location for non-invasively and conveniently tracking changes in equine CBT. Further investigations over multiple successive 24-h periods will be necessary to better understand this technology and fully validate its use to study changes in circadian phase and waveform of the equine BT rhythm and, more broadly, to detect temporal changes reflective of health and disease states.

Research Funding: This study was funded in part by a SEED grant from UCD to B.A. Murphy and the equipment was funded by Equilume, Ltd.

M68

Meta-Analysis of Transcriptomic Datasets Identifies Genes Enriched in the Circadian Pacemaker

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Abstract: The Suprachiasmatic Nuclei (SCN) are the site of the master circadian pacemaker in mammals, regulating physiology and behaviour as well as coordinating a multitude of clocks throughout the body. Investigating the function of these nuclei has often focused on rhythmic expression, but an alternative strategy to identify mechanisms critical for SCN function is to characterise those genes that are selectively enriched in the SCN.

To achieve this aim we examined the transcriptome of the SCN and whole brain (WB) of mice using publically-deposited data. We carried out a meta-analysis across a range of microarray platforms and incorporating recent RNA-Seq data. A total of 85 microarrays were obtained (24 SCN and 61 WB samples), from four different microarray platforms, alongside 17 RNA-Seq data files (7 SCN and 10 WB). 31224 MGI gene symbols had data for at least 1 platform (using BioMart mouse build 72). The meta-analysis was carried out using a random effects model (REM) for weighting individual effects scores; scores that were themselves derived from the differences in expression between the SCN and WB samples for each platform (log2-transformed fold-changes).

By using a meta-analysis across multiple microarray and RNA-Seq platforms, transcripts relevant to the function of the SCN can be reliably detected, avoiding many of the problems typically associated with transcriptomic studies, such as low power due to small sample sizes. The method used may downplay the importance of rhythmic transcripts, as well as those expressed highly in the SCN, but also in other discrete brain regions. Nevertheless, both SCN-enriched and depleted transcripts give insights into the unique functional characteristics of the SCN, in particular where classes of proteins such as G-protein coupled receptors and GABA-A receptors are known to be vital, the enrichment of different isoforms and subunits can aid further understanding of the SCN and complement the study of rhythmic transcripts.

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M69

Real-Time Ticking of a Biological Clock Assembled in a Test Tube

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Abstract: Life evolved under environments that incessantly oscillate on different time scales: circadian, seasonal, and tidal. The presence of biological clocks that adapt organisms' metabolism, physiology, and behavior to these different cycles attests to the fundamental importance of biological timing. However, no clock mechanism is well understood at the molecular level. Arguably, such ignorance impinges when deciding when to conduct experiments on live organisms. A model system that is uniquely suited for detailed mechanistic studies has been established in cyanobacteria, as its core oscillator is composed of only three proteins, KaiA, KaiB and KaiC, and can be reconstituted in vitro.[1] We have successfully developed a new real-time assay based on fluorescence anisotropy to monitor in vitro oscillations for more than two weeks with time resolution increased by 10x over the standard non-real time gel-based technique. In addition we have achieved oscillations at NMR relevant protein concentrations. Combining these techniques allows the visualization of circadian protein dynamics with respect to time at atomic resolution.

Research Funding: NIH and United States Airforce grants

M70

Hyper-Flexible and Light-Driven Rest/Activity Rhythms Under non-24h Conditions

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Abstract: Life evolved to match rest-activity cycles to environmental rhythms of day and night. Modernization and artificial lighting, however, have transformed the human environment and our behavior. Many people work outside traditional hours with detrimental consequences for behavior, cognition and health. Acute negative effects of shift work have been associated with reduced quality and quantity of sleep.

To adapt to life and work in modern schedules, a highly plastic biological clock would be beneficial. Recently, we discovered that simple manipulations of the light environment enable rodents to adopt markedly more adaptable activity-rest cycles than ever expected. Mice successfully adapted to a bifurcated light:dark:light:dark cycle and light regimes of 18 and 30 hour periods, which could lead to efficient rotating between day- and night-time shifts.

To test if activity rhythms can be mainly light-driven as opposed to oscillator-driven, C57Bl/6J mice adapted to light-cycles well beyond conventional limits of entrainment (e.g. 18h, 30h), were repeatedly exposed to 2 and 4 h phase advances and delays of their respective photocycles. Specifically, mice were entrained to LDim14:10, LDim7:5:7:5, LDim10:5:10:5, or LDim13:5:13:5. After stable entrainment was established, animals were exposed to repeated phase shifts in only one of the two night episodes, while the presence and magnitude of re-entrainment transitions were measured. While expected transitions were observed in LDim14:10, transitions were reduced in LDim7:5:7:5, and

almost completely eliminated in LDim10:5:10:5 and LDim13:5:13:5 (i.e. mice followed the light-cycle immediately). We propose that facilitation of behavioral entrainment to extreme light cycles involves reduction of oscillator strength and is mainly directly driven by light.

Additionally, we investigate sleep quality in mice exposed to our rodent shift work models with a combination of video recording and sleep-EEG/EMG. Total duration of REM and nonREM sleep as well as overall sleep architecture will be compared across different lighting conditions.

Research Funding: ONR grant N00014-13-1-0285

M71

The Evolution of Neural Circuitry Regulating Sleep and Arousal in the Blind Mexican Cavefish

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Abstract: Sleep is an essential behavior exhibited by nearly all animals, and disruption of this process is associated with an array of physiological and behavioral deficits. Independent populations of the blind Mexican cavefish Astyanax mexicanus have converged on sleep loss as a consequence of life in the subterranean environment, providing a system to examine the effect of evolutionary history and environmental perturbation on sleep-related processes. Our findings suggest the wake-promoting neuropeptide Hypocretin (Hcrt)/Orexin underlies sleep loss in cavefish. Expression of hcrt is increased in cavefish compared to surface fish and pharmacological inhibition of HCRT signaling restores sleep duration in cavefish to levels equivalent to the ancestral surface fish. We are currently investigating the evolutionarily derived changes in HCRT circuitry between cave and surface-dwelling fish. In addition, we have employed tol2 transgenesis to generate A. mexicanus expressing the calcium indicator GCaMP5.0 in peptidergic neurons. Subsequent comparisons of calcium imaging during sleep and wake states in whole brain or HCRT neurons will provide insight into the neural processes governing sleep loss in cavefish.

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M72

Regulation of Mitochondrial Dynamics by the Circadian Deadenylase Nocturnin

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Abstract: Nocturnin is a circadian deadenylase belonging to the endonuclease/exonuclease/phosphatase superfamily, traditionally characterized as a circadian posttranscriptional regulator that acts mainly in the cytoplasm. However, analysis of its mRNA sequence reveals that Nocturnin has two translation initiation sites, both with degenerate Kozak consensus sequences. When alternative translation initiation sites are used, one form of Nocturnin would contain a putative mitochondrial-targeting signal (MTS), and another would not, and therefore be cytoplasmic. Publicly available

ribosomal profiling data show that there is an enrichment of ribosomes at both initiation sites when cells were treated with puromycin. We confirmed the presence of Nocturnin inside the mitochondria in mouse liver, performing a mitochondrial fractionation assay followed by western blotting. To confirm the different localization pattern of Nocturnin when alternative translation initiation sites are used we performed immunofluorescence imaging on HEK-293 cells. We transfected Noc-/- HEK-293 cells with either wild type or mutant Nocturnin constructs, in which either the first or second ATG was mutated to force translation initiation at a particular start site. Results revealed different localization patterns for the two forms. The form that contains an MTS clearly co-localizes with the mitochondria, while the form that does not contain an MTS is cytoplasmic. To begin exploring Nocturnin's role in mitochondrial function we stained and visualized mitochondria via confocal microscopy in cells lacking Nocturnin. Immortalized MEF cells derived from either WT mice or mice lacking Nocturnin (Noc-/-) showed distinct morphological differences pointing towards altered mitochondrial dynamics (fission vs. fusion). Mitochondria from Noc-/- MEFs exhibit a more fused state and this was confirmed in an independent HEK-293 cell line lacking Nocturnin. Mitochondria undergo dynamic morphological changes in response to a variety of conditions (e.g. cellular stress, nutrient variability) and it appears that Nocturnin may play a critical role in this process. Further study into Nocturnin's role in the mitochondria will provide a novel insight into the circadian regulation of mitochondrial function.

Research Funding: NIGMS

M73

Aging and the Gastrointestinal Clock: Influence of Melatonin on the Gut Microbiome

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Abstract: The process of aging affects several aspects of physiology including the circadian clock. One area where data are lacking is of gastrointestinal function. Previously, our lab has shown that the gut expresses its own clock that can be reset by sympathetic signaling as well as restricted feeding. Here we present transcriptional profiling data of colon tissue across time of day from young (~6 months old), middle-aged (~1 year old), and elderly (~2 year old) CBA/J mice. In addition to clock genes, we assayed several genes involved in inflammation, gastrointestinal motility, and melatonin biosynthesis. Our data show that circadian rhythms in clock gene as well as other transcripts is largely maintained across all ages. However, circadian expression of the melatonin biosynthesis genes mAanat and mHiomt changes throughout the aging process. To determine if these differences affected gut microbiome levels, qPCR against 16s rRNA from fecal samples was used to quantify microbial abundance across time of day. A parallel study comparing microbe abundance in C57BL/6 vs. C3H mice also shows a significant difference across time of day. These data show an effect of age on circadian rhythms on bacterial abundance and diversity and provide a correlative relationship of melatonin levels in the gut and its resident microbiome. Currently, we are investigating the effect of melatonin supplementation on the gut microbiome with the hypothesis that entrainment to melatonin will correspond to a reinstatement of microbial abundance/diversity rhythms. This study is funded by NIH RO1 AG045833-01.

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M74

Isolated Retina Müller Cells Exhibit Sustained Circadian Rhythms in Culture

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Abstract: The mammalian retina contains an endogenous circadian clock that regulates retinal physiology and function. It is a highly organized tissue composed of three nuclear layers: the outer nuclear layer (ONL) composed of rod and cone photoreceptors, the inner nuclear layer (INL) composed of bipolar, horizontal and amacrine cells and the ganglion cell layer (GCL) composed of ganglion cells and displaced amacrine cells. Moreover, across the thickness of the retina are located Müller cells, the main glial cells of the retina. How cells are organized within the mammalian retinal clock is still unclear. We previously provided strong evidence that the retina clock consists of multiple autonomous oscillators located within each cellular layer, indicating that rhythmic properties in mammalian retinas proceed from multiple, interacting oscillators, with a major contribution from the ganglion cell and inner nuclear layers. Here we focus on the clock at cellular level, to better characterize the contribution of specific neuronal populations to the retina clock. We started investigating clock properties in Müller cells, using purified primary cultures from the mPer2Luciferase mouse. We, first, assessed the expression of circadian clock proteins such as Per2 and Bmal1 by immunofluorescence. Then, to characterize the endogenous oscillatory capacity in these cells, we used real-time recorded bioluminescence. We also confirmed the rhythmic expression of clock genes by quantitative PCR. Thus, we demonstrate that primary Müller cells exhibit robust free-running rhythms in vitro.

Research Funding: The University of Strasbourg Institute for Advanced Study (USIAS)

M75

Effects of Chronic Alcohol + Binge on Liver Rhythms and Bile Acid Metabolism in Mice

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Abstract: Introduction: Dysregulation of circadian rhythms (e.g., disruptive sleeping patterns, shift work, etc.) negatively impacts human health and is associated with increased incidence of inflammation and symptoms of metabolic syndrome, including T2 diabetes and obesity. Alcohol consumption disturbs circadian rhythms of bile acid and lipid synthesis and may contribute to the development of fatty liver and dyslipidemia, which are hallmark symptoms of both alcoholic liver disease and metabolic syndrome. Here, we examined the effects of alcohol on rhythms in gene expression and bile acid and lipid metabolism in the liver. Methods: Wild type male C57BL/6J mice were isocalorically pair-fed liquid Lieber-DeCarli control or ethanol diet for ten days and were given either maltose (control) or ethanol by gavage (representing a binge drinking episode) on day 11 at ZT 2. Mice were sacrificed at 6 time intervals over 24 hr to obtain lipid, bile acid and gene expression profiles. Results: ALT and AST increased, particularly at ZT 10 and 14. Circadian rhythms of liver clock genes were suppressed, including Clock, Bmal1, Per1 and Per2 and expression of the clock-controlled bile acid regulator Dbp was suppressed. Ethanol diet-feeding shifted triglyceride metabolism

towards increased storage in the liver. Ethanol-fed mice also showed an overall decrease in bile acid production. Conclusion: Chronic ethanol + binge suppressed clock gene expression in the liver and may drive a shift toward increased triglyceride storage and decreased free fatty acid content in the liver. Disruption of peripheral rhythms by alcohol may contribute to the metabolic syndrome-like symptoms of alcoholic liver disease.

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M76

The Role of Melatonin in the Photoperiodic Control of Bird Song Distribution and Repertoire in the House sparrow, Passer Domesticus

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Abstract: Melatonin's 'night-only' expression profile makes it an intellectually appealing molecule for transmitting calendar information to organisms. This is the case in seasonally breeding mammals, as melatonin receptors in the pars tuberalis allow seasonal dynamics to primary gonadal function. However, melatonin has only been demonstrated to affect vocalizations in seasonally breeding birds. In house sparrows, long melatonin durations simulating winter nights are sufficient for preventing the expansion of song control nuclei, the parts of the brain involved with song learning in juveniles (Cassone et al, 2008) Additionally, our lab has shown removal of the pineal gland affects the frequency and diversity of songs and calls, and the timing thereof, with a stimulating increase in photoperiod (Wang et al, 2014). We aimed to determine if short melatonin duration, simulating spring, is sufficient for causing the documented, properly timed, increases in vocalization frequency and complexity. Twenty four male house sparrows were placed in winter-like photoperiods (10:14 light-dark, LD) and were pinealectomized; then, subsets were subjected to daily long-duration and short-duration melatonin, mimicking winter and spring nights, respectively, or vehicle. While continuing this melatonin regimen, the photoperiod was expanded to equinoctial conditions (12:12 LD). The birds' locomotor activity was constantly monitored, and vocalizations were sampled weekly for the duration (10 weeks) of the experiment. Birds were sacrificed, and brains were collected for immunocytochemistry to look at the size of the song control nuclei and expression of vocal learning-related genes.

Analyzing the types of vocalizations produced are in progress, predicting that long duration melatonin prevents the seasonal expansion of vocal repertoire and complexity. Upon photostimulation, the birds receiving shot-duration melatonin and vehicle developed a tri-modal circadian distribution of song production. Birds receiving long duration melatonin failed to develop this distribution, singing less in the morning and lacking a singing bout at lights off. A long duration of melatonin is sufficient for preventing a spring-like circadian distribution of vocal occurrences.

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M77

Urokinase Plasminogen Activator (uPA) Regulates Phase Resetting of the Mammalian Circadian Clock

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Abstract: The primary mammalian circadian clock located in suprachiasmatic nucleus (SCN) responds differentially to stimuli depending on the time of day a stimulus is presented, a form of metaplasticity. Glutamate modulates light-induced phase shifts in the SCN in part through activating NMDA receptors (NMDAR). These phase shifts require concurrent activation of TrkB receptors by brain-derived neurotrophic factor (BDNF). Tissue-type plasminogen activator (tPA) contributes to differential responsivity in the SCN by activating plasmin to generate m(ature)BDNF, which binds TrkB receptors allowing clock phase shifts. Here we continue our investigation of tPA using tPA knockout (tPA-/-; B6.129S2-Plattm1Mlg/J) mice, and we identify urokinase plasminogen activator (uPA) as an additional regulator of the circadian clock. tPA-/- mice behavioral activity rhythms entrain to an LD cycle and phase shift in response to a light pulse at ZT16 or ZT22 (Zeitgeber Time: ZT0=lights-on, ZT12=lights-off) with no apparent loss in sensitivity. When the light cycle is inverted tPA-/- mice take significantly longer to re-entrain than C57BL/6 wild-type (WT) mice. The extent to which this involves decreased masking remains to be determined. Similarly, SCN brain slices from tPA-/- mice exhibit entrained neuronal activity rhythms, and glutamate treatment at ZT16 and ZT23 induces phase delays and advances, respectively with no change in dose-dependency. The lack of severe deficiencies in clock phase regulation in tPA-/- mice could reflect redundant mBDNF-generating pathways. Pre-treating slices with the tPA/uPA inhibitor, plasminogen activator inhibitor-1 (PAI-1), inhibits glutamate-induced phase resetting in tPA-/- slices, consistent with what is seen in WT slices. PAI-1 alone has no effect on clock phase. Selective inhibition of uPA with UK122 prevents glutamate-induced phase resetting in tPA-/- SCN slices but not in WT SCN slices. Western blotting shows both tPA and uPA expression in the SCN and casein-plasminogen zymography indicates time-in-vitro dependent changes in uPA activity. Collectively, these results support redundant and/or compensatory mechanisms allowing phase shifts in the SCN of tPA-/- mice and indicate that uPA contributes to the processes allowing phase resetting in the tPA-/- SCN.

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M78

Polymorphisms in the Human Clock Gene Period3 Are Associated with Diurnal Preference, Subjective Sleepiness and the Response to Morning Light

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Abstract: A length polymorphism in the human clock gene Period3 (hPer3), consisting of either 4 or 5 copies of a tandem repeat (54-bp), has been associated with diurnal preference, with the longer allele Per5 linked with morningness (Archer et al., 2003). In addition, Per5/5 subjects seem particularly

sensitive to light at night (Chellappa et al., 2012). Very recently, a SNP (C2590G, rs228697) in hPer3 that causes the amino acid substitution P864A has also been associated with morningness-eveningness (Hida et al., 2014). The aim of our study was to confirm the above associations in a large sample of healthy subjects, and to test the effect of morning light administration in relation to genotype. 1070 healthy subjects (708 females) donated buccal DNA samples and completed the Horne-Östberg diurnal preference questionnaire. A subgroup of 388 (256 females) also completed 12-day sleep logs and hourly assessments of subjective sleepiness (KSS) over one waking day. Finally, 11 Per5/5 and 13 Per4/4 homozygotes performed timed urine collections for 6-sulphatoymelatonin (aMT6s) measurement before/after two weeks of morning light treatment (10,000 lux for 45 min, 10-15 min after wake up).

829 subjects (530 females) out of 1070 were genotyped for the variable number tandem repeat (VNTR). We confirmed the trend of Per5/5 being linked to morningness previously reported; moreover Per5/5 subjects were significantly less sleepy in the morning hours compared to Per4/4 and Per4/5. After light administration, 14 subjects (11 Per4/4, 3 Per5/5) showed an advance in their aMT6s peak time, 4 a delay (all Per5/5) and 6 no significant change (2 Per4/4, 4 Per5/5). Regardless of direction, the change was more pronounced in the Per5/5 (43±8 vs. 35±14 min).

A subgroup of 194 subjects (120 females), chosen among the morning and evening types, was also genotyped for the P864A polymorphism, revealing a significant association between diurnal preference and genotype.

Taken together, our results confirm that genetic variations in hPer3 account for individual differences in human diurnal preference.

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M79

Binge Eating Behavior for Sucrose is Time-of-Day Dependent: Effects on Reward Brian Areas

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Abstract: Binge eating (BE) is characterized by the consumption of big amounts of food within a discrete interval, surpassing the expected amount for the same interval and in similar circumstances. In rodent models of binge eating highly palatable foods, as well as restricted food access promote compulsive ingestive responses and result in sustained dopamine stimulation and activation of the glutamatergic system. In this study we investigated the role of the circadian system in BE and how BE can impair the normal rhythm of neurotransmitters in the reward system. We explored the effect of sucrose intake during the rest phase vs. the active phase on BE, the influence of restricted food access on the development of BE and its effects on circadian rhythms of general activity and body core temperature. The temporal expression of dopamine D1 receptor (DR1), GluR1 subunit of AMPA receptor were assessed in the Nucleus Accumbens and TH in the VTA. Fifty six adult Wistar rats were assigned to Control conditions, Restricted access to sucrose during the day (ZT5; SUC-D) or night

(ZT17; SUC-N), restricted food access to day (RF-D) or night (RF-N) or the combination of restricted food and sucrose access in the day or night. The experimental protocol had a duration of 5 weeks with 6 days access to sucrose per week. RESULTS: BE was 250% stronger in the SUC-N vs SUC-D and was not dependent of RF. RF induced overconsumption of regular chow food, an increase in locomotor activity and temperature in anticipation and response to chow access. Food restriction combined with access to sucrose increase the anticipatory response observed in general activity and core temperature. The expression of DR1 in nucleus Accumbens was increased in the groups who had access to sucrose at night but not in the day. GluR1 was not modified between groups in the night vs. light phase. Overall our results show that BE is influenced by the time of the day, with a higher response in the night; RF can potentiate anticipatory locomotor activity and body core temperature when it is combined with sucrose access. This may depend on a circadian response of D1 receptors. Present findings will provide a better insight of factors eliciting BE and other compulsive disorders.

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M80

Removing the Brakes on Photic Entrainment in the Circadian System

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Abstract: The serotonergic (5-HT) agonists/antagonists, NAN-190 and BMY7378, potentiate photic phase shifts by about three fold. Recent studies from our lab suggest that the mechanism of action is to prolong light induced gene expression. This is consistent with the observation that these same drugs can still potentiate photic phase shifts even when given many hours after the end of the light pulse. Together, these findings suggest that these 5-HT agonists/antagonists do not alter the initial response to light, but rather later downstream events. Light-induced expression of Period1 and Period2 in the suprachiasmatic nucleus (SCN) is regulated by CREB. In other CREB-related gene expression systems, gene expression is terminated by CREB2/ATF4. It is possible that the potentiation observed with these 5-HT agonist/antagonists is due to disruption of a negative limb of the light-induced gene expression response. Here we examine the involvement of CREB2/ATF4 in circadian responses to light. First light-induced expression of CREB2/ATF4 after a phase shifting light pulse is examined to test the hypothesis that this pathway is activated by light. Next, we test the hypothesis that inhibiting expression of CREB2/ATF4 will enhance phase shifts to light. This will be examined through a behavioural analysis of locomotive wheel running activity. Small interfering RNA targeting CREB2/ATF4 (CREB2-siRNA) will be injected intracranially to the SCN or Syrian Hamsters 24hrs prior to a phase advancing light pulse to down regulate CREB2/ATF4 expression. Lastly, CREB2-siRNA will be injected into the SCN to confirm that regular light-induced CREB2 expression is truly being down regulated by the CREB2-siRNA. Preliminary results suggest that intraSCN injection of CREB2-siRNA almost significantly potentiates photic phase advances. Light induced expression studies are ongoing.

Research Funding: This research is currently being funded by NSERC.

M81

JmjC Domain Protein JMJD5: A Repressor of the Mammalian Circadian Clock and a Potential Mediator of Circadian Control of Energy Metabolism

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Abstract: Recently, a few members of the JumonjiC (JmjC)-domain-containing family have been reported to be a part of the circadian machinery. Our work is focused on understanding one such protein Jmjd5 and its role in linking the circadian clock to the genomic control of lipid and cholesterol metabolism. The precise role of Jmjd5 in the mammalian circadian oscillator is largely unknown. In this study we show that Jmjd5 acts as a repressor of the mammalian circadian clock and suppresses CLOCK and Bmal1 mediated activation of canonical circadian genes Per1 and Per2. JMJD5 is also shown to localize at critical regulatory regions of the Per2 gene promoter in a circadian manner and in addition associates with core clock components, especially with Cryptochrome1 (CRY1). JMJD5 affects the stability of CRY1 protein and regulates its degradation. Furthermore, to understand the transcriptomic networks regulated by Jmjd5 in vivo we performed microarray analysis from livers of wild type and JMJD5 Liver specific knockout (LKO) mice collected from two anti-phasic time points. Apart from altered mRNA levels of circadian genes, we observed reduced mRNA levels of critical regulators of bile acid and cholesterol synthesis in the knockouts compared to wild type. Hepatic bile acid levels in the knockouts were about four fold lower than that in the wild type mice. Jmjd5 also localizes at the promoter regions of some important regulators of lipid homeostasis. Taken together, our data enables us to hypothesize that JMJD5 has a potential role in connecting the circadian clock and lipid homeostasis.

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M82

Beyond Drosophila: Analysis of Cycling Genes in the Jewel Wasp Nasonia, an Emerging Model Organism

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Abstract: Circadian clock research in insects has traditionally focused on using Drosophila as a model for the animal clock. Research into other insects has revealed potential clock models with a deeper similarity to mammalian clocks. Nasonia vitripennis is one such emerging model organism. We here take a key step towards advancing Nasonia as a circadian model organism by using whole transcriptome RNA-seq to characterise clock-controlled genes (CCGs) in both constant darkness (DD) and constant light (LL, unlike Drosophila, wasps are rhythmic in LL with a shortened period). Adult male wasps were entrained for 4 days in LD 12:12, then collected during 2 days of constant conditions, in either LL or DD. Samples were collected every four hours over a period of 48 hours (12 samples), each sample consisting of 50 pooled wasp heads. To capture the complete range of expression patterns, we employed fuzzy c-means clustering alongside traditional algorithms such

as RAIN and JTK cycle. Cycling transcripts showed low-amplitude oscillations and a bimodal phase distribution. Clusters were assessed for rhythmicity, identifying 7 rhythmic clusters in DD, and 5 in LL. Overrepresented functions associated with these expression clusters include genes involved in catalytic activity (q < 9e-08), signalling (q < 8e-15), response to light (q < 3e-03), and a variety of regulatory and neural functions. Genes involved in catabolic activity show peak expression in the morning whilst genes involved in anabolic activity peak at dusk, demonstrating temporal separation of metabolic processes, a conserved feature of circadian clocks. The set of cycling genes includes genes known to be involved in circadian/locomotory processes (e.g. period, slowpoke, hyperkinetic, Shaker) and light response (e.g. all four Nasonia opsins). Transcriptional differences between DD and LL are analysed, identifying significant up-regulation of catabolic genes in DD, and of light-responsive genes in LL. Comparisons are drawn between Nasonia and Drosophila, identifying similarities and differences that underscore the importance of multiple model organisms for clock research.

Research Funding: Midlands Integrative Bioscience Training Programme funded by the Biotechnology and Biological Sciences Research Council (BBSRC).

M83

Zebrafish Liver Diurnal Gene Expression and Comparative Transcriptomics

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Abstract: From photosynthetic bacteria to mammals, the circadian clock evolved to track diurnal rhythms and enable organisms to anticipate daily recurring changes such as temperature and light. It orchestrates a broad spectrum of physiology such as the sleep/waking and eating/fasting cycles. While we have made tremendous advances in our understanding of the molecular details of the circadian clock mechanism and how it is synchronized, we still have rudimentary knowledge of its connections to diurnal physiology. One reason for this lack of understanding is the sheer size of the output network. Transcriptomic studies have identified more than 2000 clock-controlled genes (CCGs) with rhythmic expression patterns. Toward exploring this network in vertebrates, we selected Danio rerio as model system. As an initial step, by combining liver tissue sampling in a 2-days time series, transcription profiling using oligonucleotide arrays and bioinformatics analysis, we profiled rhythmic genes and identified several thousands rhythmic genes including ~200 clock-controlled transcription factors (CCTFs). Comparative transcriptomics between Zebrafish, mice and human datasets revealed interesting features of the output network. The results from a global analysis of the union and intersection between the datasets suggest that a large portion of the CCTFs may be involved in circadian gating. Undoubtedly, the Zebrafish model system will help identify new vertebrate outputs and their regulators and provide leads for further characterization of the cis-regulatory network.

Research Funding: Departmental funds

M84

An Assay to Characterize the Dampening Tendency of the Photo-Periodic Oscillator

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Abstract: Insects inhabiting the temperate zones measure seasonal changes in day or night-length (also called photo-periodic time measurement (PTM)) to enter the overwintering diapause. The diapause induction in many insects occurs only after the duration of night exceeds a particular length termed as critical night length (CNL). Our understanding of the time measurement mechanisms (clocks) has been continuously evolving since Bünning's proposal that circadian systems play the clock role in PTM. In early days, the photo-periodic clocks were believed to be either based on circadian oscillators or simple hour-glasses depending on the 'positive' or 'negative' responses respectively, to Nanda-Hamner and Bunsow experiments. The modelling efforts however showed that a mechanism based on damped circadian oscillator may explain the two purportedly distinct clock types. Although the low and high degree of oscillator dampening successfully simulated 'positive' and 'negative' responses respectively; the model still awaits experimental validation. Here we propose an experimental assay to characterize the dampening tendencies of the photo-periodic oscillators by studying the effect of entrainment to non-24 light-dark cycles (T-cycle) on the CNL. We predict change in the CNL as function of the T-cycle period in sustained oscillator based clocks and a fixed night-length measurement (no change in CNL) in damped oscillator based clocks. We tested our assay in diapausing northern Drosophila species D.ezoana. The D.ezoana showed CNL of about 7 hours irrespective of T-cycle period suggesting the damped oscillator based photo-periodic clock. However, the Nanda-Hamner response was found to be neither positive nor negative, contrary to the expectations from damped oscillator model of photo-periodic clock indicating the inadequacy of the model to accommodate the findings in D. ezoana. Our observations in D. ezoana therefore recommend the similar analysis in Nanda-Hamner 'positive' and 'negative' species to test the predictions of the damped oscillator model.

Research Funding: German Research Foundation's Collaborative Research Center (SFB) "Insect timing: mechanisms, plasticity and interactions (SFB 1047)"

M85

Quantitative Analysis of mRNA-Protein Flux in Circadian Rhythms by Ribosomal Profiling and Mass Spectrometry

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Abstract: Life is remarkably adapted to the 24-hour rotational movement of the earth. In mammals, the molecular time-keeping mechanism for circadian rhythms relies primarily on transcription activators and repressors. While much work has been devoted to understanding the systems regulation of the transcriptional network, less is known how the process of translation itself influences biological rhythms.Translation efficiency has been shown to affect circadian rhythms in bacteria and neurospora,

but it is unclear if similar mechanisms apply to mammals. We are taking a systems approach using next-generation sequencing of ribosome-protected mRNA fragments and mass spectrometry to understand the timing of translation of a circadian mRNA into protein. We isolated ribosomes from mouse liver at different circadian times, generated cDNA libraries from ribosomal-bound mRNA, and use high-throughput sequencing to characterize rhythmically-translated transcripts. For 16 circadian mRNAs, we also measured mRNA expression by qPCR and total protein abundance by mass spectrometry (MS) in the same liver samples to understand translation timing. To quantify absolute amount of protein, we developed a novel method termed MS-based quantification by isotope-labeled cell-free products (MS-QBiC) that uses high-throughput synthesis of internal standards from a reconstituted cell-free protein synthesis system. Our results suggest that ribosomal profiling can be used as a quantitative measure of absolute protein abundance. Our work ultimately aims to understand how modulation of mRNA translation might affect the clock, which will be important for reducing the symptoms of jet-lag and in understanding and treating circadian-related diseases such as cancer and depression.

Research Funding: RIKEN Foreign Postdoctoral Fellowship

M86

Long Term High-Fat Diet Consumption and Wheel-Running Access Produces Alterations in Circadian Locomotor Activity

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Abstract: Consumption of high-fat diets can lead to alterations to a variety of different behaviors, in addition to producing obesity and other health problems. Alterations to locomotor activity, even under entrained 24 h LD cycles, can produce alterations to feeding behaviors and energy balance, which can exacerbate the negative health consequences of a poor diet. This study investigated the effects of running wheel access and high-fat diet consumption and its subsequent removal on circadian locomotor activity patterns in C57BL/6J mice. Mice were placed into either running-wheel cages or cages without a running-wheel, and initially, mice were given access to either regular chow or 60%-fat diets and measurements of their active bouts and their average activity, light activity, and dark activity per 10 min bin was calculated. After five weeks on the high-fat diet, half of the mice given 60%-fat diets had their diets replaced with regular chow, and after another five weeks, their circadian locomotor activity parameters were measured again. Regardless of diet, animals with wheel-running access produced increased levels of locomotor activity and improved light to dark activity ratios compared to mice without a wheel. Prior to the switching of the high-fat diet to regular chow, all high-fat diet mice exhibited similar levels of circadian locomotor activity to each other and exhibited similar activity levels to mice consuming regular chow. As time progressed, animals continuously consuming high-fat diets showed decreasing levels of circadian activity, but mice with high-fat diet removed did not show decreasing levels of activity. Additionally, mice continuously fed high-fat diet exhibited reduced dark activity (but not light activity), bout length and counts per bout compared to both regular chow controls and mice with high-fat diet removed. Wheel-running mice exposed to high-fat diet did not exhibit reductions in wheel-running behaviors compared to controls or mice with high-fat diet removed. In summary, the mice with high-fat diet replaced with regular chow exhibited similar locomotor activity behaviors compared to regular chow controls. These results also

suggest that voluntary wheel-running can improve locomotor activity behaviors regardless of diet consumed, which can lead to improvements in overall health.

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M87

A Model-Based Analysis of Light-Induced Circadian Arrhythmia in the Siberian Hamster

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Abstract: In the Siberian hamster, light pulses delivered at specific times during the biological night induce permanent circadian arrhythmia. This phenomenon is peculiar, since in most other species a light pulse delivered during the biological night causes a temporary reduction in circadian amplitude but does not affect long-term rhythmicity. The physiological mechanism underlying light-induced arrhythmia is unknown. Here, we used mathematical models of the circadian clock to investigate candidate mechanisms. First, we considered the hypothesis that light-induced arrhythmia may be due to arriving at the phase singularity (i.e., zero amplitude rhythms). In other species, including humans, the phase singularity is an unstable fixed point, meaning the system spontaneously recovers its amplitude. If, however, the phase singularity were a stable fixed point, the system could be captured there, resulting in long-term arrhythmia. We tested this hypothesis using a limit-cycle model of the circadian pacemaker that included a stable fixed point at the phase singularity. This model was tested against a variety of light schedules known to either induce circadian arrhythmia or to cause a phase shift with normal rhythmicity thereafter. The model was not able to reproduce all observed patterns, suggesting light-induced arrhythmia cannot be explained by the dynamics of a single oscillator. We therefore also investigated the dynamics of a set of four coupled oscillators, representing left/right, ventral/dorsal SCN. We included differential effects of light on these oscillators based on known physiology. Previously it has been shown that activity splitting in Siberian hamsters occurs due to left and right SCN oscillating in antiphase. Using the four-oscillator model, we tested the hypothesis that light-induced arrhythmia is due to a change in normal phase relationships between all four oscillators. Preliminary results show that light patterns that induce circadian arrhythmia can disrupt normal phase relationships in this system.

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M88

CalfluxVTN, A New, Bright Bioluminescent Ca2+ Sensor that Can be Coupled with Excitatory, Optogenetic Stimulation

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Abstract: Calcium dynamics have been well-studied in various circadian-relevant brain regions using fluorescent dyes and GFP variants. However, light sensitive tissues (like the retina) and tissues that readily absorb light present obstacles for fluorescent probes. The incident, fluorescence, excitation light could either stimulate the process while it is being observed (in the light-induced cells) or obscure cells because of high background autofluorescence. Additionally, being able to both optically excite and inhibit subsets of neuronal cells (via channel- and halorhodopsins), while simultaneously recording Ca2+ transients, would expand our experimental repertoire. But unfortunately, significant spectral overlap among the stimulus and observational probes have hampered our ability to make these kinds of measurements. Bioluminescence can circumvent these issues because the light generated comes from an enzymatic process, internal to the system, instead of an external, excitation light source. By taking advantage of bioluminescence resonance energy transfer (BRET) between Nanoluc luciferase and Venus fluorescent protein, we developed the brightest-to-date, ratiometric, bioluminescent, genetically encoded Ca2+ indicator, named CalfluxVTN. In cell culture, it can report cytosolic Ca2+ fluxes in conjunction with optical stimulation of Melanopsin (Opn4) and CheRiff (a ChR2 variant).

Research Funding: NIDA 5R21DA034446-02 entitled: Coupling Optogenetic Neural Stimulation with Novel Reporters of Synaptic Activity

M89

Exploring Physiological Changes Underlying Protection from Severe Sleep Restriction in Migrating Birds

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Abstract: Small passerine birds are typically diurnally active, however, during migratory periods they have evolved to migrate at night. Nighttime flights are thought to be advantageous due to reduced metabolic costs and lessened risk of predation. Despite the high-level of nocturnal activity, birds still remain active during the day to feed. This results in severe sleep restriction or even complete sleep deprivation during the days-to-weeks long periods of migration. Interestingly, during this time birds do not show reductions in cognitive ability or metabolic performance. To date, few studies have examined molecular changes that occur during this period and so very little is known about how this protection from sleep restriction arises. In attempt to uncover the molecular underpinnings regulating changes in physiology, we captured white-throated sparrows and housed them in 12.5:11.5 L:D conditions. Once animals had habituated to the environment, we monitored behavior for the presence of zugenruhe (nocturnal migratory restlessness) in order to classify migratory status. We collected tissue at ZT 6 and 18 from migratory and non-migratory animals - time points

corresponding to mid-day when both groups would be awake and feeding, and the middle of the night when non-migratory birds would be sleeping while migratory birds are awake and flying. Total RNA was extracted and next-gen sequencing was run on an Illumina HiSeq platform. Because white-throated sparrows are a non-model organism, we first performed a de novo assembly by pooling one individual from each experimental condition. Each sample had approximately 40 million raw, 150nt, paired end reads. Following trimming for quality and adapter sequences, assembly was completed with Trinity, resulting in 921,784 identified transcripts, covering 74% of the vertebrate orthologs as measured by BUSCO. We filtered this assembly using TransRate to give a final number of 200,992 transcripts and then annotated using dammit. A further 5 samples per group were subjected to 100nt single end reads to assess differentially expressed genes using the de novo assembly. This technique allows for a hypothesis-free exploration of all genes that may be changing in transcription during migration and allows for identification of novel genes that underlie protection from sleep restriction.

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M90

Ultradian Feeding in Mice not only Affects the Peripheral Clock in the Liver, but also the Master Clock in the Brain

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Abstract: Background: Restricted feeding during the resting period causes pronounced shifts in a number of peripheral clocks, but not the central clock. On the other hand, timed hypocaloric feeding does impact the master clock in the suprachiasmatic nucleus (SCN), as indicated by shifted oscillations of clock (PER1) and clock-controlled (vasopressin) proteins.

Objectives: To determine if these changes in the SCN are due to the metabolic- or time-cues associated with caloric restriction.

Methods and Results: Male C57BL/6J mice were challenged with an ultradian 6-meals-a-day schedule (1 food access every 4 h) and control animals with food ad libitum. Mice on the ultradian feeding schedule that lost >10% body mass (i.e., hypocaloric) became diurnal, hypothermic in late night, and displayed a 3.5-h advance of their body temperature rhythm, plus also up-regulated vasopressin and down-regulated PER1 and PER2 expression in the SCN. Glucose rhythmicity was profoundly affected by ultradian feeding, but by contrast, daily variations in liver glycogen and plasma corticosterone showed the same phase under food ad libitum and ultradian feeding. Furthermore, expression of Per2 was down-regulated and phase-advanced, whereas Rev-erbα was phase-advanced in the liver of hypocaloric and isocaloric mice fed with ultradian feeding. Hepatic expression of Pgc-1α and Fgf21 was up-regulated and phase-shifted by ultradian feeding, while that of Pparα was down-regulated.

Conclusions: When the daily feeding rhythm is abolished by introducing an ultradian periodicity of food intake, daily rhythmicity in the liver clock as well as in the central SCN clock is affected. In addition, hypocaloric feeding without daily time cues is sufficient to cause a phase-advance of the central clock. Hence metabolic cues due to either hypocaloric conditions or ultradian periodicity of the meals cause significant changes in the SCN clock.

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M91

Circadian Rhythms in Actin Dynamics and Wound Healing

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Abstract: During wound healing fibroblasts migrate into wound areas where they secrete matrix proteins to allow repopulation of the wound area with normal tissue. Fibroblast cells have been successfully employed as models in the study of wound healing and more recently have also been used to study cell intrinsic timekeeping by the circadian clock. However, the influence that the circadian clock has over cell motility and wound healing is unknown. Here we show that healing in human burns patients is affected by the time of infliction, with more efficient healing elicited by daytime wounds. This observation correlates to increased fibroblast invasion of wounds in mouse ex vivo models when wounds occur in the active phase, and a rhythm in wound healing in cellular models. This rhythm is driven by circadian regulation of actin cytoskeletal dynamics, dependent on Cryptochrome:Period3 (CRY:PER) clock gene expression cycles and the control they impose on actin regulators such as Cofilin. Our findings demonstrate a functional consequence for cell autonomous circadian regulation of the actin cytoskeleton: that the efficacy of wound healing depends on when the wound occurs.

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M92

Crystal Clear: Solving the Structures of Cyanobacterial KaiABC Subcomplexes

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Abstract: The cyanobacterial clock is a powerful model organism for studying circadian rhythms as it only requires three key proteins (KaiA, KaiB, and KaiC) with ATP to maintain a 24-hour cycle in vitro (1). A dynamic interplay of autophosphorylation, protein complex assembly, and fold switching allow KaiA, KaiB, and KaiC to act as a biological timekeeper; however, many important details of how they control timekeeping are unclear due to a lack of structural insight. While individual crystal structures of the three Kai proteins have been solved, no one has been able to solve a high-resolution structure of these assembled complexes. KaiB undergoes a slow transition from its ground state tetrameric form to a rare fold-switched monomer that is needed to interact with KaiC (2). Utilizing a mutant KaiB locked into this active signaling state (KaiB*), we solved two crystal structures of KaiB* bound to the C1 domain of KaiC (at 1.8Å resolution) and of the KaiB*-KaiC C1 domain bound to a dimer of KaiA (at 2.6Å resolution). The structures of these Kai subcomplexes reveal why the metamorphosis of the KaiB protein fold is necessary to bind to KaiC and how the KaiB*C complex sequesters KaiA in the evening. Elucidating the complexities of these subcomplexes lends new insight to the molecular mechanism of day-to-night transitions in the cyanobacterial circadian oscillator.

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M93

Feedback Loops of the Mammalian Circadian Clock Constitute Repressilator

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Abstract: Mammals evolved an endogenous timing system to coordinate their physiology and behaviour to the 24h period of the solar day. While it is well accepted that circadian rhythms are generated by intracellular transcriptional feedback loops, it is still debated which network motifs are necessary and sufficient for generating self-sustained oscillations.

Here we systematically explore a data-based circadian oscillator model with multiple negative and positive feedback loops and identify a series of three subsequent inhibitions known as "repressilator" as a key design principle of the mammalian circadian oscillator. The central role of the repressilator motif is consistent with time-resolved ChIP-seq experiments of circadian clock transcription factors and loss of rhythmicity in core clock gene knockouts.

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M94

Investigating the Role of Zinc in the Circadian System

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Abstract: Zinc is released from glutamatergic synaptic terminals, where it can act as a co-transmitter or as a neuromodulator of postsynaptic receptors. The transporter that packages zinc into synaptic vesicles (ZnT3) has been found in the retina. This information raises the possibility that zinc may modulate circadian responses to light. To test this hypothesis, we first confirmed that ZnT3 was found in melanopsin-containing intrinsically photosensitive retinal ganglion cells (ipRGCs). Next we investigated the presence of zinc in the circadian brain areas. Syrian hamsters received an intraperitoneal injection of sodium selenite, and then their brains underwent an autometallographic staining to label synaptic zinc. Zincergic fibers were visualized throughout the IGL while zincergic fibers were fairly sparse in the SCN. Enucleation surgeries for the removal of the retina followed by visualization of zinc in the circadian brain areas are ongoing to confirm the retinal origin of this zincergic staining. We also investigated the role of zinc in photic entrainment by modifying its levels

prior to exposure to a phase-shifting light pulse. In separate experiments, either the SCN or IGL received drug infusions before a light pulse for three separate conditions: A vehicle control, a zinc donor (ZnCl2), and a zinc chelator (TPEN). No significant phase shifts to light were observed for animals receiving the zinc donor or chelator in the SCN or the IGL.

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M95

Variability of Behavioral Chronotypes of 16 Mammalian Species Under Controlled Conditions

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Abstract: Background: Human chronotypes (differences in preference for early or late rising each day) have been extensively studied in recent years, but similar studies have not been conducted in other animal species.

Methods: We evaluated behavioral chronotypes in 16 mammalian species along a body size gradient of five orders of magnitude (from mice to cattle). Individuals of all species were housed under a 12L:12D photoperiod in a thermoneutral environment with food and water available at all times. Rhythms of locomotor activity were analyzed for onset time, acrophase, and robustness.

Results: None of the rhythmic parameters was significantly related to body size, but onset time and acrophase varied considerably from species to species, thus characterizing diurnal and nocturnal species. Chronotype spreads ranged from less than an hour in sheep to almost 24 hours in cats, thus extending both below and above the human chronotype spread of 6 hours. The variability of chronotype (as quantified by the standard deviation of group means) was much larger between species than within species and also larger between individuals of a species than within individuals on consecutive days.

Conclusion: These results help situate the matter of human chronotypes within the broader context of variability in the phase angle of entrainment of circadian rhythms in animals.

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M96

Circadian Clock Control by Polyamine Levels through a Mechanism that Declines with Age

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Abstract: Polyamines are essential polycations present in all living cells. Polyamine levels are maintained from the diet and de novo synthesis, and their decline with age is associated with various pathologies. Here we show that polyamine levels oscillate in a daily manner. Both clock- and feeding-dependent mechanisms regulate the daily accumulation of key enzymes in polyamine biosynthesis through rhythmic binding of BMAL1:CLOCK to conserved DNA elements. In turn, polyamines control the circadian period in cultured cells and animals by regulating the interaction between the core clock repressors PER2 and CRY1. Importantly, we found that the decline in polyamine levels with age in mice is associated with a longer circadian period that can be reversed upon polyamine supplementation in the diet. Our findings suggest a crosstalk between circadian clocks and polyamine biosynthesis and open new possibilities for nutritional interventions against the decay in clock's function with age.

Research Funding: The work performed in the G.A. laboratory was supported by the Israel Science Foundation , the Abish- Frenkel Foundation, the HFSP Career Development Award.

M97

TTDP, a Primate-Specific Gene Exclusively Expressed in Testis which Regulates the Circadian Rhythms

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Abstract: The evolution of circadian clock is very interesting but poorly understood. Through bioinformatic prediction and experimental validation, we identified an evolutionarily young gene, named TTDP, which only occurs in a subgroup of primates including old world monkeys and hominoidea. In human, TTDP mRNA is exclusively expressed in testis amongst a number of adult and fetal tissues. TTDP was originated from and reorganized via splicing. TTDP encodes a protein with 183 aa containing no known domains, which is predominantly located in the nuclei. Stable expression of TTDP in U2OS cells enhanced the amplitude of the circadian rhythm of luciferase reporter gene driven by BMAL1 promoter. Similarly, an increase in the amplitude of luciferase signal by expressing the Gorilla version of TTDP in U2OS cells was observed. Moreover, overexpression of TTDP represses the mRNA levels of PER1 and PER2 in human cells. These data suggest that the circadian clock is still under evolution and TTDP might be a novel component implicated in the regulation of circadian rhythms in primate testis. The mechanisms underlying its role of TTDP in regulating circadian clock remains further investigated.

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M98

Diel Flight Activity Behavior of Wild Caught Anopheles Farauti S.s. and An. Hinesorum Malaria Mosquitoes from Northern Queensland, Australia: Temporal Differences that Might Contribute to Speciation

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[1]*University of Notre Dame*

Abstract: Species in the Anopheles farauti complex are the major malarial vectors in the Asia Pacific region. Anopheline mosquitoes exhibit several circadian/diel rhythms including nocturnal sugar and blood feeding (biting), flight activity, egg laying, and in some species, temporally discrete and short-lived dusk/early night associated swarming behavior during which mating occurs. A behavioral study of trap-caught mosquitoes in Queensland, Australia was conducted to investigate the differences in diel flight activity between two species and several reproductive states. 24-hour flight activity was monitored in individual adult female mosquitoes under light:dark cycle conditions using an infrared beam break method. For statistical analysis, data were arranged into time bins, plots of activity accumulation, and Z-scored. Species-specific differences and a species difference at one reproductive state were observed. Compared to An. farauti ss, An. hinesorum mosquitoes had an earlier dusk activity onset, an earlier peak in nocturnal activity, and a higher level of activity at the onset of darkness. A second nocturnal peak in inseminated nulliparous An. hinesorum was also observed. The species differences between these two major malarial vectors of the An. farauti complex might contribute to subtle differences in habitat adaptation and/or reproductive isolation. This study provides baseline data for analysis of populations of mosquitoes from other geographic regions, such as the Solomon Islands. This is important as selective pressures have and continue to occur using residual insecticides and insecticidal-treated bednets, and can shape the nocturnal profile of biting behavior.

Research Funding: Supported by the Eck Institute for Global Health, University of Notre Dame, and NIGMS R01-GM087508 (to G.E.D.); Bill & Melinda Gates Foundation (Grant No. 45114), and NIAID ICEMR in the southwest Pacific SU19AI089686 (to T.R.B.); and a NHMRC Senior Research Fellowship 1044698 (to S.A.R.).

M100

Natural Variation and Co-Expression Network Approaches to Assess the Contribution of the Circadian Clock to Plant Fitness

Kathleen Greenham[1], Ping Lou[1], Joshua R Puzey[2], Malia Gehan[3], Hany Farid[1], John H Willis[4], Todd C Mockler[3], C. Robertson McClung[1], Ganesh Kumar[5], Cindy Arnevik[5]

[1]*Dartmouth College*, [2]*College of William & Mary*, [3]*Donald Danforth Plant Science Center*, [4]*Duke University*, [5]*Monsanto Company*

Abstract: The widespread occurrence of an endogenous circadian oscillator across diverse taxa suggests that there is a fitness advantage in entraining internal processes to the external environment. Many of the flowering time differences among plant cultivars selected during crop domestication contain mutations in circadian clock genes, thereby expanding the geographic range of these crops.

Using a new automated motion estimation system to measure circadian parameters by leaf movement, we have uncovered latitudinal clines in circadian period in natural populations of Mimulus guttatus (Monkeyflower) and elite cultivars of Glycine max (Soybean). These results suggest that fine-tuning the circadian clock in these species may provide a fitness advantage at various latitudes. Soybean breeders appear to have selected for circadian traits during the development of these elite cultivars providing further support for exploring circadian clock diversity in crops.

The genus Brassica, comprised of important vegetable, oilseed and forage crops, is the closest crop relative of Arabidopsis making it an excellent model for identifying targets for marker assisted breeding. A recent examination of the Brassica rapa genome revealed preferential retention of circadian clock genes following genome triplication and fractionation. Whole genome duplication followed by preferential retention of dose-sensitive circadian clock genes may have driven the evolution of increasingly complex circadian clock mechanisms. To identify the consequences of an expanded circadian clock gene set in B. rapa, we performed RNAseq over a 48 h circadian time course with 2 h sampling resolution following both photocycle and thermocycle entrainment conditions. Comparative co-expression network analysis has revealed differences in expression profiles among duplicate circadian clock gene pairs consistent with the gene dosage hypothesis and suggestive of possible subfunctionalization.

Research Funding: NSF NPGI Postdoctoral Fellowships to K.G. (IOS-1202779) and J.R.P. (IOS-1202778) and NSF grants to H.F. (CNS-0708209) C.R.M. (IOS-1025965) and J.H.W. (IOS-1024966)

M101

Chloride Cotransporter KCC2 Essential for GABAergic Hyperpolarization in the SCN

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Abstract: In mammals, the central circadian clock is located in the suprachiasmatic nucleus (SCN). The most abundant neurotransmitter in the SCN is γ-aminobutyric acid (GABA). There is mounting evidence that GABA, which is known as an inhibitory neurotransmitter, can also have excitatory effects in the SCN neurons. For example, we demonstrated in a recent study that seasonal changes in day length adaptation to long photoperiod can increase the amount of GABAergic excitation. This will affect the balance between excitation and inhibition (E/I balance) which is known to play a crucial role for the functioning of our brain and may underlie some of the seasonal modulations of the SCN neuronal network function.

The sign of the GABAergic response (inhibitory versus excitatory) depends on the intracellular Cl- concentration ([Cl-]i), which can be regulated by Cl- cotransporters NKCC1 and KCC2 in the membrane. NKCC1 activity causes a high [Cl-]i, whereas KCC2 reduces the level of [Cl-]i. The prevalent view is that GABA-induced excitation is the result of elevated expression of the NKCC1 cotransporter and lower expression of the KCC2 cotransporter. However, it is also possible that cotransporter activity is regulated (e.g. by phosphorylation) or that other mechanisms, like immobile intracellular anions, contribute to the regulation of [Cl-]i in the cell, and therefore influence the response to GABA.

To investigate the underlying mechanism that determines the [Cl-]i, and thus the sign of the GABAergic response, we manipulated KCC2 and NKCC1 with pharmacological blockers and studied the GABAergic responses in brain slice in vitro. Using Ca2+ imaging, we showed that application of the newly developed KCC2 blocker ML077 caused an increase in GABAergic excitation and thus a

shift in E/I ratio from 0.95 to 3.41 ($p < 0.0001$). Bumetanide, which is often used to block NKCC1, did not change the E/I ratio, but reduced the amplitude of the GABAergic evoked elevations in [Ca2+] i. Clearly, manipulating the Cl- cotransporters influences the GABAergic response, suggesting that the KCC2 and NKCC1 play a role in the determination of the intracellular Cl- concentration in SCN cells. Our present data show that modulation of KCC2 can play a prominent role in the shift towards elevated GABAergic excitation as observed in long-day photoperiod.

Research Funding: This work was supported by The Netherlands Organisation for Scientific research/ Netherlands Organisation for Health Research and Development Grant TOPGo 91210064.

M102

Does the Polarity of SCN GABAaR Signaling Regulate Phase Advances of the Behavioral Clock?

John McNeill[1], James Walton[1], Elliott Albers[1]

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Abstract: γ-aminobutyric acid (GABA), its receptors, and associated synthesis and uptake proteins are expressed in most neurons of the suprachiasmatic nucleus (SCN). GABAA receptor signaling regulates firing rate rhythms of SCN neurons in vitro as well as behavioral rhythms in vivo. Despite GABA's entrenched role as the primary inhibitory CNS neurotransmitter, in vitro studies have identified excitatory GABAAR responses in the adult SCN. The ratio of excitatory to inhibitory responses to GABA depends on chloride influx by Na+/K+/Cl- co-transporter 1 (NKCC1). Inhibition of NKCC1 abolishes excitatory responses to GABA in the adult SCN. We have shown that the inhibition of NKCC1 enhances phase delays to light. The following experiments aimed to characterize the role of NKCC1 activity in both photic and non-photic advances of the behavioral rhythm. Adult male Syrian hamsters were housed in constant dark conditions and on test day received injections of bumetanide (a NKCC1 inhibitor) or vehicle into the SCN region immediately followed by phase advancing (CT 19) light pulses. To explore the role of excitatory GABAAR signaling in non-photic advances of the rhythm in the subjective day, hamsters received phase advancing (CT 6) injections of muscimol or a cocktail of bumetanide and muscimol into the SCN region. Phase shifts were calculated by fitting regression lines to activity onsets before and after test days. NKCC1 inhibition in the late subjective night decreased photic phase advances and produced a phase delay when administered without light. Further, co-administration of the NKCC1 inhibitor with a phase advancing injection of muscimol appeared to block phase advances during the subjective day. These data suggest that inhibiting excitatory SCN responses to GABA produces delays late in the subjective night and inhibits advances in the late subjective night and subjective day.

Research Funding: Funding: Supported by NIH NS078220 and NIH NS092545

M103

Phase It! Strength Isn't Everything, at Least in the Mammalian SCN

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Abstract: Background: In the mammalian suprachiasmatic nucleus (SCN), robustness and precision emerges out of a network of sloppy neuronal oscillators by means of inter-neuronal coupling. This coupling is mediated by a collection of neuropeptides and neurotransmitters, and possibly gap junctions. However, these coupling factors and their receptors are heterogeneously distributed within the SCN and overlay the neuronal oscillators that themselves have spatial distributions of intrinsic properties. Experimental studies have revealed a hierarchy of coupling factors based on the effect of perturbations on rhythms in in-vitro, ex-vivo or in-vivo settings. Interpretations of these experiments and their associated modeling studies have principally relied on changes in the strength of coupling to explain the observed phenotypes.

Results: We show using computational studies that changes in circadian phase of coupling can indeed explain many observed phenotypes equally well and that in fact, the phase of action of a coupling agent is as important as its strength (Ananthasubramaniam et al. 2014). In particular, we can generate spatial phase-differences and waves within the SCN, different re-entrainment rates in response to jet-lag and altered phase configuration in summer and winter SCNs with minor changes in coupling phase. We present two case-studies where the phase of coupling provides a plausible explanation for the experimental observation: (a) the change in the nature of the neurotransmitter GABA from opposing to aiding synchrony in very long day SCN, where the phase configuration between the core and shell has been altered (Evans et al. 2013). (b) the surprising observation of a synchronized SCN in neonatal Cry1,2-/- mice that are arrhythmic in adulthood (Ono et al. 2013, Tokuda et al. 2015).

Conclusions: Quantification of coupling requires characterization of both strength and phase of action of the coupling factor. Thus, we can place most experimental observed phenotypes of the master circadian clock in a space characterized by just three system parameters: robustness of single oscillators, strength of coupling and phase of coupling.

Research Funding: Deutsches Forschungsgemeinschaft (DFG SPP InKomBio), Alexander von Humboldt Foundation, Bundesministerium fuer Bildung und Forschung (BMBF 01GQ1503)

M104

Stem-Like Cell Cultures of the Adult Mouse Suprachiasmatic Nucleus

Dilshan Beligala[1], Astha Malik[1], Arpan De[1], Michael Geusz[1]

[1]*Bowling Green State University*

Abstract: Cells with stem-like properties have been described in adult rodent SCN. These cells express SOX2, an important component of embryonic and adult neurogenesis. In addition, some SCN neurons express doublecortin, a protein present in differentiating cells committed to becoming neurons. To determine whether SCN stem-like cells would persist under conditions that favor culture of neural stem cells rather than mature neurons or glia, we maintained SCN explant cultures in serum-free stem cell medium containing fibroblast growth factor 2 and epidermal growth factor.

Because neurogenesis continues throughout the lifetime, we examined the SCN of mice at 57 days to 14 months of age. The optic chiasm and ependymal cell layer were surgically removed from the nearby SCN. Cell proliferation was assayed indirectly by measuring areas of SCN explants in digital images. During the first five days in culture, explants showed an average daily increase of 15.23% (SD ±2.98, n=7 explants). Explants continued to expand for at least 22 days and developed neurosphere-like structures associated with neural stem cell proliferation. After seven days in culture, immunocytochemistry and confocal imaging revealed cellular SOX2 expression. Fewer cells expressed GFAP, a marker for mature astrocytes. These results suggest that the mature SCN may have unique regenerative properties useful for adjusting circadian timing throughout the lifetime.

Research Funding: Supported by a grant for faculty research from Bowling Green State University.

M105

Vasopressin Mediates Clock-driven Anticipatory Thirst

Claire Gizowski[1], Cristian Zaelzer[1], Charles W. Bourque[1]

[1]*Research Institute of the McGill University Health Centre*

Abstract: Clock-driven circadian rhythms adapt behaviours to the physiological demands of a 24 hour activity cycle; however, it is unclear how the central clock mediates these effects. As previously shown, we found that mice ingest significantly more water prior to sleep (PS; ZT 21.5-23.5) compared to baseline (ZT19.5-21.5). We found that this behaviour is not driven by physiological stimuli for thirst such as increased body temperature, serum osmolality or hematocrit. Restricting water access during the PS period resulted in a significant increase in serum osmolality, indicating that this anticipatory thirst is physiologically relevant. In vitro recordings of thirst neurons in the Organum Vasculosum Lamina Terminalis (OVLT) showed increased activity during the PS period compared to baseline, suggesting these neurons may mediate anticipatory thirst at this time. Injection of fluorescent beads into mouse OVLT retrogradely labelled vasopressin (VP) cells in the Suprachiasmatic Nucleus (SCN), but not in other brain regions containing VP neurons. Visually identified VP SCN neurons in vitro showed a significant increase in activity during the PS period compared to baseline. In vitro electrical stimulation of the SCN caused detectable VP release in the OVLT by engineered HEK sniffer cells. Moreover, stimulation of the SCN excited OVLT thirst neurons via V1a receptors (V1aR). The role of this SCN-OVLT pathway was further explored using transgenic mice expressing accelerated channelrhodopsin (ChETA) or archaerhodopsin-3 (ArchT) in VP neurons. Application of blue light (473 nm) to the OVLT during the baseline period caused local VP release as detected by sniffer cells, and excited thirst neurons via V1aRs. In contrast, yellow light (589 nm) applied to the OVLT inhibited VP/V1aR dependent firing during the PS period. To investigate whether these effects contribute to anticipatory thirst, we examined the effects of light delivered to the OVLT via fiberoptic cannula in vivo. Yellow light application during the PS period suppressed anticipatory water intake, whereas blue light application during the baseline period promptly stimulated water intake. Collectively, these findings indicate that anticipatory water intake is mediated by VP release from the axon terminals of SCN clock neurons that project to the OVLT.

Research Funding: Canadian Institutes of Health Research Foundation Grant FDN-143337, McGill University Health Centre Research Institute Studentship, James McGill Research Chair Program

M106

Manipulating the Cellular Circadian Period of AVP Neurons Alters the Behavioral Circadian Period

Michihiro Mieda[1], Hitoshi Okamoto[2], Takeshi Sakurai[1]

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Abstract: The suprachiasmatic nucleus (SCN) is the primary circadian pacemaker in mammals and entrains to the environmental light/dark cycle. It is composed of multiple types of neurons, and neuronal network properties are critical for the normal function of the SCN. However, mechanisms underlying the SCN neuronal network have remained elusive.

We previously disrupted cellular clocks by deleting Bmal1 specifically in the neurons producing arginine vasopressin (AVP), one of the primary neuronal types in the SCN, and reported that cellular clocks in AVP neurons of the SCN are critical for interneuronal coupling regulating circadian behavior rhythm. Here, we tested whether AVP neurons act more actively as pacemaker cells to regulate the circadian period of behavioral rhythm. For this purpose, we manipulated the period of cellular clocks specifically in these neurons by selective deletion (Avp-CK1Δ-/- mice) or overexpression of casein kinase 1 delta (CK1Δ). Phosphorylation of PER proteins by CK1Δ is thought to enhance their degradation and to consequently accelerate the speed of cellular clocks. CK1Δ deletion lengthened the free-running period of circadian behavior by ~50 min while CK1Δ overexpression shortened it by ~30 min. Thus, manipulation of CK1Δ expression levels in AVP neurons bi-directionally changed the period of circadian behavior rhythm. When the 8-hr phase advance of LD cycle was given, control mice advanced while Avp-CK1Δ-/- mice delayed their activity rhythm to reentrain to the new LD cycle, which is consistent with the longer free-running period of Avp-CK1Δ-/- mice. PER2::LUC imaging in slices confirmed that cellular circadian periods of the SCN shell, where AVP neurons predominate, were lengthened by ~90min in Avp-CK1Δ-/- mice. Collectively, we conclude that AVP neurons are an essential component of circadian pacemaker cells in the SCN.

Research Funding: Grants-in-Aid for Scientific Research (B) (24390052) from JSPS

M107

Rhythms in VIP Cell Output within the Mouse Suprachiasmatic Nuclei

Timothy Brown[1], Sasha Kulick[1], Lydia Hanna[1]

[1]*University of Manchester*

Abstract: The suprachiasmatic nuclei (SCN) comprise a neurochemically and functionally diverse group of neurons that, together, generate precisely timed neural output to coordinate downstream physiology. Cells expressing vasoactive intestinal polypeptide (VIP) are known to play an essential role in synchronising network activity yet the nature of the daily output signals supplied by VIP cells are largely unknown. Indeed, while it is clear that VIP cells are involved in processing visual input to the SCN, the extent to which VIP cells actually convey rhythmic output signals remains controversial. Here we use multielectrode array recordings from mouse SCN slices, coupled with optogenetic identification of VIP cells, to address this issue. We show that the intrinsic response of VIP cells to channelrhodopsin2 activation are enhanced during the early projected night, suggesting

a rhythm in intrinsic excitability that matches temporal gating of circadian light responses. We next show that putative VIP cells show pronounced and relatively tightly coordinated day-night rhythms in spontaneous activity that match those observed in untargeted electrophysiological recordings. By contrast, our data suggest that electrophysiological rhythms in non-VIP cells are more broadly distributed across the circadian day. In summary, our data provide new insight into SCN network organisation and the roles of VIP cell signalling.

Research Funding: Funded by a BBSRC (UK) David Phillips Fellowship to TMB.

M108

BK Channels are Activated by Distinct Calcium Sources During Day and Night in SCN Neurons

Josh Whitt[1], Andrea Meredith[1]

[1]*University of Maryland School of Medicine*

Abstract: BK K+ channels are regulated by membrane depolarization and increases in the local intracellular Ca2+ concentration ([Ca2+]i). Across neurons and muscle, BK channels have been shown to differentially couple to the voltage-gated Ca2+ channels L-, P-, Q-, and N-type , as well as to ryanodine receptor- (RyR) mediated release from intracellular Ca2+ stores. In the suprachiastmatic nucleus (SCN), both intracellular free Ca2+ and voltage gated Ca2+ channel (VGCC) currents are regulated in a circadian manner, with greater [Ca2+]i found during the day. We used a pharmacological approach to identify the Ca2+ sources for BK current activation in day and night SCN neurons. We found a 70% reduction in BK current with nimodipine during the day, with less effect during the night (15% reduction), suggesting that L-type VGCCs are the primary Ca2+ source for BK activation during the day. Conversely at night, when VGCC currents are reduced, we found a significant decrease in BK current using dantrolene , blocking Ca2+ release from RyR (70%). Thapsigargin, which depletes Ca2+ from intracellular stores caused a 75% reduction in BK current, demonstrating that nighttime BK activation is primarily driven by Ca2+ release from intracellular stores through RyR. The voltage dependence of BK activation was shifted to more positive potentials with nimodipine during the day and both dantrolene and thapsigargin at night. Lastly, BK currents were reduced by conotoxin MVIIC to a similar extent between day and night (25% and 22%, respectively), suggesting that a fraction of BK channels maintain stable Ca2+ channel coupling over the circadian cycle. These data demonstrate diurnal regulation of the coupling between BK channels and their Ca2+ source and suggest circadian changes in Ca2+ coupling could contribute to BK current regulation in the SCN and its role in circadian rhythmicity.

Research Funding: This work was supported by NHLBI R01-102758 (ALM) and NIAMS T32-AR007592 (JPW).

M109

Sex Differences in Age-Related Sleep Changes in Mice

Martha Vitaterna[1], Peng Jiang[1], Christopher Olker[1], Eun Joo Song[1], Vance D Gao[1], Fred W Turek[1]

[1]*Northwestern University*

Abstract: Background/objectives: Age-related changes in sleep and circadian rhythms have been examined in model organisms, but relatively little is known of how sex differences may impact this. This experiment sought to determine whether sex differences exist in aging-related sleep changes in mice.

Methods/results: C57BL/6 mice were surgically implanted with ElectroEncephaloGram (EEG) and ElectroMyoGram (EMG) electrodes, under ketamine/xylazine anesthesia. Young (10 week old) and Old (16 month old) mice of both sexes were recorded. An unperturbed 24-hr baseline period was monitored, as well as the 24-hr response following a 6-hr sleep deprivation period. Both age and sex differences in baseline sleep were observed. In addition, age and sex differences in the response to sleep deprivation, with males exhibiting a greater response.

Conclusions: Sex differences and age differences in sleep are evident in mice. The mouse is a useful model organism to examine mechanisms underlying these phenomena.

Research Funding: NIH P01 AG 11412

M110

Identifying Neurons that Regulate Plasticity in Sleep Duration

Seana Lymer[1], Justin Blau[1]

[1]*New York University*

Abstract: Despite the universality and importance of sleep, we still do not understand how the brain determines sleep length. Multiple factors influence sleep duration, including age, light exposure, and prior waking experience. Drosophila sleep shares many characteristics with mammals, suggesting that the underlying mechanisms are evolutionarily conserved. We are currently screening for neurons that are responsible for plasticity in sleep duration. By activating neurons for 24 hours, we have identified several GAL4 lines that persistently change sleep length after the end of neuronal activation. These neurons overlap in critical central brain areas, such as the mushroom body and ellipsoid body. Further experiments will demonstrate how these neurons regulate plasticity in sleep duration.

Research Funding: This research is funded by the NIH, NYU's Graduate School of Arts and Science, and NYU Abu Dhabi.

M112

Fields of Iteration – A Novel Concept of Entrainment in Neurospora

Manfred Goedel[1]

[1]*Institute of Medical Psychology, University of Munich*

Abstract: Seminal entrainment studies have been done on the model Neurospora crassa.

So far, the entrainment process was considered finished as soon as a distinct phase relationship of the internal phase–markers to zeitgeber cycles is established.

This view is at least incomplete:

Applying temperature cycles, the phase of entrainment depends on the cycle pattern. The resulting phase relationships differ when applying a cold:warm (DD22/27°C 6:6h) cycle or a worm:cold (DD27/22°C 6:6h) cycle, respectively, even if the transient of the phase marker crosses the same zeitgeber phase. From that I concluded, that before–conditions have an impact on entrainment, even in identical further T–cycles.

Second, analysing the proteome of Neurospora using SELDI-TOF mass spectrometry in a timeseries, one can detect an ongoing synchronization among the proteins, although there is a stable phase of entrainment in clock markers.

Based on this, I hypothesize, that an increasing stabilisation of entrainment is achieved by the identical in phase-iteration of oscillations.

Experiments applying transitions between diverging entrainment conditions prove this hypothesis valid: The more often the Neurospora cells have gone through an entrained cycle (with a constant phase angle) in the condition before the transition, the more entraining cycles are necessary to shift the clock to a different phase of entrainment in the second condition. Similarly, the perturbation of the entrainment process by constant conditions is less effective on a system entrained for 3 cycles in comparison to one entrained for 1 cycle. The iteration of oscillations in free run conditions, although a stable state of rhythm, too, seem to not to deploy a comparable effect.

These stabilizing effects cannot be explained by the established models of entrainment. The physical correlate of that stability in the cell, is still elusive. Based on physico-chemical considerations a novel model of entrainment as a minimum of Gibbs free energy is developed. In addition, an approach, how to test this model by applying synthetic zeitgebers using the expression of clock–genes and calorimetric measurements is suggested.

Research Funding: Private Funding

8:30 pm - 10:30 pm

Poster Session III

T1

The Circadian Clock Proteins BMAL1 and CIOCK control G2/M Cell Cycle Transition

Elham Farshadi[1], Jie Yan[2], Pierre Leclere[3], Ines Chaves[1], Albert Goldbeter[4], Gijsbertus van der Horst[1]

[1]*Erasmus University Medical Center,* [2]*Center for Computational Biology, Soochow University,* [3]*Université Nice Sophia Antipolis, CNRS, INSERM,* [4]*Université Libre de Bruxelles, Brussels*

Abstract: Gating of cell division by the circadian clock has been observed in various studies. There is significant evidence that in mammals circadian rhythms affect the timing of cell division in vivo. Circadian clock disruption, either by light or genetic mutations, is linked to alterations in the rate of cell proliferation, apoptosis, DNA damage and metabolism, and to cancer predisposition. Using the NIH3T33C mouse fibroblast line (carrying fluorescent reporter genes for clock and cell cycle phase) we have previously identified a tight coupling between the circadian clock and the cell cycle (Feillet et al. 2014, PNAS 111:9828-33). However, still little is known on how these two oscillators interact.

In order to understand the mechanism underlying this bidirectional coupling, we have studied the impact of circadian clock deregulation on cell cycle progression of NIH3T33C cells. To this end, we have knocked down either Bmal1 or Clock, and analyzed timing of cell division at the single cell level (confocal microscopy), as well as at the population level (FACS analysis). Inactivation of either Bmal1 or Clock resulted in a lengthening of the cell cycle, notably S/G2/M phase. Further molecular analysis revealed reduced levels of Cyclin B1, an important G2/M regulator molecule upon inactivation of Bmal1 or Clock gene expression. Thus, the observed effect is not specific for BMAL1 or CLOCK but for the whole positive limb of the circadian core oscillator.

In agreement with these observations, a mathematical simulation of the effect of Bmal1 or Clock knockdown on the coupling between cell division and the circadian clock also revealed a lengthening of the cell cycle with decreasing mRNA levels.

In conclusion, in the absence of the positive limb of the circadian clock, cell division takes longer and this observed effect is attributable to a delay in G2-M transition. Ongoing experiments will delineate the mechanism behind the coupling between the two cycles.

Research Funding: The Netherlands Organization for Scientific Research, the Netherlands Genomics Initiative/Netherlands Toxicogenomics Center, ERASysBio+ project C5Sys

T2

Cardiovascular Dysfunction in a Mouse Model of Huntington's Disease.

Tamara Cutler[1], Saemi Park[1], Dawn Loh[1], Cristina Ghiani[1], Christopher Colwell[1]

[1]*University of California, Los Angeles*

Abstract: While Huntington's disease (HD) is classified as a neurological disorder, HD patients exhibit a high incidence of cardiovascular events leading to heart failure and death. Our prior work has established that the Q175 mouse model exhibit disrupted rhythms in circadian and sleep behavior early in the disease progression. Furthermore, the SCN neurons of the mutants show pathophysiology with decreased spontaneous firing and altered potassium currents. In the present study, we sought to better understand the consequence of the circadian disruption on the cardiovascular system. Echocardiograms indicate that the ventricular mass and other morphological parameters of the Q175 hearts are smaller compared to WT starting at 6 months of age. Functionally, the ejection fraction of the homozygote mutant is reduced by 12 months of age. Telemetry recordings indicate that activity rhythms are significantly reduced by 4 months of age while core body temperature (CBT) rhythms are altered by 9 months. Interestingly, the Q175 mutants exhibit intervals in which they are unable to maintain CBT. These hypothermic events are a clear sign of metabolic dysfunction. While the rhythms in heart rate (HR) are not lost in the Q175 line, the mutants show high resting HR during sleep. Baroreceptor evaluations were carried out to determine the autonomic responsivity to blood pressure perturbations. Strikingly, the Q175 mice had very blunted HR responses to nitroprusside i.e. the HR did not rise very much in response to a drop the pressure. There is an abnormal response to angiotensin in that the HR goes out of control oscillating up and down. Thus, there is clear evidence for diminished autonomic function in the HD mutants. Finally, the Q175 hearts show histopathology with localized points of fibrosis that resemble those caused by myocardial infarction. Thus, the Q175 model of HD exhibit a number of changes in cardiovascular function that start early in the disease progress and may provide an explanation for the higher cardiovascular risk in HD.

Research Funding: CHDI Foundation

T3

Endogenous Circadian Rhythm in Vascular Function and Cardiovascular Risk

Saurabh Thosar[1], Alec Berman[1], Sally Roberts[1], Noal Clemons[1], Miki Morimoto[1], Maya Herzig[1], Jonathan Emens[1], Matthew Butler[1], Steven Shea[1]

[1]*Oregon Health and Science University*

Abstract: Introduction: Adverse cardiovascular (CV) events occur more frequently in the morning especially within three hours of awakening. It is possible that these events could be triggered by the CV changes associated with: (1) the long period of immobility during sleep; (2) the CV stresses associated with awakening and initiation of active behaviors; or (3) the endogenous circadian system. Vascular endothelial function (VEF) is an excellent prognostic marker of CV function.

Methods: We tested whether there is an endogenous circadian rhythm in VEF. So far, data have been collected in 6 healthy participants (age 50±6 yr, BMI 25±4 kg/m^2, 3 men) throughout a 5 day 'forced desynchrony' protocol. The protocol incorporated 10 cycles of identical, recurring 5h 20m behavioral cycles (2h 40m sleep opportunity; 2h 40m wake periods) in dim light and constant temperature. All

activities and meals were the same throughout each wake period. At each scheduled awakening (i.e., every 5h 20m), VEF was estimated from brachial artery flow mediated dilation.

Results: The preliminary results in the 6 participants suggest that the circadian system contributes to lower VEF during the susceptible morning period.

Conclusion: If these preliminary results hold with a larger subject group and in individuals with preexisting CV vulnerability, we would conclude that the endogenous rhythm in vascular function may contribute to the observed early morning peak in CV events.

Research Funding: National Space Biomedical Research Institute through NCC 958, Medical Research Foundation of Oregon, NIH/NHLBI R01HL125893-01, and CTSA grant UL1TR000128

T4

Determining the Ontogeny of Synchronization in the Xenopus Laevis Embryo Using Gene Expression and Behavior

Kristen Curran[1], Amber Hibben[2], Betsy Beck[2], Matt Redmann[3], Morgan Hadley[4], Garrett Whitehead[1]

[1]*University of Wisconsin Whitewater*, [2]*Marquette University*, [3]*University of Alabama*, [4]*Vermont College of Medicine*

Abstract: How does a vertebrate embryo become synchronized to external environmental cues? We found that specific embryonic organs display rhythmic expression of select oscillator and output genes (mature rhythm) at different embryonic stages in LD. The eye displays a mature rhythm 66 hours post fertilization (hpf; qPCR), but detectable time of day dependent changes in gene expression begins 53 hpf. Preliminary findings show that the pronephros may attain a mature circadian rhythm after 5.5 days of development while the embryonic heart does not. Although fully differentiated, the ten day old heart only displays a significant rhythm in xRev-erba and xPeriod1 ($p<0.01$). The peak of gene expression in each organ is behind that of similarly aged eyes suggesting that, similar to mammals, organs of the tadpole are not directly light entrainable. Recent findings suggest that background preference in premetamorphic and metamorphic tadpoles may be under circadian control. Premetamorphic tadpoles (5.5 dpf) prefer a white background during the daytime and choose a dark background at night when maintained in LD ($p<0.01$). Addition of melatonin (0.1mM) one hour after dawn increased the number of tadpoles present on the white background during the day and night. Tadpole preference for a white background peaks prior to lights out suggesting a possible explanation for the effects of melatonin addition.

Research Funding: National Science Foundation, Beta Beta Beta National Honors Society, University of Wisconsin Whitewater Undergraduate Research Program

T5

MicroRNA-92a Acts as a Circadian Regulator of Neuronal Excitability in Drosophila

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Abstract: Many biological and behavioral processes in animals are governed by a circadian clock. Although most previous studies have focused on transcriptional regulation, we are focusing here on i) how post-transcriptional regulation affects Drosophila circadian rhythms, specifically how miRNAs contribute to rhythms, and ii) how the circadian clock regulates miRNA metabolism. We show that expression levels of microRNA-92a (mir-92a) not only oscillate "around the clock" but also respond to light pulses in PDF neurons. Multiple lines of evidence including loss-of-function and overexpression experiments indicate that mir-92a suppresses neuronal activity and is therefore an intermediary between the circadian clock and activity. First, manipulation of mir-92a levels in PDF neurons alters the voltage sensor ArcLight ex vivo and an in vivo calcium reporter CaLexA consistent with a role in suppressing neuronal activity. Second, manipulation of mir-92a levels in PDF neurons affects neuronal fasciculation, a property previously linked to the circadian clock and importantly to neuronal firing. Third, manipulation of mir-92a levels in 3 different specific neuron groups (dopaminergic neurons as well as two sets of circadian neurons, LNds and DN1s) affects behavioral outputs (fly sleep duration and quality) consistent with suppressing neuronal activity. Fourth, in vitro and in vivo assays suggest mir-92a suppresses sirt2 activity, which has been previously reported to affect neuronal excitability. Our data in sum suggest an important role of mir-92a in regulating neuronal excitability downstream of the clock.

Research Funding: HHMI

T6

Drosophila DH31 Neuropeptide and Pdf Receptor Control Night-Onset Temperature Preference

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Abstract: Body temperature exhibits rhythmic fluctuations over a 24-hour period. Body temperature decreases during the night, which is associated with sleep initiation. However, the underlying mechanisms of this temperature decrease are largely unknown. We have previously shown that Drosophila exhibit a daily temperature preference rhythm (TPR), in which their preferred temperatures increase during the daytime and then decrease during the evening (night-onset). Because Drosophila are small ectotherms, their body temperature is very close to the ambient temperature, suggesting that their TPR generates their body temperature rhythm (BTR). Here, we demonstrate that the neuropeptide Diuretic Hormone 31 (DH31) and its receptor pigment dispersing factor receptor (PDFR) control the preferred temperature decrease at night-onset. We showed that PDFR and tethered-DH31 (t-DH31) expression in dorsal neuron 2 s (DN2s), restored the preferred temperature decrease at night-onset, suggesting that DH31 acts on PDFR in DN2s. Although PDF (another ligand of PDFR) is

a critical factor for circadian locomotor activity, pdf mutants exhibit normal preferred temperature decreases at night-onset. This suggests that circadian locomotor activity and night-onset TPR are regulated by different neuropeptides that use the same receptor expressed in different clock cells. Thus, we found that DH31-PDFR signaling specifically regulates a preferred temperature decrease at night-onset. Additionally, we proposed that night-onset TPR and locomotor activity rhythms are differentially controlled not only by clock neurons in the brain but also by neuropeptide signaling.

Research Funding: JST (Japan Science and Technology)/Precursory Research for Embryonic Science and Technology (PRESTO), the March of Dimes, and NIH R01 grant GM107582

T7

Ion Channels that Regulate Neuronal Physiology and Circadian Behavior in Drosophila melanogaster

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Abstract: Circadian rhythms have been extensively studied in the fruit fly where many clock genes that interlock through negative feedback loops and generate daily oscillations have been described. Clock genes are expressed in approximately 150 clock neurons in the Drosophila brain. Among them, the pigment dispersing factor (PDF)-expressing lateral neurons (LNvs) have been found to play central roles as pacemaker (the small-LNvs) and arousal (the large-LNvs) neurons.

Still, little is known on how the electrical properties of Drosophila clock neurons are specified, and what mechanisms allow them to change their firing rate on a daily basis. We have performed a behavioral genetic screen through the downregulation of candidate voltage-gated ion channels using RNA-interference specifically in LNvs. Among the positive hits we focused our attention on the hyperpolarisation-activated cation current Ih. In mammalian neurons, this channel is involved in complex neuronal behaviors such as bursting, the same firing pattern that LNvs display. Here, we show that Ih expression is important for the behaviors that LNvs command. Moreover, using genetics and pharmacology coupled to whole-cell patch clamp electrophysiology in ex vivo Drosophila brains, we show that Ih is necessary to achieve the high frequency bursting firing pattern of LNvs. Since bursting firing has been associated to neuropeptide release, we hypothesized that Ih would be important for PDF-mediated communication. This is indeed the case; we found that constitutive downregulation of Ih affects sLNvs development and adult-specific downregulation of Ih affects PDF levels and structural plasticity of sLNvs dorsal projections.

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T8

Regulation of Chromatin Accessibility on Clock/Cycle Direct Targets in Drosophila

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Abstract: Circadian rhythms in both flies and mammals are controlled by a transcriptional feedback loop including the heterodimeric transcription activators CLK:CYC in flies and its ortholog CLK:BMAL1 in mammals. Recent experiments in mammals suggest that the CLK:BMAL1 functions as a pioneer transcription factor, which temporally opens chromatin on clock-controlled genes (CCGs) to facilitate circadian transcription (Menet and Rosbash, 2014). To explore whether CLK:CYC might have a similar activity in Drosophila, we have used ATAC-seq to probe chromatin structure in fly brains as well as in S2 cells. At the peak of circadian transcription (ZT14) in fly brains, the profile shows regions of open chromatin that correspond almost perfectly to locations of CLK binding defined by ChIP-seq. Surprisingly, the ATAC-seq profile remains static and open "around the clock" despite the dramatic changes in CLK binding and circadian transcription. Moreover, the same open chromatin pattern is observed in a mutant that abolishes CLK/CYC binding (cyc01) as well as mutant completely lacking CLK (CLKout). Yet preliminary results in S2 cells indicate that CLK induction leads to more open chromatin surrounding the promoters of CLK:CYC-controlled genes, consistent with the original suggestion (Menet and Rosbash, 2014). Experiments are underway to synthesize these results and to identify other factors involved in establishing and maintaining chromatin accessibility on CCGs in Drosophila.

Research Funding: Howard Hughes Medical Institute

T9

Integration of Clock and Temperature Circuits Drives Pre-Dawn Temperature Preference in Drosophila

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Abstract: Although animals have sophisticated sensory systems, the behavioral phenotypes vary depending on the animals' internal time of day. The circadian clock is one of the factors that influence behavioral and physiological outputs. We previously found that Drosophila exhibit circadian rhythms of temperature preference, but how temperature and central clock information are integrated and how it alters behavioral outputs remains largely unclear. Here, we demonstrate that the Transient receptor potential A1 (TrpA1) expressing thermosensing Anterior Cells (ACs) and the small ventral lateral clock neurons (sLNvs) are important for temperature preference behavior before dawn. GFP reconstitution across synaptic partners (GRASP) experiments indicate that TrpA1 expressing neurons and pigment dispersing factor (PDF) expressing clock neurons contact in the dorsal protocerebrum area. AC neurons are serotonergic neurons, and 5HT1B serotonin receptor expression in sLNvs is important for temperature preference before dawn, suggesting that serotonin is involved in the

transmission from ACs to sLNvs. sLNvs contact and activate Dorsal Neurons 2 (DN2s), the main clock cells responsible for regulating temperature preference rhythm. The number of these contacts dramatically increases during the night and peaks before dawn, suggesting that time-dependent neuronal plasticity correlates with pre-dawn temperature preference. While AC neurons are not important for temperature entrainment of the circadian clock, our results suggest that sLNvs may function as a gate to incorporate ambient temperature information into the central clock circuits. Thus, it raises the possibility that ambient temperature might influence clock-dependent events before dawn, such as arousal or body temperature fluctuation.

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T10

The Expression of Period and Timeless in the Early Development of Drosophila Melanogaster

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Abstract: How the clock develops throughout life is not fully understood. In this study, the initial patterns of clock gene expression of period and timeless were recorded continuously in Drosophila from embryo to early adult, using transgenic luciferase reporter lines. All of the transgenic XLG flies (n=79) kept in constant darkness (DD) showed an increase of period expression from embryo, which subsequently peaked in larvae with a majority (63%, n=50) occurring on day two post-hatching. Period expression then returned to relatively low level. Under light cycles (LD 12:12) a similar pattern was elicited with 49 XLG flies (n=50) showing the first period peak during larval stage, the majority of which (64%, n=32) peak on day two post-hatching. No oscillations were evident in this first rise of period clock gene expression, and timeless showed no noticeable increase in embryonic or larval stages. Both period and timeless were expressed rhythmically from the pupal stage onward for four to eight oscillations with periods close to 24 h according to ClockLab analysis. (100% in XLG flies (n=27) in DD, with an average period of 24.09±0.90 h; 97% in XLG flies (n=29) in LD cycles, 24.04±0.52 h; 95% in tim-luc flies (n=22) in DD, 24.17±0.71 h; 83% in tim-luc flies (n=12) in LD cycles, 24.15±1.28 h). This early developmental pattern of the clock gene expression does not require entrainment by light. Light does not affect the establishment or speed of the development process of the circadian clock. In conclusion, we have analysed the temporal expression pattern of the key clock genes period and timeless in pre-adult stages of Drosophila. The mature circadian clock starts to function before the animal reaches adulthood but in the pupal stage, rather than the larval stage, as suggested by previous studies.

Research Funding: University of Auckland

T11

A Drosophila Apterous Mutation Uncouples Locomotor Activity from Circadian Clock Control

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Abstract: The Drosophila apterous gene is a member of the highly conserved LIM-homeobox gene family encoding proteins important for central nervous system development and wing development. We investigated the effect of the (ap56f) allele on circadian rhythms for locomotor activity and eclosion in Drosophila melanogaster. Analysis of circadian activity in F1 and F2 flies derived from crossing apterous with wild-type flies, and comparison to wingless vestigial mutants, showed that the apterous mutation significantly impairs circadian locomotor rhythms in a genetically recessive manner and is not caused by the absence of wings. The apterous mutation did not significantly alter the circadian rhythm for eclosion. We conclude that apterous is not a "clock gene" but is essential for linking locomotor activity to circadian clock control.

Research Funding: Skidmore College, Biology Department

T12

How Does Electrical Activity Regulate Circadian Gene Expression in Drosophila Pacemaker Neurons?

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Abstract: Activity-dependent gene expression has an important role in mammalian circadian rhythms since genes such as mPer1 are rapidly induced by light to reset the clock. We recently found that electrical activity regulates as many as 50% of genes expressed with a circadian rhythm in Drosophila pacemaker neurons, leading to the idea that many rhythmically expressed genes are driven by neuronal activity rather than by the core molecular clock.

How does electrical activity regulate circadian gene expression in Drosophila LNv pacemaker neurons? To answer this, we generated fluorescent reporter genes to follow the transcription of two rhythmically expressed genes in vivo. Our studies show that Hr38 expression peaks at ZT2 and its transcription is activity-dependent, while expression of Pura peaks at ZT18 and is repressed by electrical activity. Interestingly, we found that Pura expression is induced by hyperpolarization, which is the first example to our knowledge of hyperpolarization inducing transcription in neurons. The timing of peak Hr38 and Pura expression fits with the idea that LNvs are more excitable in the morning than the evening. Therefore, Hr38 and Pura represent two distinct categories of genes controlled by electrical activity. Since activity-dependent genes have been extensively studied in many systems, we decided to focus on identifying the mechanisms of transcriptional regulation of Pura. We are currently dissecting the ~50kb Pura locus to identify the cis-regulatory elements that respond to hyperpolarization and using genetics to find the relevant transcription factor(s).

Research Funding: NIH, NYU and NYU Abu Dhabi

T13

Ovarian Hormones Prevent Disruption of Daily Rhythms from High-Fat Feeding in Female Mice

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Abstract: The loss of circulating estrogen after menopause increases the prevalence of obesity and the risk of life-threatening, obesity-related disorders such as cardiovascular disease and stroke in women. Shift-work also increases the risk of obesity in women, implicating circadian disruption as a contributing factor. We sought to determine how estrogen interacts with the circadian system to protect females from obesity and related disorders. C57BL/6J male mice develop profound diet-induced obesity. In our previous study, we found that after only one week of high-fat diet consumption, daily rhythms (eating behavior and the phase of the liver clock) were altered in male mice. In contrast to males, C57BL/6J female mice are resistant to diet-induced obesity. In this study, we sought to determine if daily rhythms in female mice are resistant to disruption from high-fat diet feeding. We fed female PERIOD2:LUCIFERASE mice 45% high-fat diet for 1 week and measured daily rhythms. Female mice retained robust rhythms of eating behavior during high-fat feeding that were similar to chow-fed females. In addition, the phase of the liver PER2::LUC rhythm was not altered by high-fat diet consumption in females. To determine if ovarian hormones protect female mice from disrupted daily rhythms from high-fat diet, we analyzed daily rhythms in ovariectomized mice. Daily rhythms in ovariectomized females, like males, were susceptible to disruption with high-fat diet. During high-fat feeding, ovariectomized females had low-amplitude or arrhythmic eating behavior rhythms and their liver PER2::LUC rhythms were advanced ~4 hours. Together, our studies show that circulating ovarian hormones protect against changes in daily rhythms from high-fat diet in female mice.

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T14

Oscillations in Circadian Gene Expression in Liver and Brain in Response to Scheduled, Calorie Restricted Feeding

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Abstract: All cells are capable of maintaining rhythmic gene expression. The oscillation of genes in the mammalian circadian system is normally tightly coupled to the rising and setting of the sun. Interestingly, peripheral tissues can have their clocks reset by scheduled feeding independent of the light:dark cycle. It has been reported that most of the brain continues to track the light:dark cycle in animals on a restricted feeding schedule. We conducted a study of circadian regulated genes, comparing their expression in live as well as three brain regions—cortex, striatum, and cerebellum. In this study mice were either fed ad libitum or were restricted to 60% of their normal diet for four

weeks. Mice were sacrificed at 6-hour intervals and the expression of Rev-erbα, Bmal1, and Dbp in the liver and Per2 and Cry1 in the brain were assessed using qPCR methods. In the liver we observed that circadian gene oscillation shifted to scheduled feeding. Between the cortex, striatum, and cerebellum there were clear changes caused by scheduled feeding, and much to our surprise, it appears that all of these structures have their circadian gene expression changed. Future experiments will explore the transcriptional changes occurring in discrete brain regions in response to scheduled feeding as a means to identify which molecular components of the circadian system are causal in mediating food entrainment.

Research Funding: CSU Program for Education and Research in Biotechnology (New Investigator Award), Kellogg Undergraduate Scholars Program, California State Polytechnic University, Pomona

T15

A Piece of Chocolate in the Dark Phase Prevents Circadian Desynchrony and Overweight in Male Shift-Worker Rats

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Abstract: Desynchronization models, where conflicting conditions arise between the LD cycle and general activity, food is an excellent Zeitgeber to reinstate temporal order to the body by resetting the activity and gene expression of peripheral oscillators.

We've demonstrated that chocolate entrains neuronal activity in several areas in the brain and most importantly that the presentation of chocolate during the activity phase of the body can increase the amplitude of the neuronal activity of the SCN.

In the present study we explored whether chocolate presentation during the active phase can prevent circadian desynchrony. In an experimental model of night work we observed that the presentation of 5g of chocolate coupled to the night onset increased the amplitude of the rhythm of physiological variables such as core temperature, serum glucose and triglycerides. This effect was not observed in animals that received chocolate coupled to the onset of the light phase, just before the beginning of their working schedule.

More over night time presentation of chocolate had an important effect on body weight regulation. Here we observed that worker rats that received a piece of chocolate during the night developed a lower body weight gain as compared to worker rats that received chocolate in light phase. This effect in body weight is correlated with a higher posprandial thermogenesis in response to chocolate when presented at night due to an increased response in brown adipose tissue thermogenesis as determined by UCP1 in the interescapular brow adipose tissue.

The present results demonstrate that chocolate ingestion at the proper time prevents desynchrony and induces efficient energy expenditure thus preventing body weight gain.

Research Funding: This study was supported by grants CONACyT 239403 and PAPIIT-UNAM: IG200314.

T16

Relationship between Timing of Food Intake and Insulin Sensitivity

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Abstract: Introduction: Previous research on the effect of food intake on metabolism in humans has focused primarily on quality (macronutrient type) and quantity (calories consumed). The fasting/feeding signal is one of the strongest zeitgebers of metabolic peripheral clocks besides the central clock. Therefore irregularities in timing of feeding can lead to misalignment causing disturbances in metabolic homeostasis. The goals of this study were (1) to generate a summary measure that characterizes the timing of food intake and (2) to determine if this measure is associated with insulin sensitivity, one measure of metabolic function.

Methods: This ongoing study is enrolling non-diabetic adults. They complete a 3-day food diary that asks for both the amount and types of food eaten and time of these meals and snacks. They also spend one night in the laboratory at the University of Chicago and undergo a 5-hour 12-sample oral glucose tolerance test (OGTT) in the morning. Insulin and glucose are assayed on each sample and the Matsudo Index of insulin sensitivity (MI) is calculated. To characterize the timing of food intake we calculated the clock times at which 25%, 50% and 75% of total calorie intake (TCI) were reached. Using Pearson's correlation, the associations between average calorie intake, the timing measures and MI were estimated. Regression models were used to assess these associations adjusting for TCI and waist circumference.

Results: There were 33 subjects (15 women) who had completed the OGTT and food diary. Mean (SD) age was 28.7 (7.9) years. The mean (SD) was 8.6 (4.3) for MI. The average times at which 25%, 50% and 75% of TCI were reached were 11:50 (2:15), 15:14 (2:36), and 18:56 (1:40) hours respectively. The Pearson correlations between the MI and the timing measures were: r= -.35, p=0.048 for 25% TCI, r=-.24, p=.18 for 50%TCI and r=-.21, p=.23 for 75% TCI. In the regression models, the significant association between insulin sensitivity and 25% TCI persists (Beta = -0.76, p=0.04).

Conclusion: The average time participants reached 25% of their daily calorie intake was most strongly associated with insulin sensitivity. Those who reached this point earlier in the day had higher insulin sensitivity in this sample of non-diabetic adults.

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T17

Circadian and Feeding Rhythms Differentially Affect Rhythmic Mrna Transcription and Translation in Mouse Liver

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Abstract: Rhythmic gene regulation in mouse liver originates from an intricate interplay between the circadian clock and feeding rhythm. While such interactions have been extensively studied, a complete picture of the resultant diurnal transcription-translation process is still lacking. Using RNA-

Seq and Ribosome profiling of mouse liver under physiological light–dark conditions and ad libitum or night-restricted feeding in wildtype and Bmal1-deficient animals, we quantified temporal mRNAs transcription, accumulation, and translation.

We found that rhythmic transcription is the main driver underlying rhythmic mRNA accumulation and translation for a majority of genes. On the other hand, translation efficiency is rhythmically regulated for genes with 5′-Terminal Oligo Pyrimidine tract (5′-TOP) sequences or a Translation Initiator of Short 5′-UTR (TISU) motif. This diurnal regulation is mainly driven by feeding rhythms although Bmal1 deletion slightly affects amplitude and phase of translation for some genes.

Finally, we developed computational methods to infer ribosome residence time and a proxy for translation initiation rate from the ribosome profiling data sets to evaluate more precisely the translation regulation landscape in mouse liver. We found that ribosome residence time is amino acid specific and span a threefold range from the fastest to slowest codon. Moreover, this analysis undercover a dozen of new candidate genes rhythmically regulated at the translation level.

Research Funding: ERC, Nestlé Institute of Health Sciences

T18

Circadian Fluctuations in Hemodynamic of Surgeons Under 24-Hour Duties

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Abstract: Higher levels of blood pressure among surgeons are more common compared to other medical professionals. Under 24-hour duties they do not show normal circadian fluctuations in blood pressure that is known risk factor for hypertension development. The purpose is to reveal both the circadian fluctuations and the specific features in hemodynamic parameters of surgeons working 24-hour duties.

Methods. 33 surgeons (27-74 y.o.) were observed 4 times (8:00, 16:00, 22:00, 8:10) during the 24-hour duties and 66 surgeons (23-74 y.o.) - at the duty beginning (24-h duty (8:00-8:00) followed by 72-h off). Registered: heart rate (HR), blood pressure (BPs, BPd), height and weight. Hemodynamic parameters were calculated.

Results. Due to the mean-group data of 33 surgeons, the lowest BP was found at 16:00 (BPs: $p<0,01$ compared to 8:00; $p<0,05$ - to 22:00; $p<0,06$ - to 8:10; BPd: $p<0,01$ compared to 8:00 and 8:10), the lowest HR - at 22:00 and 8:10 ($p<0,01$ compared to 8:00), the highest circulatory insufficiency (BPs/HR) - also at 22:00 ($p<0,04$ compared to 8:00, 12:00) that did not change at 8:10. Kerdo's vegetative index at 16:00 tended to be higher (then at 22:00: $p<0,09$), peripheral vascular resistance - at 22:00 tended to be higher (then at 16:00 - $p<0,10$). Broken (vascular) type of bloodcirculation selfregulation was registered at any time. Due to the mean-group data of 66 surgeons, majority hemodynamic parameters shown normal levels. However, peripheral vascular resistance (2391±116 kPa*s/l) shown the below middle level, Kerdo's vegetative index (-13±2,9) - the vagotonia, insufficient circulatory index (1,82±0,05) - the strain regulation. Hypertension was found in 38% of surgeons, broken bloodcirculation selfregulation – in 94% of surgeons, blood pressure asymmetry higher than 10 Hg mm – in 50%.

Conclusion. The results reflect the unfavorable changes in circadian rhythms and mean-group levels of hemodynamic parameters of surgeons working 24-h duties that manifests the need for preventive measures aimed primarily to the maintenance of both normal circadian rhythms of heart rate, blood pressure and normal bloodcirculation selfregulation.

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T19

The Effect of Daytime Napping under Bright Light Condition After Simulated Night Work on Biological Rhythm in Healthy Human

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Abstract: Night working under bright light delays the phase of biological rhythms in human. On the other hand, daytime bright light exposure advances the rhythms. The guideline for shift workers nursing on Japanese hospitals indicated that shift workers should be exposed to bright light after night work, in order not to disturb the biological rhythms. In addition, it is recommended that irregular shift workers should take a nap under dark conditions after night work. The aim of this study is to evaluate the effect of napping under bright light after simulated night work on phase of biological rhythm.

Twelve subjects (aged 24.8±4.3 with BMI of 22.1±2.3 (Mean ± SD)) participated in experimental sessions for 3 days under 2 conditions in random order in a laboratory. First day, subjects came laboratory at 16:00 and collected saliva samples every 1 hours (18:00-24:00). And on the second day, they conducted the simulated night work (0:00-8:00) and took a napping under different light intensities: >3000 lux (Bright light condition) and <50 lux (Dim light condition) (10:00-16:00). After napping, they collected saliva samples again in the same way as first day. Saliva samples were collected for measuring the concentration of melatonin hormone. Dim light melatonin onsets (DLMOs) were determined at both first and second day in both light conditions. Change in DLMO was compared between both light conditions by paired t-test. This study protocol was approved by the ethics committee of Kyoto University graduate school and faculty of medicine.

The changes in DLMO were significantly different between conditions ($p<.05$). DLMO on the napping under bright light was advanced for 11±17 (SEM, n=7) minutes, on the contrary, that under dim light was delayed for 7±14 (SEM, n=7) minutes.

In conclusion, DLMO in humans was changed by the light environment on napping after night work. Bright light could affect the biological rhythm through closed eyes. Therefore, we recommend that irregular shift workers should select bright or dim light environment on napping after night work depend on their next shift schedule.

Research Funding: The study was supported by JSPS KAKENHI grant number 15J08227.

T20

Insights into the Human Chronobiome

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Abstract: Physiological function and drug effects vary by time of day. Dysfunction of the molecular clock associates with increased disease incidence and severity as well as altered therapeutic safety and efficacy. What we know of the molecular clock in human biology is largely derived from forced

desynchrony experiments performed in highly controlled settings. Here we wished to perform a pilot study in n=6 healthy volunteers as a prelude to characterization of the human chronobiome "in the wild". This involves integration of remote sensing (activity, sleep, light exposure, communication, mobility, and dietary intake), clinical (blood pressure), and 'omics (metabolomics, proteomics, microbiomics, and transcriptomics) outputs under basal and perturbed conditions in apparently healthy individuals as a prelude to seeking alterations in patients who exhibit a time dependence in the incidence or severity of their disease. We find i) outcomes according to expectations, such as nocturnal dipping in blood pressure or oscillating cortisol levels in plasma and saliva; ii) internal consistency, for example, times of being active versus non-active correspond to diurnal rhythms of blood pressure, heart rate, communication, mobility and self-reported sleep; and iii) evidence of time-of-day dependent differences in the 'omics assessments iv) despite the observed inter-individual variability and the small sample size. This proof-of-concept study demonstrates the feasibility of characterizing the time dependent expression of the human chronobiome in free-ranging healthy volunteers. Such reference data are the prerequisite to parse patient phenotypes for evidence of temporal disruption.

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T21

Human Circadian Timing after Weekend Exposure to the Modern versus Natural Light-Dark Cycle

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Abstract: The invention of electrical lighting in the late 1800s revolutionized daily life. Our laboratory has demonstrated that modern daily living results in reduced natural light exposure and increased electrical light exposure. In addition, people often awaken later on the weekend reducing their exposure to morning light. This modern pattern has been shown to delay the timing of the human circadian clock. Late circadian timing has been reported to be associated with negative health outcomes such as obesity, mood disorders, and substance abuse. We therefore compared the impact of one weekend of exposure to natural light camping on the human circadian clock to a typical weekend living in the modern environment. Eleven moderately active participants (5 male) aged 25.3 ± 6.8y (±SD) participated in the 8 day protocol. Three days before an in-laboratory baseline circadian phase assessment, participants wore wrist actigraphs with photodiodes to document their habitual sleep timing and light exposure patterns. Circadian phase was then determined during a 24h melatonin rhythm assessment (< 8 lux maximum). Afterwards, participants either went camping for a weekend in the Rocky Mountains of Colorado (n=8) or spent the weekend in their typical home-social constructed environment. Circadian phase was then reassessed in the laboratory. The timing of the dim-light melatonin onset (DLMO) was used as the primary circadian phase marker. One weekend of exposure to the natural light-dark cycle while camping advanced the DLMO by approximately ~1.4h, whereas continued exposure to the habitual modern environment delayed the DLMO by ~1h ($P < 0.01$). Our findings provide further evidence that the circadian clock is timed later in modern society and demonstrate that a weekend camping successfully counteracts this delay.

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T22

Phase-Angle Differences between Dim-Light Melatonin Onset and Sleep Onset in Patients Diagnosed with Delayed Sleep Phase Syndrome

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Abstract: Background: In current literature, the majority of the population shows a dim-light melatonin onset (DLMO) around 10pm and a phase angle difference to sleep onset of approximately 2h. But until now no study has investigated the phase angle difference between these values for extreme evening types, namely patients diagnosed with delayed sleep phase syndrome (DSPS). Therefore, we collected DLMO as well as sleep diaries of those patients.

Methods: We compared 79 patients diagnosed with DSPS at a Sleep Medicine Centre and 9 controls. The mean age was 38.4 (SD=14.8); 46.6% were female. We collected saliva, melatonin and sleep diaries from all participants. The patient group was split into three subgroups according to their DLMO; G1: late bedtime, DLMO before midnight (n=17); G2: late bedtime, DLMO between 0am and 2am (N=26); G3; ultra late bedtime, DLMO after 2am (n=36). ANOVA (w/ Bonferroni correction) was used for statistical analysis.

Results: Controls mean DLMO was 10:21pm, the patients subgroups DLMO was 10:49pm (G1); 0:48am (G2) and 3:25am (G3); the groups differed significantly from each other (except G1 and controls). The reported sleep onset was 23:59pm in controls, 03:36am (G1); 02:20am (G2) and 03:34am (G3). The groups were all different between them with the exception of G1 and G3. The phase angle differences were 1:41h (controls), 4:46h (G1); 1:33h (G2) and 00:08h (G3). Phase angle differences differed significantly between groups, with the exception of G2 and controls. There were no significant group differences for age.

Conclusions: G1 showed late sleeping schedules but showed a DLMO close to the controls. We therefore considered them a "behavioural evening group". G3 showed the latest DLMO and the least phase angle difference, therefore called "sleep phase delay". The phase angle difference was only similar between G2 and the healthy group. For the extreme sleep onset groups (G1, G3), there were statistically significant differences in DLMO and sleep onset phase angle. This study enhances the importance of DLMO measurement for therapeutically intervention, suggesting the sleep diary as a poor indicator of circadian phase in DSPD patients. Furthermore, melatonin DLMO does not explain all late bedtimes. Diagnosis should be complemented by objective sleep onset measures, such as actimetry or polysomnography.

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T23

Glucocorticoid Signalling is Disrupted by Mistimed Sleep

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Abstract: Background. The 24-hour production of cortisol by the adrenal glands is strongly circadian, being driven, in part, by a rhythmic signal from the suprachiasmatic nuclei via the hypothalamic-pituitary-adrenal axis. In forced desynchrony protocols where sleep is mistimed relative to the central clock, as occurs during shift work, cortisol rhythms do not shift, although their amplitude can be somewhat reduced. Cortisol drives the glucocorticoid receptor signalling pathways, which regulate the expression of thousands of genes in peripheral tissues by both direct DNA binding and indirectly by modulating transcription factors. These pathways regulate many processes including peripheral circadian clocks, metabolism, cell development, immune responses and inflammation.

Methods/objectives. In a forced desynchrony study, we collected seven, four-hourly blood samples from 22 volunteers across a 28-hour day while they slept both in-phase and subsequently out-of-phase with their biological clock. We performed whole-genome transcriptome analyses on RNA extracted from these samples and compared expression profiles of components of the glucocorticoid signalling pathways during both sleep conditions using mixed-model ANOVA.

Results. During the forced desynchrony protocol, the phase of the central circadian clock, as indexed by the plasma melatonin rhythm, remained largely unchanged by sleeping out of phase, in agreement with previous studies. However, when we examined the expression profiles of genes involved in both the direct and indirect glucocorticoid signalling pathways, we observed significant temporal disruption of many components (e.g. HSP70, HSP90, P300, CBP, NCOR1, ERK2, P38, NFKB2) but not all (e.g., SRC, PCAF, ELK1, FKBP4). By contrast, in a separate study where RNA samples were collected during 40h of total sleep deprivation with or without one week of prior sleep restriction, the temporal organisation of these components was not affected.

Conclusions. These data show that downstream glucocorticoid signalling molecular components can be disrupted by mistimed sleep. Because glucocorticoid signalling regulates the expression of many genes associated with biological pathways linked with health, this has implications for the increasing numbers of shift workers.

Research Funding: UK Biotechnology and Biological Sciences Research Council (BBSRC; BB/F022883), USA Air Force Office of Scientific Research (AFOSR; FA9550-08-1-0080)

T24

Identifying Circadian Transcripts in Human Subcutaneous Adipose Tissue

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Abstract: Circadian rhythmicity is seen in a host of biological processes, including the transcriptome. In humans, blood has been analysed for circadian variation in the transcriptome under different

conditions, but serial sampling of other human tissues for transcriptome analysis is difficult. Using a biopsy technique developed by our group, subcutaneous adipose tissue was taken in healthy males (n=5) under the highly controlled laboratory conditions of a constant routine (37 hours, constant wake, semi-recumbent posture, dim light <8lux, hourly isocaloric meals, social isolation).Five biopsies from each participant were taken at six hourly intervals for microarray analysis.

Transcriptome analysis revealed 448 circadian probes (1% of 41619 total probes in the array), which are defined as fulfilling the dual criteria of being circadian (having an R2>0.8 for the circadian model) in 3 or more individuals as well as under a single fit model (model is fitted to all 5 subjects at once where you have subjects as random effects, R2>0.8). The most robustly circadian transcripts were the canonical clock genes ARNTL, NPAS2, PER1, PER2, and PER3, CRY2, NR1D1 and DBP which were circadian in all participants. The peak timing of all circadian probes showed bimodal distribution with morning and evening peaks. Cluster analysis of circadian probes identified 302 as evening peaking and 146 as morning peaking. Circadian genes PER1, PER2, and PER3, CRY2, NR1D1 and NR1D2, and DBP peaked in the early morning, and ARNTL peaked in the late evening, consistent with previous studies in our laboratory and elsewhere. Gene ontology enrichment analysis identified a separation of biological processes in morning and evening peaking clusters. Evening peaking probes were primarily involved with fatty acid and coenzyme metabolism.The dominant cluster of morning peaking probes was involved in circadian rhythm regulation, as well as involvement in RNA and nucleic acid metabolism.

This work shows the key metabolic processes under circadian variation in the human adipose tissue transcriptome. It represents the first circadian transcriptome analysis from a key metabolic tissue in humans.

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T25

Sleep-Wake Rhythms and Safety: Using a Meta-Analytic Risk Index Model to Predict Occupational Injuries

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Abstract: Introduction. Fatigue is a major risk factor for occupational accidents and injuries, and is largely due to insufficient, non-recuperative or mistimed sleep. Laboratory-based bio-mathematical fatigue models have often failed to accurately predict real-world accident risk leading to the proposal of a "Risk Index" to estimate the risk of human error on different work schedules based on the empirical risk of accidents and injuries. There are four different components of work schedules included in the "Risk Index" (i.e. duty length, timing and number, and rest break interval), which showed good predictive power for aggregated data. To further improve the individual -level predictive value, we aim to include relevant individual characteristics, such as sleep duration and preferred sleep timing ('chronotype').

Methods. Currently, we are conducting a systematic literature search on each model component using PubMed and Embase. Cochrane Collaboration directives and guidelines for meta-analysis of

observational studies in epidemiology (MOOSE) are followed. An inverse variance approach to meta-analysis will be used to synthesize effect sizes and estimate between-studies variance ('heterogeneity').

Results. Parameter values of the original Risk Index model will be updated and the new variables added (i.e. individual level components of sleep duration and chronotype). Relative risks will be presented across a range of pre-defined values of each parameter in the form of a 'heat map' visualizing regions of high and low injury risk as a function of sleep length and timing.

Conclusion. Workers' safety is a major challenge to industrialized societies. Sleep-wake behavior that is disrupted by unusual or extended work times plays an important role in safety management. The Risk Index integrates both, occupational and individual characteristics to predict empirically work-related accident and injury risk and can serve as a tool for researchers and practitioners to evaluate hazards and maximize safety across different work schedules.

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T26

Inflammatory Markers during Night Work and Vacation

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Abstract: A recognized consequence of night shift work on employees is the loss of internal temporal order, which results in pathophysiological changes. This study aimed to evaluate the effects of night shift work and its recovery during downtime on the levels and circadian rhythmicity of inflammatory cytokines. Fifteen volunteers from day shift and 15 from night shift and the same 15 from night shift during vacation participated in this study. During seven consecutive days, sleep and wakefulness patterns were recorded. In addition, saliva and urine samples were collected during this 7-day period on one work day and one day off. Melatonin, IL-1β, and IL-6 concentrations were quantified in saliva samples every three hours while subjects were awake. Furthermore, urine levels of 6-sulphatoxymelatonin were collected after the main sleep episode. Nested ANOVA followed by Dunnet's test for multiple comparisons; individual and population Cosinor were used. Night shift workers had a lower amplitude rhythm of salivary melatonin, higher concentrations of salivary IL-6, reduced concentration of urine 6-sulphatoxymelatonin than day shift workers. Its last concentration was higher during vacation than work time, but no significant results were observed for the cytokines. During vacation, night shift worker main sleep episode duration was 128,1 minutes longer than during shift work days. In conclusion, night shift workers were synchronized and showed no signs of sleep deprivation but higher inflammation level compared with day shift workers. However, during vacation, sleep duration and melatonin secretion during the main sleep were increased. These findings highlight the negative health consequences that night shift work can cause on the immune system and show that there are partial reverse effects of these changes during a short-time vacation, suggesting the need for an increase in time off and vacations.

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T27

Photoperiod Influences Circadian Rhythms of Adaptive and Innate Immune Responses of Male Siberian Hamsters

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Abstract: Changes in photoperiod markedly affect innate and adaptive immune function in Siberian hamsters and other species. Most reports of photoperiodic effects on immune function are derived from interventions performed once, at a specific phase of the light-dark cycle (e.g., midpoint of the light phase, shortly before dark onset, etc.). Circadian rhythms in a wide array of immune responses are well documented. In any photoperiodism study, there can be no perfect matching of circadian phase if waveforms differ (e.g., in long vs. short photoperiods). These experiments examined whether photoperiodic changes in immunity reflect seasonal phenotypic changes that persist throughout the circadian cycle, or instead are influenced by photoperiodic differences in circadian waveforms. Adult male Siberian hamsters were exposed to long (15L:9D; LD) or short (9L:15D; SD) photoperiods. In experiment 1, skin inflammatory responses (delayed type hypersensitivity reactions; DTH) were examined; in experiment 2, innate immune responses (sickness responses to i.p. LPS treatment) were assessed. In both experiments, separate groups of hamsters were immune challenged at 3 h intervals across the circadian cycle. Data were aligned relative to light-onset, dark-onset, and light-midpoint, to determine if phase alignment influenced effects of photoperiod on these immune responses. DTH reactions varied markedly over the course of the circadian cycle: DTH was significantly greater in SD relative to LD hamsters at select times-of-day, but not at others. Data to be presented will specify whether the typical SD-attenuation of sickness responses to LPS is present throughout the circadian cycle, or only manifests at select time points. Together, these experiments offer insights into the relative contributions of photoperiod per se and photoperiod-driven changes in circadian waveform on photoperiodic changes in immunity.

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T28

Circadian Control of CD8+ T Cell Response

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Abstract: Circadian clocks are located in most tissues, including secondary lymphoid organs. Circadian clocks control different aspects of the immune system such as cytokine secretion by various innate immune cells. However, the links between circadian rhythms and the adaptive (antigen-specific) immune response remains poorly understood. Previous work in the laboratory showed a circadian rhythm in T cell proliferation after a stimulation of the antigen-specific T cell receptor (TCR). We hypothesized that the response of T cells to antigen presentation by dendritic cells (DCs) presents a circadian rhythm and that this rhythm is controlled by a circadian clock within the T cells. To test the first hypothesis, we immunized mice every 4 h over 24 h in constant darkness with DCs loaded with a

peptide antigen from ovalbumin (OVA), and measured OVA-specific T cell responses after 7 days, the time at which the peak of T cell response occurs. We found higher T cell responses after immunization during the middle of the subjective day (CT6) than the rest of the day, and a consistent rhythm of effector functions. Indeed, interferon gamma (IFNγ)-producing T cells showed a 24 h rhythm, but interestingly, there was no rhythm for the frequency of CD8+ T cells expressing interleukin-2. To test the involvement of the T cell clock in the rhythm of response to antigen presentation, we used the Cre-lox system to knock out the Bmal1 gene (an essential clock gene) specifically in CD8+ T cells. We crossed E8I-Cre mice, expressing the Cre recombinase only in mature CD8+ T cells, with mice with loxP site-containing Bmal1 gene. As expected, there was a rhythm in CD8+ T cell expansion and INFγ production after immunization every 6 h over 24 h in wild type mice. Importantly, the rhythms were lost in the CD8+ T cell Bmal1 knock out mice. Thereby, the CD8+ T cell-intrinsic clock is essential for the circadian rhythm of T cell response to immunization. Understanding how the clocks regulate T cell functions could lead to improvements of vaccination as well as better understanding of how the adaptive immune response can be tuned to more efficiently fight pathogens and tumor cells.

Research Funding: Canadian Institutes of Health Research

T29

Cyclically Expressed Heme Oxygenase Protects the Fruit Fly's Retina against Light-Induced Damage

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Abstract: Heme oxygenase (HO) is an enzyme that degrades heme to carbon monoxide (CO), ferrous ions, and biliverdin. In mammals there are two HO isoforms, which differ in distribution and functions, but they both act as cytoprotective and anti-apoptotic agents by scavenging reactive oxygen species (ROS). In Drosophila melanogaster there is only one gene encoding HO that plays an important role in development and in controlling the signaling pathway of DNA damage. In our previous study we showed that ho is rhythmically expressed in the retina, with two peaks at the beginning of the day and during the night. We have also found that HO affects the circadian clock in the retina.

In our present study we investigated the role of HO in the retina in the morning, during the first peak of ho mRNA rhythm. This peak is maintained in light/dark (LD12:12) and in constant darkness (DD) conditions. We found that 1h light pulse in DD enhances the level of ho expression. This effect was observed only at CT1 but not at other time points. Interestingly, the canonical phototransduction pathway is not involved in this process, since in norpA mutants the level of ho mRNA was still increased after light exposure at CT1. On the other hand, this effect was depended on PER because in per0 the expression of ho was activated by light not only at CT1 but also at other time points.

To conclude, ho gene expression is clock-controlled and the rhythm of ho mRNA is bimodal in the retina of wild-type flies. Moreover, the expression of ho is enhanced by light in the morning and this process is PER-dependent. The role of HO in the retina is unknown but there are evidences that it may protect photoreceptors from the oxidative stress caused by daylight in the morning.

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T30

Beta-Arrestins Shape Melanopsin-Dependent Responses to Light

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Abstract: Melanopsin is an opsin class of G-protein coupled receptor (GPCR) expressed in a small subset of retinal ganglion cells which are intrinsically photosensitive (ipRGCs). ipRGCs contribute to the brightness perception of vision, while they are indispensable for non-image forming visual responses including light modulation of pupil constriction, circadian rhythm, sleep and mood. However, mechanisms behind unique responses properties of ipRGCs (slow activation, light integration and delayed deactivation) are still poorly understood.

Here we explored the role of β-arrestins in melanopsin signaling. Arrestins are multifunctional adaptor proteins that, once recruited by an activated GPCR, promote signal termination and receptor internalization. Only β-arrestin 1 and 2 are expressed at detectable levels in the ipRGCs. We first showed in vitro that melanopsin functionally interact with both of them upon light stimulation and subsequent phosphorylation of melanopsin C terminus tail. We then tested the role of the two β-arrestins by measuring ipRGCs electrophysiological responses in either β-arrestin deficient mice retina or model where we over-expressed β-arrestins. Finally, we showed that alterations observed in vitro translated in vivo by assaying negative phototaxis and pupillary reflex to light.

This study identified the key and distinct roles of β-arrestin 1 and 2 in melanopsin signaling and shows that both are critical for proper adaption of the animal behavior to its environment. Melanopsin has recently catalyzed general attention on the importance of the light input to the brain and its impact on health. Knowledge of this novel photopigment functioning will enable the design of better therapeutic strategies.

Research Funding: Fyssen foundation, Catharina foundation

T31

Colour Processing in the Non-Image Forming Visual System

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Abstract: Information about daily changes in light levels regulate a range of physiological and behavioural parameters via the 'non-image forming' (NIF) visual system, a diverse set of brain regions that receive dense input from melanopsin-expressing retinal ganglion cells (mRGCs). We recently demonstrated that a major component of this system, the suprachiasmatic circadian clock, receives chromatic input and is thus sensitive to changes colour of daylight. Here we set out to investigate the contribution of colour signals to visual processing in the other major NIF system components in mice: the intergeniculate leaflet/ventral lateral geniculate nucleus (IGL/vLGN) and the pretectal olivary nuclei (PON). We first establish and validate a modification of our previous approach for manipulating cone photoreception (which relied on animals with altered cone sensitivity), allowing us to study chromatic processing in wildtype animals. We next use multielectode recordings to show that blue-ON chromatic signals are highly enriched in both and IGL/vLGN region and especially the

PON, relative to neighbouring visual regions. Finally, in a subset of recordings we use an optogenetic-based strategy to identify the neurochemical phenotype and possible roles of chromatic units. Together our data are consistent with the notion that a subset of mRGCs convey chromatic signals that are used to regulate a range of NIF visual functions in addition to circadian photoentrainment, such as control of pupillary responses.

Research Funding: BBSRC

T32

Differential Roles for Mammalian Cryptochromes in the Retinal Circadian Clock

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Abstract: Cryptochromes (CRYs) are key components of the negative limb of the mammalian circadian clock. As in most tissues, CRY1 and 2 are expressed in the retina where they are thought to play a role in regulating rhythmic processes, including the retinal clock. Studies differ in consensus as to their localisation and function, with CRY1 thought not to be expressed in the retina. However, many commercially available CRY antibodies do not provide specific staining, and produce a similar signal in retinae from mice lacking CRY.

Here, we describe a novel pattern of CRY1 and CRY2 expression using newly raised CRY antibodies that have undergone extensive validation (Anand et al., 2013, Maywood et al., 2011), showing that CRY1 is indeed expressed throughout the mammalian retina, whereas CRY2 is restricted to the outer retina. Colocalization studies were performed with CRY1 and CRY2 and an extensive set of retinal cell markers to determine CRY1 expression in various retinal cell types. Colocalization data between CRY1/2 and clock proteins CLOCK and PER1, and the circadian photopigment OPN4 (melanopsin) are also reported.

We then examined the role of CRY1 and CRY2 in retinal circadian rhythms. Cry1-deficient mice were found to have reduced retinal PER2::LUC circadian rhythm amplitude and stability whilst Cry2-deficient mice exhibited retinal rhythms comparable with wildtype animals. Finally, using in vivo assays, we go on to show that rhythms in retinal physiology, including the photopic electroretinogram, contrast sensitivity and pupillary light response are all attenuated or abolished in Cry1-deficient mice. By contrast, these physiological rhythms are largely unaffected in mice lacking Cry2. Together, our data suggest that CRY1 is an essential component of the mammalian retinal clock, whereas CRY2 appears to be functionally redundant.

Research Funding: Electric and Magnetic Fields Biological Research Trust

T33

Are Intrinsically Photosensitive Retinal Ganglion Cells (ipRGCS) Necessary for Light Entrainment of Peripheral Clocks?

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Abstract: Background: The suprachiasmatic nucleus (SCN) in the hypothalamus houses the central circadian pacemaker and orchestrates autonomous circadian clocks in peripheral tissues to generate rhythms of metabolism and physiology. While the SCN biological clock follows an intrinsic period of ~24 hours, neurochemical signals from the retina provide photic information for its precise alignment to environmental light-dark cycles. This photic information is conveyed by a subpopulation of retinal ganglion cells (ipRGCs) that express the photopigment melanopsin (Opn4) and therefore respond to light even in the absence of rod and cone photoreceptor input. It is still unclear whether ipRGCs are the sole pathways for photic entrainment of the peripheral clocks present in nearly every body cells.

Objectives/methods: In this study we have assessed the role of ipRGCs in synchronizing peripheral clocks to environmental light-dark cycles in mice. ipRGC neurotransmission was silenced by expression of tetanus toxin light chain fragment (TeNT) by crossing the Opn4Cre/+ with R26RTeNT/+ mouse line that have Cre recombinase-controlled expression of TeNT. The rhythmicity of peripheral clocks were monitored by intercrossing Opn4Cre/+ , R26RTeNT/+ with mPer2Luciferase (mPer2Luc) knockin mice.

Results: non-image-forming visual behaviors such as pupillary light reflex, and photoentrainemnt of circadian locomotor activity were effectively abolished in Opn4Cre/+ , R26RTeNT/+ mice. Moreover, light-induced c-fos expression in the SCN were largely reduced in the mutant mice in comparison to littermate controls. Both SCN and peripheral tissues in explant cultures from Opn4Cre/+ , R26RTeNT/+, Per2Luc/+mice show robust and self-sustained circadian rhythms for several days which followed the circadian locomotor activity but not the environmental light-dark cycles. Likewise, measurements of corticosterone secretion using subcutaneous microdialysis probes and sampling over several days shows that adrenal glucocorticoid secretion to be “free running” in the mutant mice.

Conclusions: Overall these results indicate that ipRGCs are required for the synchronization of circadian clocks in peripheral tissues to environmental light-dark cycles.

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T34

Circadian Forced Desynchrony Leads to Behavioral Manifestations of Depression in Rats

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Abstract: In mammals, circadian rhythms are coordinated by a master circadian clock within the suprachiasmatic nucleus (SCN) of the hypothalamus that governs a wide array of behavioral and physiological rhythms either directly or through inputs to extra-SCN circadian oscillators that locally

time tissue-specific processes. Thus, the SCN master clock is responsible for maintaining phase coherence within a complex network of body oscillators. Affective disorders are typically associated with disruption of this fine-tuned 'internal synchronization' and it is believed that the internal misalignment of circadian rhythms may represent a contributing factor for the onset of mood disorders. To date, depressive-like behavior in animal models has been induced by aversive or invasive methods that do not necessarily concur with the etiology of affective disorders in humans; moreover, they fail to specifically target the circadian system as the mode to generate phenotypic manifestations of mood. We have developed 'forced desynchrony' protocol in rats, where mere exposure to a symmetric 22 light-dark (LD) cycle (LD22) leads to the uncoupling of two distinct populations of neuronal oscillators within the SCN. Forced desynchronized rats present all key properties of human forced desynchrony including desynchronized circadian regulation of temperature, sleep stages, melatonin and cortisol. Hence, this genetically, neurally and pharmacologically intact animal model represents a unique opportunity to assess the effect of a systematic challenge to the central circadian pacemaker on behavioral manifestations of mood disorders. We show that forced desynchrony of circadian rhythms in rats induces depressive-like phenotype including anhedonia, sexual dysfunction, and increased immobility in the forced swim test, as well as changes in the levels and turnover rates of monoamines within the prefrontal cortex. Our results support the notion that the internal misalignment of circadian rhythm induced by environmental challenges to the circadian system constitutes part of the etiology of depression.

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T35

Circadian Clocks Modulate Huntington's Disease via Stress Response Pathways

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Abstract: Neurodegenerative disease is frequently associated with disrupted sleep and circadian rhythms. Yet it is unknown if the clock regulates neurodegenerative disease and if so, which molecular pathways link the clock to neuronal function and survival. To address this question, we are using a model of Huntington's disease (HD) in the fruit fly Drosophila. Huntington's disease is caused by a polyglutamine expansion in the Huntingtin protein (HTT) and this expansion is

accompanied by disrupted circadian rhythms and a reduction in clock neurons in affected individuals and in mammalian models. When we expressed pathogenic human Htt (HttQ128) in a subset of circadian clock neurons, transgenic flies lose their normal 24 h rhythm of locomotor activity as well as loss of a subset of their clock neurons, the small ventral lateral neurons (sLNv). To test the role of the circadian clock in this process, we examined mutants of the core circadian gene Clk. Surprisingly, we found that neurodegeneration was reduced in poorly rhythmic Clk mutants. To independently perturb clock function, we exposed flies to 10 h light: 10 h dark cycles instead of their normal 12:12 cycles. Contrary to expectation this circadian "misalignment" was neuroprotective in our HD model. These data further support the notion that modulation of circadian clocks alters neurodegeneration in a fly model of human disease. To identify the clock effectors of these neurodegeneration effects we employed FACS sorting of clock neurons in combination with RNA-seq at different times of day. We then systematically knocked down the expression of these genes using RNAi and looked for suppressors of the HttQ128 induced behavioral arrhythmia. Using this approach, we have identified

and validated multiple cycling suppressors including protein chaperones and stress granule components including the translational regulator Ataxin2 (Atx2), the latter is also a key activator of period translation. Detailed phenotypic analysis suggests that suppressors of HttQ128 affect Htt levels, aggregation, cell loss and/or cell function. Taken together these studies reveal the molecular pathways linking the clock to neurodegeneration and suggest that oscillating genes may impact early stages of the disease.

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T36

Selective Inhibition of Casein Kinase I Delta Enhances Hippocampal Dependent Learning and Alters Expression of Circadian Clock Proteins in the Hippocampus

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Abstract: The circadian clock may be essential for learning and memory, an idea supported by the presence of an autonomous circadian clock in the hippocampus. Therapeutics that target circadian dysfunction may therefore help treat associated cognitive decline in disorders such as Alzheimer's Disease and schizophrenia. Casein kinase 1 (CK1) is part of a family of kinases with a variety of roles within and outside of the circadian clock. Its delta isoform (CK1Δ) functions in part to release the "brake" of the molecular clock by phosphorylating Period 2 (PER2) in the cytoplasm, marking it for proteasomal degradation and allowing a new 24-hour cycle to occur. The goals of this study were to test whether pharmacological inhibition of CK1Δ could improve performance in learning and memory tasks, and whether this improvement would be associated with changes in clock protein expression in the hippocampus.

C57BL/6J wild type (WT) mice, and mice with mutations in the two major period genes (PER1PER2) were treated with CK1 inhibitor PF-670462 at a dose that specifically inhibits the CK1Δ, or vehicle. PER1PER2 mutant mice have disrupted circadian rhythms but have not been shown to have learning and memory deficits. During treatment, Morris Water Maze (MWM) and Fear Conditioning (FC) performance were tested to assess the impact of treatment and genotype on learning and memory, and Elevated Plus Maze (EPM) and Forced Swim Test (FST) performance were tested to assess and control for differences in anxiety, affect, and locomotor performance. After testing, hippocampal and hypothalamic tissue from study animals was subjected to western blotting to assess the effect of Ck1Δ inhibition on clock protein expression. In vitro, we exposed HT22 hippocampal neurons to the same inhibitor and measured changes in the cycling levels of negative arm clock proteins.

In WT but not PER1PER2 mutant mice, CK1Δ inhibition improved performance in contextual FC and 72h MWM. PER2 showed a trend toward upregulation in the WT hippocampus ~24 hours after treatment.

The enhancement in hippocampal-dependent learning and increase in hippocampal PER2 in WT but not PER1PER2 mutant mice treated with a CK1Δ inhibitor supports the hypothesis that memory can be improved by altering the molecular clock in the hippocampus.

Research Funding: Departmental funds, USF Health Byrd Alzheimer's Institute grant

T37

Behavioral and SCN Neurophysiological Disruption in the Tg-SwDI Mouse Model of Alzheimer's Disease

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Abstract: Alzheimer's disease (AD) is a progressive neurodegenerative form of dementia associated with elevated levels of the amyloid β (Aβ) peptide and the formation of pathological Aβ plaques and neurofibrillary tangles. Patients with AD often exhibit a constellation of symptoms known as "sundowning syndrome," as well as misaligned core body temperature and activity rhythms, suggesting a dysregulated circadian network. In mammals, the primary circadian pacemaker is located in the suprachiasmatic nucleus (SCN). SCN neurons exhibit daily rhythms in spontaneous action potential (AP) firing which are critical for robust and consolidated circadian behavior. Loss or dampening of SCN neuronal activity rhythms has been described in animal models of other neurodegenerative disorders and aging; however, changes in SCN neurophysiology in AD models have not yet been examined. In this study we examined circadian behavioral and electrophysiological changes in a mouse model of AD, using the Tg-SwDI line which expresses human amyloid precursor protein with the familial Swedish (K670N/M671L), Dutch (E693Q), Iowa (D694N) mutations. The free-running period of wheel-running behavior was significantly shorter in Tg-SwDI mice compared to wild-type (WT) controls at all ages examined (3, 6, 8 & 10 months; $p < 0.05$). At the SCN level, the day/night difference in spike rate was significantly dampened in 6-8 month-old Tg-SwDI mice ($p < 0.001$), with a decrease AP firing during the day and an increase in neuronal activity at night. The dampening of SCN excitability rhythms in Tg-SwDI mice was not associated with changes in input resistance, resting membrane potential, or AP waveform properties; however, SCN neurons from female Tg-SwDI mice had significantly reduced A-type potassium current (IA) compared to WT cells during the day and at night ($p = 0.05$). Ongoing experiments will examine IA in male Tg-SwDI and WT mice of the same age. Taken together, these results provide the first evidence of SCN neurophysiological disruption in a mouse model of AD, and highlight IA as a potential target for AD treatment strategies in the future.

Research Funding: This work was supported by NIH 1R01NS082413 to KLG, F31NS086282 to JRP, and P30 NS47466.

T38

Circadian Abnormalities in the Btbr Mouse Model of Autism Spectrum Disorder

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Abstract: Sleep difficulties are common in children and adolescents with autism spectrum disorder (ASD) and have been proposed, in part, to be caused by impairments in the underlying circadian system. Children with ASD often exhibit abnormal circadian-related hormonal profiles. Moreover, there is a higher incidence of mutations in genes involved in circadian function in ASD patients compared to controls. Given these observations, we asked whether there are alterations in circadian rhythms in a commonly used mouse model of ASD. BTBR mice, which display all core features of ASD,

were examined under entrained and free-running conditions. BTBR mice had a significantly shorter circadian period ($p<0.001$) and a briefer alpha segment ($p<0.001$) compared to C57Bl/6 controls. No significant differences, however, were found when comparing phase angles ($p>0.05$), or phase shifts to 15 min light pulses ($p>0.05$). Thus, despite changes in the circadian period and alpha segment, the entrainment of BTBR mice to the external light-dark cycle appeared preserved. Further work examining suprachiasmatic nucleus anatomy is ongoing. Finally, as a high-fat, low-carbohydrate ketogenic diet has been shown to ameliorate behavioural features in BTBR mice, we are determining whether such a ketogenic diet can reverse the circadian abnormalities observed herein.

Research Funding: NSERC

T39

Alcohol Abuse in Circadian Desynchrony: Impact of Age, Genetics, and Environment

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Abstract: Background: Circadian desynchrony (CD), the stressful state that occurs when one's internal clock is out of sync with the external time, exists in many populations, including teenagers waking early for school, shift workers such as health care professionals and truck drivers, and patients with diseases like schizophrenia and depression. All of these populations show increased rates of alcohol abuse and dependence, and mutations or polymorphisms in key circadian genes are also associated with increases in alcohol abuse. Thus, understanding how CD impacts alcohol intake may provide novel pathways to treat alcohol use disorders. Nonetheless, it has proven difficult to model CD-induced changes in alcohol consumption in mouse models, which is an important step toward identifying the mechanisms by which CD increases alcohol intake. In our laboratory, we have examined the effects on alcohol intake of both genetic CD, in mice mutant for the Period1 and Period2 genes, and environmental CD, using light- and temperature-controlled housing with 24-hour recording of activity and fluid intake. We have also begun to assess how chronic stress interacts with CD to alter alcohol intake. Methods: In Study 1, we examined free access alcohol, water, and food intake in adult wild-type and Per mutant mice, as well as alcohol preference, sensitivity, and metabolism during a normal light:dark cycle (LD). In Study 2, we assessed free access alcohol and water intake, and wheel-running activity in adult and adolescent wild-type mice during normal LD and during CD. In Study 3, we assessed free access alcohol, water, and food intake in adult and adolescent wild-type and Per mice during normal LD and chronic stress. Results: Both genetic and environmental CD increase alcohol intake, although this effect is more pronounced in adult mice. This increase may be explained by changes in alcohol reinforcement and metabolism. In addition, stress interacts with CD to further increase alcohol intake. Conclusions: Using both genetic and environmental models of CD, we demonstrate that CD can increase alcohol intake, and push mice to a binge-like pattern of alcohol intake, especially in the presence of other stressors. Furthermore, adults are more sensitive than adolescents to the effects of CD and stress on alcohol intake.

Research Funding: Departmental Start-up funds; Byrd Institute Seed grant; University of South Florida New Investigator grant.

T40

Transgenerational Epigenetic Effects of Cocaine on Circadian Behavior and Cocaine Reward

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Abstract: Cocaine irreversibly lengthens circadian period (tau), which could underlie the significant health issues of cocaine addiction. Others have reported that rewarding effects of paternal cocaine use are transgenerational. We hypothesize that the disruptive effects of cocaine on tau may also be transgenerational, causing altered subjective cocaine reward response in offspring (F1). Male C57Bl/6J mice ~6 wks were exposed to forced cocaine-water (0.5 mg/ml; experimental) or water (control) for 6 wks. Immediately following this treatment, the mice were harem-mated with cocaine naïve females. Offspring (22 male, 21 female) were weaned after three weeks and then behaviorally phenotyped for cocaine or sucrose (to test for reward specificity) preference using a dual bottle (water and drug [0.15 mg/ml cocaine] or water and sucrose [2%]) free-choice regimen. Tau was analyzed in individual mice by measuring circadian activity using passive infrared activity sensors and ClockLab analysis. A long-term (possibly permanent) lengthening of tau after drug withdrawal was evident in sires with forced cocaine compared to water controls (24.18± 0.17 h vs. 24.07±0.02 h; $p<0.05$). Tau and alpha were not altered in the F1's. However, preference for cocaine was decreased in cocaine-sired F1 males compared to those from control sires (49.36±0.67 vs. 54.929±0.78; $p<0.05$). In contrast, cocaine preference in cocaine-sired F1 females was increased compared to those from control sires (57.74±0.77 vs. 53.21±0.74; $p<0.05$).). There were no such differences in F1 sucrose preference at this dosage. These data reveal that there is no transgenerational transmission of a cocaine-lengthened tau phenotype. Significantly, however, paternal cocaine exposure significantly altered F1 preference for cocaine, but not sucrose, suggesting a selective effect on cocaine reward mechanisms. Thus, cocaine addiction could be influenced by a transgenerational paternal mode of epigenetic inheritance.

Research Funding: Department of Biological Sciences at Kent State University

T41

Measuring Time in Adipocytes-The Effect of Insulin on Clock Gene Expression

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Abstract: Obesity is a critical risk factor for a number of chronic diseases including diabetes mellitus type 2, cardiovascular diseases and cancer. Accordingly, adipose tissue biology has received increased attention. White adipose tissue (WAT) is now identified as a dynamic organ regulating body weight-, glucose homeostasis and systemic inflammation. Importantly, WAT underlies the hierarchy of the circadian system. It is known that feeding is a major synchronizer of the circadian clock in peripheral tissues. Little is known, however, about the underlying mechanisms. We tested, whether the postprandial hormone, insulin, can influence circadian dynamics in adipose tissue and in adipocytes specifically. Using epididymal fat of PER2::LUCIFERASE knockin reporter mice we show a substantial increase of PER2 protein expression after insulin stimulation. Further, we observe a subsequent shift in the circadian rhythmicity of PER2, whose extent is dependent on the phase of

insulin stimulation. We also show that per2 mRNA expression is up-regulated upon insulin treatment in 3T3L1 adipocytes. This implies that insulin synchronizes the clock on both protein and mRNA level. We hypothesized that insulin acts on Per2 expression via the cAMP response element (CRE) located in the per2 promoter. Preliminary data show, however, that insulin mediated induction of per2 expression also is likely be mediated by other factors. Using reporter constructs we will test which of the per2 promoter regions are important for the insulin-mediated induction. Identifying the relevant reporter regions will allow to asses which transcription factors are involved in per2 activation and will illuminate feeding mediated clock-synchronizing pathways.

Research Funding: DFG funding and scholarship for the accomplishment of the PhD thesis.

T42

Circadian Rhythms DISTURBANCES, Depression and Type 2 DIABETES: Possible Interrelationships

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Abstract: We explored the effect of short photoperiod (SP) acclimatization on the development of diabetes and depressive-like behavior utilizing a non-traditional diurnal animal model.

It is widely accepted that circadian rhythms disruption is associated with a number of disorders, including type 2 diabetes and depression. Diabetic patients are 2-3 times more likely than the average person to have depression and people with depressive disorders have a 65% increased risk of developing diabetes. This alludes to disruption of circadian rhythms as a common risk factor and a possible underlying mechanism for the development of these disorders.

We hypothesize that basic changes, which accompanied the switch from the ancestral nocturnal activity pattern of mammals to the current diurnal one, reduced the robustness of the daily rhythms of diurnal species, and consequently, increased their susceptibility to circadian rhythm-related diseases such as diabetes and depression.

Understanding the involvement of circadian rhythms disturbances in the development of diabetes and mood disorders will offer new avenues aiming at resetting and enhancing the circadian clock, thereby alleviating symptoms and improving the patient's quality of life

Using the diurnal fat sand rat - Psammomys obesus as an animal model we conducted 2x2 experiments, with day length (SP vs. neutral photoperiod) and diet (standard rodent diet (SRD) vs. low energy diet) as variables.

Both SP acclimatization and SRD resulted in significantly reduced glucose tolerance and increased fasting blood insulin levels, with a significant interaction. SP acclimatization, but not SRD, resulted in increased despair behavior in the forced-swim test. Both SP and SRD resulted in significantly increased systolic blood pressure with no interaction.

SP acclimatization accelerated the onset of diabetes and resulted in depressive-like behavior. We suggest that reduced robustness of the circadian system of diurnal species increases their susceptibility to diabetes and depression. We believe that using diurnal animal models to study circadian rhythms related diseases might lead to the development of more effective treatments and recommendations for better artificial lighting usage, which will enhance daily rhythms.

Research Funding: The research is funded by the Israel Endocrine Society.

T43

Effects of a Forced Desynchrony Protocol on Feeding Patterns and Glucose Tolerance in C57BL/6J Mice

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Abstract: In humans, chronic disruption of the circadian system leads to negative health outcomes, including increased incidences of diabetes and obesity. The aim of this study was to determine how uncoupling central and peripheral circadian rhythmicity would affect the health of male C57Bl/6J mice on either a high fat or low fat diet. The central circadian pacemaker (suprachiasmatic nucleus) is synchronized by environmental light/dark cycles while rhythmicity in peripheral metabolic tissues (liver, pancreas) tend to be synchronized by feeding rhythms. In an attempt to produce desynchronization between central and peripheral rhythms, mice were subjected to 24-hour feeding rhythms (via 12 hour daily food restriction; T24) and 23 hour light/dark cycles (T23). Locomotor activity was monitored by infrared motion detectors. Control animals were subjected to either 24-hour light rhythms and 24-hour feeding rhythms (restricted feeding); T23 light cycle with ad lib food availability; or T24 light cycle with ad lib food availability. Locomotor rhythms entrained to both T24 and T23, albeit with slightly delayed phase angles. Feeding activity was expanded more into daytime hours in mice fed ad libitum. High fat diet significantly increased body weight and impaired glucose tolerance in the mice, regardless of light cycle. In mice on low fat diet there was a trend toward better glucose tolerance in restricted versus ad lib food availability, while differences were less prominent in mice on a high fat diet. Mice on the T23 light cycle had slightly lower glucose tolerance than their T24 counterparts. Mice on the forced desynchronization protocol tended to have a lower glucose tolerance (not statistically significant). In summary it cannot be concluded that the forced desynchronization protocol yielded significant changes in glucose regulation.

Research Funding: Rider University Department of Biology

T44

Oscillations in Bat Thermogenesis are Independent of the Adipocyte Circadian Clock

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Abstract: The misalignment between the internal circadian clock and the timing of behavior/ activity has been implicated to changes in energy homeostasis that lead to obesity in both humans and mice. Brown adipose tissue (BAT) thermogenesis produces heat used for the maintenance of body temperature and contributes to total energy expenditure. Here we investigate the dependence of diunal oscillations in BAT thermogenesis on functional circadian clocks. Global post-natal deletion of Bmal1 in adult mice abolishes the rhythms of core body temperature and reduces mean temperature both at room temperature and thermoneutral environmental temperature, while animals maintain normal locomotor activity under regular 12h light 12h dark schedule. Knockout animals show normal body heat loss and fur, skin insulation suggesting a defect in heat production. Mice with adipocyte

specific deletion of Bmal1 (Ad-Bmal1-/-) in contrast, maintain both rhythms and normal levels of body temperature under room temperature and thermoneutral environmental temperature. Deletion of Bmal1 has no effect on the induction of uncoupling protein 1 (UCP1) in BAT of mice exposed to cold. The weight, morphology and mitochondrial content of brown adipose tissue of knockout mice is indistinguishable to wild-type mice both at thermoneutrality and after prolonged exposure to cold. Global Bmal1 knockout mice show increased drop of body temperature in response to cold exposure during both peak and trough BMAL1 activity. In contrast, Ad-Bmal1-/- mice are able to defend body temperature in response to cold exposure. Shivering in response to cold appears normal in both groups. Our findings suggest that BMAL1 in adipocytes is not required for BAT thermogenesis and diurnal oscillations of BAT thermogenesis are driven by clocks outside the adipocyte.

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T45

Circadian Rhythms of Triglyceride Accumulation in NIH3T3-L1 Cells

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Abstract: Studying lipid metabolism is important not only to understand the mechanism of obesity and diabetes, but also to produce better food in the future. It has been widely studied using various experimental animal models and cell lines. Recently, several studies have reported lipid metabolism disorders in mice lacking clock genes, suggesting that the circadian time-keeping system relates to coordinating the daily energy balance (intake, storage, and utilization) in organs involved in lipid metabolism, such as adipose tissues and liver. However, the presence of a circadian rhythm of triglyceride (TG) accumulation in adipocytes has not been elucidated.

Here, to investigate the mechanism by which the molecular clock regulates lipogenesis and lipolysis in adipocytes, we measured circadian rhythms of TG accumulation in the NIH3T3-L1 (L1) cell line. To measure TG accumulation, L1 cells were grown to 100% confluence in regular medium comprising DMEM supplemented with 10% FBS, 1x penicillin-streptomycin-glutamine, and 1 μM sodium pyruvate (Day 0). On Day 2, these cells were fed with induction medium (regular medium with 1 μM dexamethasone, 0.5 mM isobutylmethylxanthine, and 2.5 μg/ml insulin). On Day 4, these cells were transferred to maintenance medium (regular medium containing 2.5 μg/ml insulin). Day 6 onwards, these cells were grown in regular medium. Once these cells were fully differentiated into adipocytes, they were subjected to a serum shock (50% fetal bovine serum), and sampled every 6 hours for 30 hours. Only lipids were extracted from these cell samples and the intracellular TG content was measured by GPO/DAOS method using LabAssayTMTriglyceride (Wako, Japan).

A significant circadian rhythm was observed for intracellular TG accumulation, which peaked at 12 hours after the serum shock. Moreover, L1 cells transfected with luciferase reporters (either Bmal1-Luc or Per2-Luc) displayed circadian rhythms for bioluminescence. The peak phase of Bmal1-Luc was consistent with that of TG accumulation. These results suggest that the molecular clock regulates circadian rhythms of TG accumulation in the L1 cell line.

Research Funding: None

T46

Nocturnal Light Exposure Acutely Disrupts Glucose Metabolism

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Abstract: Study Objectives: Life on earth has evolved itself with the rhythmic exposure to light and darkness due to sunrise and sunset. Since the invention of artificial light, over 200 years ago, exposure to light at night (LAN) has increased dramatically, hypothetically disrupting circadian physiology. Not only shiftworkers are frequently exposed to LAN, but also the rest of the population is increasingly exposed to nocturnal light due to light pollution. Only recently it was realized that LAN may be detrimental to human health, including a higher risk to develop metabolic disorders such as obesity and diabetes mellitus type 2. Animal studies with chronic LAN exposure showed increased bodyweight and disrupted glucose metabolism. Furthermore, studies with acute LAN exposure demonstrated alterations in hormone secretion (e.g. melatonin, corticosterone), gene expression (e.g. clock and metabolic genes in liver, muscle and pineal after 30-60 minute LAN) and autonomic nervous system activity. These effects raised the question whether glucose homeostasis would be affected by LAN acutely.

Methods: In the current study we exposed freely moving male Wistar rats to intravenous glucose and insulin tolerance tests during 2-hour LAN exposure at different times of day (ZT15 and ZT21). The effects of different light intensities (ranging from 5 to 150 lux) and wavelengths (red, blue, green or white spectrum) were investigated by measuring glucose, insulin and corticosterone levels in glucose tolerance tests.

Results: Results showed that 2-hour LAN acutely induces glucose intolerance and insulin resistance, a pre-diabetic state, without changed plasma levels of corticosterone. At ZT15, LAN caused increased glucose levels without a proper insulin response, whereas at ZT21 normal glucose levels were accompanied by increased insulin secretion (i.e., reduced insulin sensitivity). Increasing light intensities at ZT15 caused a dose-dependent increase of glucose intolerance. It is known that retinal cells projecting to the SCN, among other areas, have higher sensitivity for light with wavelengths in the blue spectrum, and therefore currently the contribution of different wavelengths, with equal amounts of microwatt per squared cm, is studied in our protocol.

Conclusion: From the current results we conclude that acute light at night disrupts healthy glucose homeostasis, possibly contributing to the correlation of LAN with increased risk of metabolic disorders.

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T47

Metabolic Defects in Bmal1 KO Mice

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Abstract: The mammalian circadian clock plays a fundamental role in animal physiology and its disruption has been shown to lead to different metabolic pathologies including obesity. The aim of this study is to better understand the mechanisms involved in this circadian clock induced metabolic disorders. For this purpose, we first induced obesity in Bmal1 KO mice and control littermates with HFD (high fat diet). We show that HFD-fed Bmal1 KO mice exhibit a faster obese phenotype than WT mice as relative weight gain, BMI (Body Mass Index) and fat content increase faster in KO mice than in WT mice. In addition, HFD induces a severe impairment of the glucose homeostasis in both WT and KO mice, even though Bmal1 KO mice remains insulin sensitive. In a second time, we produced a more severe obesity model through the generation of Bmal1 KO mice harboring the Ob mutation (leptin deficiency). Interestingly, despite the fact that no embryonic lethality is observed, the ObOb. Bmal1KO mice exhibit about 60% of premature death. In addition, while ObOb.Bmal1KO mice have a body weight curve similar to the ObWT.Bmal1WT and ObWT.Bmal1KO littermates, their BMI and fat content show that ObOb.Bmal1KO mice exhibit an obese phenotype. Moreover, ObOb.Bmal1KO mice exhibit a higher fasting glycaemia and an impaired glucose clearance like ObOb.Bmal1WT despite their high insulin sensitivity. Interestingly, HFD-fed Bmal1 KO and ObOb.Bmal1KO mice present significantly lower hepatosteatosis compared to their controls and higher serum triglycerides concentration. All together, these data show that Bmal1 KO mice in obesity conditions exhibit an insulin-sensitive obese phenotype characterized by lipid and glucose storage defects.

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T48

Characterization and Behavior of Multimeric Protein Complexes of the Mammalian Circadian Clock across the Circadian Cycle

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Abstract: The mammalian circadian clock is built on a transcriptional feedback loop in which the three PERIOD (PER) proteins and two CRYPYTOCHROME (CRY) proteins inhibit CLOCK-BMAL1, the transcription factor driving their own expression. Upon nuclear entry, the PERs and CRYs recruit multiple repressors, including chromatin-modifying and nucleosome-remodeling factors, into a large complex (PER complex) that binds to DNA-bound CLOCK-BMAL1, repressing its transcriptional activity. Little is understood about how the PER complex functions over time as a macromolecular machine. To this end, we developed a high-resolution modification of blue native polyacrylamide gel electrophoresis (BN-APAGE), in which a decreasing gradient of agarose is superimposed on an increasing gradient of polyacrylamide. Using this method to analyze mouse liver nuclear extracts, we observed CLOCK-BMAL1 complexes and PER complexes over the circadian cycle. During the activation phase of the cycle, CLOCK-BMAL1 is present exclusively in a 700-kDa complex. At the beginning of the repressive phase (~CT12), the nuclear PER complex (1.9 +/- 0.1 mDa) begins to

accumulate and incorporate CLOCK-BMAL1, with a concomitant gradual reduction in the abundance of the 700-kDa CLOCK-BMAL1 complex. By CT19, the approximate time of peak abundance of the nuclear PER complex, essentially all of the CLOCK-BMAL1 is incorporated, leaving little or no detectable 700-kDa complex. During degradation/disassembly of the PER complex (~CT20-CT2), the 700-kDa CLOCK-BMAL complex reappears, having been released or reassembled. A similar analysis from mutant mice singly lacking PER1, PER2, PER3, CRY1, or CRY2 revealed distinct molecular pathologies associated with particular mutations. Nuclear PER complexes lacking PER2 or PER3 exhibited a reduction in incorporation of CLOCK-BMAL1 compared to wildtype or the other single mutants. In addition, nuclear PER complexes lacking PER1 or PER2 exhibited a reduction of Casein Kinase 1Δ (CK1Δ) in the PER complex compared to wildtype or the other single mutants. The results reveal the predominant macromolecular assemblies comprising the circadian clock feedback loop, and they point to specific defects in the assembly and behavior of nuclear PER complexes in particular circadian clock mutants.

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T49

Identification of REVERBα Degradation Mechanisms

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Abstract: The central mechanism of mammalian circadian clock revolves around the timely expression of transcription factors that comprise a set of interlocked transcriptional negative feedback loops. Transcriptional activators such as CLOCK and BMAL1 are expressed at certain time interval of the day to turn on sets of transcriptional repressors, including Per1/2, Cry1/2, REV-ERB-α REV-ERB-βb, which accumulate with time to turn off expression of BMAL1. Although the importance of the expression of these transcription factors is very well established, the timely degradation of their gene products must be important to sustain the precise timing of the circadian clock. Our laboratory has recently established a relatively simple cell-based system to screen for E3 ubiquitin ligases that target proteins of interest for degradation. SIAH2 was identified in such a screening experiment to be an ubiquitin ligase that specifically targets both REV-ERB-α and REV-ERB-βb, for degradation by the 26S proteasome. Depletion of SIAH2 results in the disruption of REV-ERB-a circadian rhythmicity and suppression of its downstream target genes, as well as overall circadian oscillator function, suggesting circadian degradation of REV-ERB-±a is important for clock function. The current study aims to identify which lysine residues on REV-ERB-±a are the targets for ubiquitination. Site-directed mutagenesis initially focused on 3 lysine residues located within the ligand-binding domain found to be ubiquitinated in HCT116 cells. The location of these potentially ubiquitinated sites within the ligand-binding domain suggests REV-ERB-αa stability may also depend on its interaction with the ligand or other modes of REV-ERB-± regulation. Moreover, identification of these sites could provide the means to directly examine the role of high-amplitude REV-ERB± rhythms to both circadian oscillator timing, as well as control of REV-ERB-± specific circadian outputs.

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T50

CNOT1 Promotes Phosphorylation of Mammalian Clock Proteins via Pka.

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Abstract: Protein kinase A (PKA) is a key component in the Neurospora circadian feedback loop, and the NOT1 protein is important for maintaining both WC-1 and WC-2 levels and promotes their phosphorylation. However, it remains unknown whether the related protein CNOT1 is associated with protein kinase A in the eukaryotic clockwork. In the present study we show that CNOT1 associates with both mammalian CLOCK and BMAL1, promotes their phosphorylation and stability, and inhibits the transcriptional activity of CLOCK/BMAL1. Expression of either CLOCK, BMAL1 or CNOT1 could interact with endogenous PKA as assessed by coimmunoprecipitation. PKA can directly phosphorylate CLOCK and BMAL1 and is promoted by CNOT1. Genetic targeting of PKA by CRISPR/Cas9 results in longer periods of the circadian rhythm; while overexpression of PKA induces shorter periods. Furthermore, we found that CNOT1 associates with CLOCK and BMAL1 in liver and promotes their phosphorylation during the activation phase. PER2, but not CRY2, is also a PKA target. Our results suggest that CNOT1 and PKA play a critical role in the mammalian circadian clock.

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T51

Cry2 Suppresses Transformation by Destabilizing c-Myc

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Abstract: The circadian clock regulates many aspects of cell physiology and increasing evidence suggests an important role in growth control and tumorigenesis. Indeed, shift workers have increased cancer susceptibility but the molecular explanation for this phenomenon is still to be determined. c-MYC is the ubiquitously expressed member of a family of bHLH transcription factors. Its expression is deregulated in up to 70% of human cancers and it is among the most highly somatically amplified oncogenes in human tumors. A tight regulation of c-Myc expression is essential for normal cell function and is controlled by phosphorylation of specific sites, especially the threonine 58 and the serine 62. We demonstrate that c-Myc phosphorylated on threonine 58 (T58) forms a trimeric complex with the circadian clock components Cry2 and Fbxl3, which promotes ubiquitination and degradation of c-Myc. c-Myc protein displays a robust circadian rhythm and is elevated in Cry2-/- or Fbxl3-depleted cells. c-Myc contributes to tumorigenesis in part by driving metabolic reprogramming to support biosynthetic processes. We demonstrate that Cry2-/- cells exhibit increased Myc-dependent gene regulation, glucose-driven biosynthetic metabolism, and susceptibility to transformation. Genetic disruption of Cry2 accelerates lymphoma development in mice expressing c-Myc in hematopoietic cells. In humans, CRY2 expression is lower in tumors than in associated normal tissues. We also demonstrate that re-expression of Cry2 in tumor-derived cell lines reduces c-MYC expression and decreases proliferation and anchorage-independent growth. Together, these results support that

Cry2 can suppress transformation by destabilizing c-Myc and unraveled a new unappreciated mode of clock output where Cry-driven cycles of proteasomal degradation impacts cancer susceptibility due to circadian disruption.

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T52

An Evolutionary Hotspot in CRYPTOCHROME's Structure Tunes the Period of the Mammalian Circadian Rhythm

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Abstract: Cell autonomous molecular clocks with robust 24-hr rhythms have evolved to coordinate physiological processes with the environment. In vertebrates, this clock is principally defined by a transcription/translation feedback loop in which CLOCK and BMAL1 form a heterodimeric transcription factor that regulates the expression of many targets including the genes encoding the repressors: Cryptochrome (Cry) 1 and 2 and Period (Per) 1, 2, and 3. CRY and PER proteins translocate to the nucleus where they bind and repress CLOCK and BMAL1 activity. Crys evolved from genes encoding a family of photoactivated DNA-repair enzymes known as Photolyases. Photolyases bind two cofactors, an FAD molecule in a central pocket, critical for the light-mediated repair of UV-damaged DNA, and a secondary cofactor, which functions as a light-harvesting antenna and is dispensable for normal function. Vertebrate CRYs are not photoactive, but they have evolved into an indispensible component of the clock. To elucidate their evolution into this central role, we created a large protein alignment of Photolyases and CRYs including bacterial, archaeal, and eukaryotic sequences and performed Statistical Coupling Analysis (SCA) to identify residues that are co-evolving and representative of protein sectors with potentially discrete roles in the biology that these family members perform. Multiple residues contributing to the pocket where secondary cofactors bind were identified in distinct protein sectors, suggesting this pocket might be an evolutionary hotspot. We mutated a number of these residues in mCRY1 to determine whether they play a role in mammalian CRY biology and used the resulting mutant CRYs in rescue experiments. Aggressive mutations resulted in a failure to rescue rhythms, caused by an inability for CRY1 to bind CLOCK. Surprisingly, subtler mutations dramatically shortened the period of the rescue while also increasing the amplitude of the oscillation. The data suggests that rather than a decrease in CRY's stability, acceleration of the clock is caused by weakened binding between CRY1 and CLOCK at this pocket, which allows CLOCK to be more transcriptionally active in the early repressive phase of the cycle until higher levels of CRY build up and the kon/koff rate reaches a point at which full repression begins.

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T53

A Functional Synthetic Hybrid Circadian Oscillator Generated through Transcriptional Rewiring

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Abstract: Circadian rhythms are self-sustained rhythms that under constant conditions (i.e constant darkness) have periods close to 24 hours. These rhythms are generated at the cellular level by a transcription-translation negative feedback loop (TTFL), where a negative element directly inhibits the activity of the positive elements that control its expression.

In the fungus Neurospora crassa the negative element is the protein FRQ, encoded by the gene frequency (frq) and the positive element is the White Collar complex (WCC), composed by the transcription factors White Collar 2 (WC-2) and White Collar 1 (WC-1), being the latter also a photoreceptor. The oscillator transmits the temporal information to various biological processes, including gene expression, through output pathways. This is mediated, in part, by a hierarchical arrangement of transcription factors, which allows the rhythmic expression of clock-controlled genes (ccgs).

To improve our knowledge of the transcriptional regulation of frq and the plasticity of the core-oscillator we adopted a synthetic biology approach to generate, by transcriptional rewiring of the core clock and the output pathways, a core-oscillator with a new topology. The blueprint of this hybrid oscillator (HO) is not a closed TTFL but instead consists of an expanded TTFL that includes additional transcriptional steps where the positive element is indirectly inhibited by the negative element. We evaluated the ability of this HO to generate and sustain rhythms, analyzing its behavior under different environmental and entrainment conditions. This HO adheres to the basic definitions of a circadian system, while presenting some interesting aspects regarding response to light and phase information. Remarkably, because of the new circuitry of this HO, there are transcription factors that are now core components of this circadian system, although they do not affect core-mechanisms in a WT strain. Therefore, we have created a synthetic hybrid circadian oscillator based on an indirect TTFL conformed by multiple new additional core-components. Such an approach will allow testing design principles of circadian systems, providing also new insights such as the relative importance of posttranslational versus transcriptional control of TTFL-based mechanisms.

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T54

Re-Evaluating the Roles of Protein Kinase A (PKA) and Camp Signaling in Circadian Core-Clock Mechanisms

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Abstract: Circadian clocks are found in a broad range of organisms, impinging close to 24-hour rhythms to diverse processes. A common design in the circuitry of core-clock oscillators involves a transcription-translation negative feedback loop, which in the case of the filamentous fungus

Neurospora crassa is composed of the transcription factors WC-1 and WC-2 that, as a complex (WCC), positively regulate the expression of the negative element FREQUENCY. The latter, in association with an RNA helicase (FRH) is able to inhibit WCC activity in a daily manner. Several kinases and phosphatases have been described as playing critical roles in the mechanisms of WCC inactivation and FRQ maturation impacting, therefore, clock function. As part of a large genetic screen, we have re-examined the importance of one of these kinases: PKA (cAMP dependent Protein Kinase A).

PKA and cAMP are known to play a central role in the regulation of developmental processes such as conidiation in Neurospora and therefore, overt rhythms in conidiation (which is under clock control in this organism) cannot be easily analyzed in PKA deficient strains. For that reason, in this study, we have utilized bioluminescent real-time reporters to analyze clock function in different mutants associated with cAMP signaling, including PKA deficient strains. These analyses have revealed rather WT circadian rhythms in each case, indicating that the "canonical" cAMP signaling is not necessary for the generation/maintenance of circadian rhythms.

Using the same approach we evaluated the effect of caffeine, theophylline and aminophylline: drugs known to inhibit phosphodiesterase (PDE) producing an increase in intracellular cAMP levels. As in previous reports, we observed that all these drugs led to significant period lengthening. Remarkably, this phenotype was still observable in all of the analyzed cAMP signaling pathway mutants, suggesting that these PDE inhibitors lead to circadian phenotypes through mechanisms different from the canonical PDE-cAMP-PKA axis. These results further question the role that this signaling molecule may play in cell-autonomous clock mechanisms, at least in Neurospora

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T55

Circadian Clock Regulation of Mrna Translation through the Eukaryotic Elongation Factor eEF-2

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Abstract: The circadian clock has a profound effect on gene regulation in diverse organisms, controlling rhythmic transcript accumulation for up to half of expressed genes in eukaryotes. Evidence also exists for clock control of mRNA translation, but the extent and mechanisms for this regulation are not known. In Neurospora crassa, the circadian clock generates daily rhythms in the activation of conserved mitogen-activated protein kinase (MAPK) pathways, including the well-characterized p38 osmosensing (OS) MAPK pathway. The clock regulates rhythms in the phosphorylation of the MAPK OS-2 (P-OS-2), such that OS-2 is phosphorylated and active during the day. P-OS-2 then phosphorylates downstream targets, including transcription factors, chromatin modification proteins, and kinases, which lead to rhythmic control of pathway outputs. We previously demonstrated that clock control of P-OS-2 leads to rhythmic phosphorylation and activation of a conserved serine/threonine kinase, RCK-2. Clock-controlled RCK-2 rhythmically phosphorylates and inactivates the highly conserved eukaryotic elongation factor-2 (eEF-2), with the peak in eEF-2 phosphorylation (P-eEF-2) occurring at mid-day. We show in vitro that rhythmic regulation of P-eEF-2 leads to rhythmic control of translation elongation rates. To determine which mRNAs are regulated at the level of translation elongation by rhythmic inactivation of eEF-2 in vivo, we have carried out rhythmic ribosome profiling in WT versus clock, or RCK-2, mutant cells. Comparison of rhythmic ribosome occupancy with transcriptome data

from the same cells provides novel insights on the extent of clock control of mRNA translation, and the impact of rhythms in the activity of eEF-2 on this process.

Research Funding: NIH Grant

T56

Novel Transcriptional Mechanisms of Muscle-Specific Clock Output

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Abstract: Background: An unresolved phenomenon in the field of circadian rhythms is the underlying mechanism(s) that allow the core molecular-clock proteins (Bmal1/Clock common across all cell types) to transcriptionally target genes in a tissue-specific fashion. The goal of this project was to use a targeted approach in skeletal muscle to identify mechanisms in which the molecular-clock regulates the transcription of skeletal muscle specific genes.

Methods: We employ circadian bioinformatics to elucidate the skeletal muscle circadian transcriptome and to identify muscle-specific clock-controlled genes. Promoter-reporter assays and lumicycle bioluminescence were performed in C2C12 myotubes to test how the molecular-clock and muscle specific factors work to promoter muscle specific circadian gene expression.

Results and Conclusions:We focused our study on the transcriptional regulation of the muscle specific z-line gene Titin-cap (T-cap) because it is highly circadian in skeletal muscle, is regulated downstream of the myogenic regulatory factor MyoD1, and loses circadian expression with loss of BMAL1 expression in skeletal muscle. Utilizing a T-cap promoter-reporter contstruct in C2C12 myotubes, we demonstrate that the T-cap promoter contains essential elements required for circadian oscillation in vitro (absence of environmental time cues) suggestive of a direct clock-controlled gene. We performed a series of transfection experiments testing T-cap promoter-reporter constructs in C2C12 myotubes, and demonstrated that T-cap is transactivated by overexpression of clock factors BMAL1:CLOCK, as well as MyoD1. Interestingly, cotransfection studies with mutant forms of Bmal1 or MyoD1, showed that BMAL1:CLOCK and MyoD1 work in a synergistic fashion and require each other to promote the circadian expression of T-cap in C2C12 myotubes. Deletion constructs and mutagenesis studies followed by ChIP assays demonstrated that both BMAL1 and MyoD1 were bound to the proximal promoter of T-cap, and we identified a tandem E-box element required for T-cap circadian expression. These results demonstrate that the molecular-clock factors, BMAL1 and CLOCK, work with MyoD1, a tissue-specific transcription factor, to promote the circadian expression of T-cap in skeletal muscle.

Research Funding: My research in funded through an R01 awarded to Dr. Karyn Esser.

T57

Circadian Rhythm of Muscle Mitochondrial Metabolism

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Abstract: The best strategy for prevention of type 2 diabetes mellitus (T2DM) is increasing physical exercise combined with reducing caloric intake. Muscle is the most important tissue for glucose uptake. Recent work suggests that targeting muscle mitochondrial functioning could prevent and treat T2DM and both exercise and caloric intake affect mitochondrial capacity of skeletal muscle. Also, it was suggested that mitochondrial functioning is under the control of the biological clock. Indeed, epidemiological studies found an increased risk of obesity and T2DM in night-shift workers. These workers tend to take meals with high fat and/or sugar content, possibly adding to the risk of T2DM.

To examine the effects of both shifted meal timing and meal composition on (mitochondrial) metabolism, male Wistar rats were subjected to ad libitum or time-restricted feeding (TRF) during the light or dark period. In combination with TRF rats received normal chow, chow and a high fat (HF), or chow and a high fat and high sugar (HFHS) diet. Whole body metabolism was evaluated using metabolic cages. Expression patterns of core molecular clock genes, as well as genes associated with mitochondrial metabolism are currently being investigated using qPCR on skeletal muscle tissue, collected at 3h intervals throughout a 24h period.

TRF significantly lowered the respiratory exchange ratio (RER) per 24h for HF and HFHS groups as compared to chow fed groups, indicating the use of fat as main energy source. Furthermore, diurnal patterns of RER were inverted between dark and light fed groups, with highest RER during the feeding period. Preliminary results show that compared to dark fed groups, light fed groups had dampened rhythmic expression of molecular clock genes Bmal1 and Per2, as well as some genes associated with mitochondrial metabolism such as PGC1α and Glut4, contrasting previous research on liver showing shifted rhythms during light period TRF.

These data indicate that a HF or HFHS diet increases fat metabolism in favor of carbohydrates as main energy source, especially during the fasting period. Also, the molecular clock in skeletal muscle is altered by TRF, but changes clearly differ from those previously described for liver. Future work should address the effects of TRF and meal composition on additional genes involved in mitochondrial metabolism.

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T58

Circadian Rhythm of Redox State in Hippocampal CA1 Regulates Neuronal Membrane Excitability

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Abstract: Circadian rhythms regulate many aspects of metabolic homeostasis. At the cellular level, metabolic state manifests as redox state. The ratio of redox cofactor pairs, such as glutathione disulfide (GSSG)/glutathione (GSH), originates from reduction-oxidation reactions. We previously reported a novel model for the interdependency of the transcriptional-translational clock machinery, redox oscillation, and neuronal excitability in the suprachiasmatic nucleus (SCN, Wang et al. Science 2012; Gillette & Wang, Antioxid. Redox Signaling 2014). Does this interrelationship exist in extra-SCN brain regions? We focused on the hippocampus, where Per 2 gene expression in CA1 pyramidal cell layers undergoes a robust circadian oscillation, 180° out-of-phase with that of the SCN. We evaluated the excitability of CA1 neurons in rat hippocampal brain slices using whole-cell patch-clamp recording. We observed oscillations in membrane potential (Vm) that varied with circadian time (CT), such that neurons were hyperpolarized during the subjective day (CT 7) and depolarized during the subjective night (CT 14). Average resting Vm in midday (CT 5-7) was significantly more hyperpolarized than during the late day (CT 10-12), early night (CT 13-15), or late night (CT 16-20). Next, we assessed time-of-day changes in redox state. Glutathiolation, the capacity of proteins to incorporate reduced GSH, was lowest in hippocampal tissue at CT 14. This corresponds to a more reduced state, opposite that of the SCN at CT 14. Experimental manipulation of redox state rapidly altered membrane excitability. Average GSH-induced changes in Vm were dependent on CT and were significantly larger at midday (CT 7) than any other time. Minimal effects were caused during early night (CT 14). In conclusion, both membrane excitability and redox state undergo circadian oscillations in CA1 neurons in hippocampal brain slices.

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T59

Altered Circadian Phenotype in Cannabinoid Receptor 1 Knockout Mice

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Abstract: The suprachiasmatic nucleus of the hypothalamus (SCN) drives daily rhythms of physiology and behavior. Previous studies indicate that pharmacological activation of the cannabinoid type 1 receptor (CB1) inhibits the phase shifting effects of light, that CB1s are present in the SCN, and that they act as presynaptic inhibitors of GABA release. To further evaluate the role of endocannabinoids in regulating circadian clock function, we screened CB1 knockout mice (CB1-/-) for altered circadian rhythms of behavior. CB1-/- mice show an increased incidence of circadian disorganization, with some, though not all animals displaying increased wheel-running activity during the light phase of the light-dark cycle. In both constant dark and in constant light, CB1-/- mice show free-running periods that are significantly shorter than in wild-type controls. CB1-/- mice also show increased

phase shifts in response to brief light pulses than control mice. Next we evaluated the response of the mice to 6 hour phase advance and delays of the light-dark cycle. Reentrainment rates appeared to be faster in the CB1-/- mice, however, this was difficult to evaluate statistically due to disruption of the rhythmic pattern of activity. Finally, we evaluated food anticipatory activity (FAA) during timed restricted feeding in mice under both a light-dark cycle and in constant light. Because CB1-/- mice show a very lean phenotype, we expected to find reduced FAA. Results suggest that CB1-/- show FAA that is equal to or greater than wild type mice. These data suggest that the loss of CB1 receptors has a substantial impact on circadian behavior, possibly through weakening the coupling between SCN neurons.

Research Funding: Kent State Department of Biological Sciences

T60

Circadian Effects of Conditional Serotonin Knockdown in the Midbrain Raphe Nuclear Complex of Adult Mice

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Abstract: The brain serotonin (5-HT) system plays key roles in regulating critical processes such as circadian clock timing, motivation, reward response and sleep. 5-HT knockout (SKO) mouse models have previously been used to study 5-HT actions, but such global 5-HT depletion is limited by non-targeted neurophysiological and developmental manifestations. Therefore, conditional SKOs are needed. Here, we performed preliminary behavioral phenotyping in a conditional 5-HT depletion model where 5-HT synthesis blockade was targeted specifically to the midbrain raphe nuclear complex (source of hypothalamic serotonergic innervation) in adult mice. MaleTph2fl/+ (Tph2-CON) andTph2fl/- (Tph2-CKO) mice were injected in the dorsal and median raphe nucleus with AAV-Cre at 7-9 weeks of age (n=13). Animals were given 1wk to recover and 2wks to acclimate prior to experimentation. General locomotor behavior was monitored for a 2wk post-injection period, during which circadian parameters (period [tau] nocturnal activity length [alpha] and daily activity bouts) were measured using overhead infrared motion detectors interfaced with a computerized data acquisition system. HLPC analyses revealed 5-HT and 5-HIAA levels in the forebrain were significantly decreased inTph2-CKO mice (9.7±0.3 vs. 0.1±0.1, p<0.0001; 5.5±0.2 vs. 0.0±0.0, p<0.0001), confirming knockdown of Tph2 expression. There was no difference in tau between Tph2-CKO and Tph2-CON mice (24.02±0.01 vs. 24.01±0.01; p=0.40). Notably, there was a large difference in alpha, with Tph2-CKO mice having longer alpha (877.9±23.7 min vs.741.2±14.6 min; p<0.0001). Further, total daily activity counts for Tph2-CKO mice were significantly higher than Tph2-CON mice (2563±385 counts vs. 1267±208 counts; p<0.01). Compared to Tph2-CON mice, Tph2-CKO mice were also more active in both the light phase (633.1±125.7 counts vs. 142.3±36.6 counts; p<0.001) and the dark phase (1930±287.5 counts vs. 1125±182.3 counts; p<0.001). Taken together, the data reveal that this conditional removal of 5-HT signaling in hypothalamic and forebrain structures significantly disrupts daytime and nocturnal activity patterns, suggesting a critical role of 5-HT in sleep and circadian regulation.

Research Funding: Department of Biological Sciences, Kent State University

T61

Transcriptomic Study of Circadian Rhythm in Astrocytes

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Abstract: Astrocytes are the most abundant cells in mammalian brain. Emerging evidences had shown that astrocytes may have circadian rhythm and play important roles in the maintenance of normal circadian function. It has been well established that the suprachiasmatic nucleus (SCN) neuron is the master circadian pacemaker in mammals. But the function of astrocytes in circadian rhythm is largely unexplored at the systems level. In this study, we first validated the existence of circadian clock in cultured astrocytes from newborn mice by showing the circadian expression of core clock genes. We then uncovered the circadian oscillating genes in the astrocytes acutely sorted from the brain tissues of circadian entrained mice by RNA-seq. We observed that the expression of several astrocyte specific genes such as Fabp7 have significant circadian rhythm. We are currently investigating the astrocyte-specific circadian functions of these genes, the circadian regulation of them, and the interplay between neurons and astrocytes in circadian rhythm.

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T62

Entrainment Pathways of C. Elegans Circadian Rhythms

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Abstract: Caenorhabditis elegans is a nematode widely used in different fields of research which has been proposed as a model organism in chronobiology. Several labs have provided evidence of rhythmicity in various variables such as osmotic stress resistance, oxygen consumption, defecation, feeding and locomotor activity.

Recently, a phototransduction pathway has been described in this organism that requires LITE-1 (candidate photoreceptor) and TAX-2 /TAX-4 (cGMP-sensitive CNG channels). Using an infrared method to measure locomotor activity in C. elegans, we found that loss of tax-2 or tax-4 results in circadian locomotor arrhythmicity, in a similar fashion but with a stronger phenotype than with the loss of lite-1.

To further characterize these phenotypes we used a long-term video recording system which allows the analysis of speed, acceleration, number of turns and distance travelled for each single nematode; in addition, short video tracks enabled us to test the response of different mutant strain to acute light stimulation, of different wavelength. We found a significant difference in the response to blue and green light in N2 nematodes, as well as different phototaxis responses in selected mutant strains.

Another element that we found to modulate the locomotor activity of C. elegans is the neuropeptide pigment-dispersing factor (PDF). There are two PDF homologs, PDF-1 and PDF-2, and a PDF receptor, PDFR-1, which had been implicated in locomotor behavior. In this work, we have studied the role

of the PDF neuropeptide in the circadian system of C. elegans and found that both pdf-1 and pdf-2 mutants affect the normal locomotor activity outputs. In particular, loss of pdf-1 induced circadian arrhythmicity under both light–dark (LD) and constant dark (DD) conditions. Our results indicate that PDF-1 is involved in rhythm generation and in the synchronization to LD cycles, as rhythmic patterns of activity rapidly disappear when pdf-1 mutants are recorded under both entrained and free-running conditions.

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T63

Daily Changes in Opsin mRNA Levels in the Antarctic Krill

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Abstract: The Antarctic krill Euphausia superba experiences almost all marine photic environments throughout its life cycle. Antarctic krill eggs hatch in the aphotic zone up to 1000 m depth and larvae develop on their way to the ocean surface (development ascent) and are exposed to different quality (wavelength) and quantity (irradiance) of light. Adults and larvae show a daily vertical migration pattern, moving downward during the day and upward during the night within the top 200 m of the water column. We have investigated the transcriptome of E. superba and identified six novel opsins. Five are r-type visual opsins (four middle-wavelength-sensitive and one long-wavelength-sensitive) and one is a non-visual opsin member of Peropsin group. It has 48-49% of amino acid identity with Chelicerata peropsins and 35-39% with deuterostome homologs.

To investigate the expression at the mRNA level of opsin genes in different tissues of E. superba, we performed qPCR using RNA from dissected brain, eye and abdomen. All these newly identified opsin genes were significantly expressed in compound eyes and brain, while only two of six (Peropsin and Rh2) were clearly detected in the abdomen.

We also study the temporal expression of the novel opsins detected. qPCR was performed on the head (including brain and compound eye) of specimens collected in the Ross Sea at different times of the day (01:00, 06:00, 10:00, 15:00, and 18:00) during the Antarctic summer, when they were exposed to an almost continuous 24 hours photoperiod characterized by daily variations in solar irradiance. The overall data suggest the existence of a temporal modulation of the amplitude and phase of expression for all the novel opsins and a statistically significant oscillations were observed in EsRrh3 and EsPeropsin.

Our results contribute to the dissection of the complex photoreception system of E. superba, which enables this species to respond to the daily and seasonal changes in irradiance and spectral composition in the Southern Ocean.

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T64

Stress Alters Adrenal Clock Function in a Sexually Dimorphic Manner

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Abstract: Glucocorticoid production is gated at the molecular level by the circadian clock in the adrenal gland. Although stress is known to influence daily rhythms in behavior and physiology, it remains unclear if stress hormones affect the function of the adrenal clock. To address this issue, we examined the influence of stress on the adrenal clock of male and female PER2::LUC mice. First, we simulated a stress response in vitro by exposing adrenal glands to the tropic pituitary hormone, adrenocorticotropic hormone (ACTH). Consistent with previous work, ACTH reset the adrenal clock in vitro in a phase-dependent manner. ACTH also increased the amplitude of PER2::LUC rhythms, with the magnitude of potentiation varying over the circadian cycle. Adrenal samples collected from female mice displayed larger ACTH-induced changes in both phase and amplitude relative to those from male mice, which mirrors sex differences in glucocorticoid production. Next, we tested whether in vivo stress exposure would influence adrenal clock function. Male and female mice were exposed to one of two different stress manipulations in vivo prior to adrenal culture. Relative to adrenals from non-stressed control mice, adrenals from stressed mice displayed marked changes in the amplitude of adrenal PER2::LUC rhythms in vitro. Interestingly, the effect of stress on adrenal PER2::LUC rhythmicity varied by sex and type of stressor. Together, these results indicate that the adrenal clock responds to stress in a sexually diergic manner, which may have implications for sex differences in stress reactivity and stress-related disorders.

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T65

A Dissociation between Diurnal Cycles in Locomotor activity, Feeding Behavior and Hepatic PERIOD2 Expression in Chronic Alcohol-Fed Mice

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Abstract: Chronic alcohol consumption contributes to fatty liver disease. Our studies revealed that the hepatic circadian clock is disturbed in alcohol-induced hepatic steatosis, and effects of chronic alcohol administration upon the clock itself may contribute to steatosis (Zhou et al 2014, Scientific Reports 4, 3725). We extended these findings to explore the effects of chronic alcohol treatment on daily feeding and locomotor activity patterns. Mice were chronically pair fed ad libitum for 4 weeks using the Lieber-DeCarli liquid diet, with calorie-controlled liquid and standard chow diets as control groups. Locomotor activity, feeding activity, and real-time bioluminescence recording of PERIOD2::LUCIFERASE expression in tissue explants were measured. Mice on liquid control and chow diets exhibited normal profiles of locomotor activity, with a ratio of 22:78% day/night activity and a peak during early night. This pattern was dramatically altered in alcohol-fed mice, marked by a 49:51% ratio and the absence of a distinct peak. While chow-diet fed mice had a normal 24:76% ratio of feeding activity, with a peak in the early night, this pattern was dramatically altered in both liquid-diet groups: mice had a 43:57% ratio, and an absence of a distinct peak. Temporal differences were also

observed between the two liquid-diet groups during late day. Cosinor analysis revealed a ~4-h and ~6-h shift in the alcohol-fed group feeding and locomotor activity rhythms, respectively. Analysis of hepatic PER2 expression revealed that the molecular clock in alcohol-fed and control liquid-diet mice was shifted by ~11 h and ~6 h, respectively. No differences were observed in suprachiasmatic nucleus explants, suggesting that changes in circadian phase in the liver were generated independently from the central clock. These results suggest that chronic alcohol consumption and a liquid diet can differentially modulate the daily rhythmicity of locomotor and feeding behaviors, aspects that might contribute to disturbances in the circadian timing system and development of hepatic steatosis.

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T66

Kaia Mutant on Oxidized Quinone Binding Site Overcomes Jet Lag Faster

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Abstract: Circadian clock systems provide many advantages for organismal survival and productivity on Earth, allowing for many organisms to adjust their metabolism to the daily oscillations of light and dark cycles. The cyanobacterial circadian clock is composed of a three part of a signaling system: input, oscillator, and output. The input pathway detects changes in light and dark from the environment, and synchronizes the phase of oscillator with day/night cycles. The central oscillator is encoded by three genes, kaiA, kaiB, and kaiC, whose protein products function together to generate a 24-hour rhythm of KaiC phosphorylation. The oscillator transmits the 24-hour KaiC phosphorylation signal to an output pathway resulting in the regulation of a wide variety of rhythmic behaviors including gene expression. The central oscillator of cyanobacterial circadian clock is encoded by three genes, kaiA, kaiB, and kaiC, whose protein products function together to generate a 24-hour rhythm of KaiC phosphorylation. KaiC has two residues (Ser431, Thr432) that can be phosphorylated and modulation of KaiC's autokinase and autophosphatase activities generates a 24-hour period phosphorylation and dephosphorylation rhythm. KaiA activates the autophosphorylation of KaiC and KaiB attenuates KaiA's function, resulting in KaiC dephosphorylation. The 24-hour KaiC phosphorylation rhythm is generated by timely association and dissociation amongst these three Kai proteins. The 24-hour rhythm of KaiC phosphorylation can be reconstituted in vitro by mixing purified KaiA, KaiB, and KaiC with adenosine triphosphate (ATP). Cyanobacteria can synchronize their circadian rhythm to the environmental light/dark cycle by sensing redox state in the cell. The transition from light to dark oxidizes plastoquinone, which is sensed by KaiA and changes the phase of KaiC phosphorylation. This redox sensing is an alternative to direct light sensing in cyanobacteria which lack of light receptors and is an important factor for entrainment. The pseudo-receiver domain of KaiA and CikA are the main components to sense redox state of quinone. We generated a KaiA mutant, which is more sensitive on the quinone signal, by structural analysis. The mutant KaiA was able to overcome jet lag faster than wild type.

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T67

Cohabiting Grass Rats Synchronize their Activity Bouts on a Non-Circadian Scale

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Abstract: Circadian rhythms can be entrained to periodic cues in the environment including the solar day, food, and temperature. Social cues may also entrain the clock, but various technical and conceptual issues have complicated this research. In previous work in the gregarious Nile grass rat, we found no evidence for synchronization of circadian rhythmicity in cohoused pairs of females, males, or female-male couples (although social history did affect free-running period, with a shortening in sexually experienced animals that likely contributed to an advanced phase angle of entrainment to the light-dark cycle in females). To determine if cohabitation synchronizes rhythmicity on a non-circadian scale, we analyzed body temperature (Tb) and activity patterns of grass rats individually implanted with temperature dataloggers, released into constant conditions for 8-9 days following recovery from surgery, then co-housed as female-female or female-male pairs for a week, and finally separated and kept in isolation for an additional week. Activity patterns were video-recorded and active-only bouts (every 5 minutes) were scored before, during, and after cohabitation (for 3, 4, and 3 nonconsecutive days, respectively). Analysis of the Tb data confirmed our previous findings of a lack of circadian rhythm synchronization during cohabitation. While cohousing did not change the proportion of the 24h day that individuals spent active (about 1/3 of the time), it did double the percentage of simultaneous activity time (regardless of pair type) from the percentage expected, suggesting that activity bouts were synchronized by their redistribution over 24 h. Our findings suggest that social interactions may induce changes in individual "time allocation" budgets across the day, which in natural settings could be of adaptive significance, especially for animals living in groups.

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T68

The Circadian Clock Regulates Autophagy Directly through Nuclear Hormone Receptor Rev-Erbα and Indirectly via C/ebpβ in Zebrafish

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Abstract: Autophagy is a highly conserved intracellular degradation system, and recently was shown to display circadian rhythms in mice. The mechanisms underlying circadian regulation of autophagy, however, are still unclear. Here, we observed that numbers of autophagosomes/autolysosomes exhibit daily rhythms in the zebrafish liver, and c/ebpβ and various autophagy genes are rhythmically expressed in zebrafish larvae but significantly up-regulated in per1b and TALEN-generated rev-erbα mutant fish, indicating that both Per1b and Rev-erbα play critical roles in autophagy rhythms. Luciferase reporter and ChIP assays show that the circadian clock directly regulates autophagy genes through Rev-erbα, and also regulates transcription of c/ebpβ through Per1b. We also found that fasting leads

to altered expression of both circadian clock genes and autophagy genes in zebrafish adult peripheral organs. Further, transcriptome analysis reveals multiple functions of Rev-erbα in zebrafish. Taken together, these findings provide evidence for how the circadian clock regulates autophagy, implicate that nutritional signaling affects both circadian regulation and autophagy activities in peripheral organs, and shed light on how circadian gene mutations act through autophagy to contribute to common metabolic diseases such as obesity.

Research Funding: National Basic Research Program of China (973 Program) (2012CB947600) and the National Natural Science Foundation of China (NSFC) (31030062).

T69

Time Restricted Foraging Activity and Clock Gene Expression in Honey Bees

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Abstract: Honey bee, Apis mellifera, colonies were kept in an outdoor flight enclosure (12m x 5m x 2.5m) and provided with sugar-water as the only food source. The colonies were exposed to three different feeding schedules: (a) ad libitum feeding, i.e. feeder present during the whole day, or (b) 2 hours feeding in the morning (8000 - 1000hrs), or (c) two hours in the late afternoon (16300-1830 hrs). Foragers were trained for 10 days to each feeder schedule and then collected at six different time points: 6AM, 10AM, 2PM, 6PM, 10PM and 2AM. We measured expression levels for two major clock genes: Cryptochrome2 (Cry2) and Period (PER) in the brain and antennae. Foragers exposed to ad libitum and morning feeding showed very similar Cry2 and PER expression cycles. In contrast, afternoon trained foragers showed a shift in the Cry2 and PER expression cycles, peak phase of the amplitude was shifted for about 6 hours. Interestingly, we did not find any shift in the expression pattern of clock genes in the antennae. As there is some evidence that antennal clocks are autonomously light entrainable, thus we hypothesize that in our experiments the antennae are not entrained by the feeding schedule.

Frisch and Aschoff (1987) demonstrated that feeding cycles of honey bee foragers are able to entrain colony activities. Our results confirm their finding and show for the first time that the entrainment leads to a shift in the brain expression pattern of two major clock genes.

Research Funding: Above work is funded by National Centre for Biological Sciences - in house funding and I am also supported by Junior Research Fellowship from Indian Council of Medical Research.

T70

The Bioclock Studio: Undergraduate Students Connecting Circadian Biology and Sleep Research from Laboratories to Classrooms and the Public

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Abstract: The BioClock Studio at the University of California, San Diego is an innovative and collaborative course focused on the topics of circadian biology and sleep research. The BioClock Studio consists of 15-20 motivated students from diverse majors (Biology, Writing, Communications, Management Science, Visual Arts, and Media) and an instructional team of 4-5 junior instructors (postdoctoral and graduate student trainees) led by HHMI Professor Susan S. Golden.

The BioClock Studio students use various media to develop creative educational materials that are based on scientific research and yet are suitable for a wide audience ranging from high school students to the general public. The inaugural BioClock Studio class in 2015 produced a video series, "Demystifying Circadian Lab Techniques," on setting up and analyzing a rodent wheel-running experiment and "Expert Interviews" of scientists who presented at the annual Center for Circadian Biology "From Cells to Clinic" Symposium. These video clips and other tutorials were deployed for ~300 students in a Circadian Rhythms-Biological Clocks course to enhance the student learning experience. With these materials, we found a significant increase in students' academic performance compared with that of the previous year's class.

The project-based nature of the BioClock Studio challenges students to create final products of excellent quality and reinforces interactions with instructors and scientific consultants. Our main goal is that students will be able to translate and communicate research findings to the public. An additional goal is that students will obtain new skills, ranging from reading primary literature and writing to time management and oral presentation. In addition, the junior instructors have unique opportunities to enhance management and communication skills that are necessary for career as scientists.

The BioClock Studio website and a YouTube channel provide a portal for free distribution of materials that are useful for the public and our scientific communities. We hope that the BioClock Studio will bridge the communication gap between scientists and the public and inspire future generations of circadian and sleep researchers.

Research Funding: This work is supported by the HHMI Professor grant to Dr. Susan S. Golden.

T71

Effects of Constant Bright Light and Heavy Water on Interval Timing in Rats

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Abstract: Mammals are thought to have evolved at least two distinct systems to time regularly recurring events: circadian rhythms for events occurring daily, and interval timing for events in the seconds-to-minutes range. Reports that mice rendered arrhythmic due to constant light exposure (LL) were incapable of timing short intervals suggest that these two systems may rely on shared

mechanisms (Agostino et al., 2011), despite evidence that animals made arrhythmic by SCN ablation or clock gene mutation show no deficits. To investigate whether these findings extend to rats, two groups of 7 rats were housed in LD 12:12 (Group 1) or LL (Group 2), mildly water restricted, and tested on two peak-interval procedures with target intervals of 15 and 30s. Rats rendered arrhythmic by LL exhibited normal temporal control to both target intervals that did not differ from rats in an LD cycle. Interpretation of the results is complicated by the appearance of a circadian activity rhythm that free-ran from the test time during a week of adlib water access without testing. Rescue of free-running rhythms may result from a feeding binge evident for ~2h following daily water access. To address this concern, a third group of 7 rats underwent the same training but with food access restricted to six 20-min 'meals' occurring every 4h to prevent consolidated food intake. These rats exhibited no circadian rhythmicity before or after training, but again exhibited normal ability to time short intervals. Finally, administration of heavy water (25% D2O) rapidly and robustly lengthens the circadian period of SCN-dependent rhythms in constant conditions and phase delays or disrupts entrainment in LD. If interval timing and circadian rhythms share common processes, deuteriation could slow or disrupt interval timing. After initial training on the peak interval tasks, Group 1 rats received 25% D2O in place of water and continued training on the 30s task. All rats showed delayed onsets in daily circadian activity, but there was no significant change in the response distributions to the interval. These results add support to the existing body of evidence that circadian and interval timing are mediated by distinct neural mechanisms.

Research Funding: Supported by NSERC (Canada)

T72

Per2 Expression Rhythms in Mice with Early Senescence and Bimodal Locomotor Rhythms

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Abstract: Aging rodents and humans often experience circadian desychronization, in part due to functional changes in the suprachiasmatic nucleus (SCN). Common age-dependent changes include decreased rhythm amplitude and a reduced capability for entrainment. The senescence-accelerated mouse (SAM) model (SAMP8) displays behavioral and pathological impairments of aging and Alzheimer's disease, including overproduction of amyloid precursor protein (APP) and elevated tau phosphorylation. Age-related cognitive deficits have been linked to "split" circadian rhythms; "split" wheel-running activity has been previously reported in 7- and 12-month-old SAMP8 mice.

The present study aims to test the hypothesis that SAMP8 mice develop bimodal locomotor rhythms independent of running wheel access at an early age, which are accompanied by changes in circadian clock gene (Period2; PER2) expression in the SCN and hippocampus.

SAMP8 and SAMR1 (control) mice were maintained on a 12:12 h light/dark cycle with Zeitgeiber time (ZT) 12 at the start of the dark phase. Locomotor activity was quantified by beam breaks and PER2 expression was assessed by immunohistochemistry. Circadian rhythmicity of general locomotor activity was found in a subset of 4-month old mice. While the majority of SAMR1 mice exhibited a single rhythmic component of ~24h, results indicated a significant secondary component with a mean tau (± SEM) of 14.0 ± 0.9 hours in all of the SAMP8 mice. Moreover, the amplitude of the 24h component was significantly lower (143.3 ± 8.0 vs. 191.2 ± 8.6) and the percentage of total activity occurring in the light cycle was significantly higher (46.0 ± 2.6% vs. 38.2 ± 1.4%) for the SAMP8

mice compared to the SAMR1 controls, respectively ($p < 0.05$). Fluorescence immunohistochemistry experiments to detect PER2 expression in the SCN and hippocampus are ongoing. Preliminary data of PER2-immunoreactivity in the SCN revealed few differences between SAMP8 and SAMR1 mice at two time points (ZT 11 and ZT 23).

Although the underlying molecular mechanisms for bimodal locomotor rhythms are unclear, these results reveal an earlier onset of age-related circadian disruption in SAMP8 mice than previously thought. Also, locomotor rhythm splitting is shown to occur in the absence of a running wheel.

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T73

Does Duper Alter Pacemaker Function or the Core Molecular Clock?

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Abstract: The duper mutation in Syrian hamsters shortens the free running period of locomotor activity in constant darkness to about 23 hours. This mutation is distinct from tau in that it: (i) is recessive, (ii) is not a change in the coding sequence of CK1, (iii) amplifies phase-shifting responses in response to 15-min light pulses, and (iv) dramatically reduces behavioral jet lag. Mathematical modeling indicates that the duper phenotype can be explained by weakened coupling between components of the circadian pacemaker (Manoogian et al., JBR 30:129-143, 2015). An alternative possibility is that duper alters an element of the transcriptional-translational feedback loops common to central and peripheral oscillators. In order to discriminate between these hypotheses, we utilized a cell-culture based bioluminescent assay. We first established that mouse Per2:luc and Bmal1:luc can report circadian rhythmicity in an immortalized hamster kidney cell line (BHK-21) after being engineered into the hamster genome using a piggyBac transposon-mediated transgenesis. mPer2 and mBmal1 promoters drove antiphase rhythms of luciferase expression with a circadian period that was 2h shorter than that of mouse Per2:luc primary embryonic fibroblasts run simultaneously as a positive control. Next, we monitored rhythms of bioluminescence in primary hamster embryonic fibroblasts (HEF) isolated from duper and WT animals that were stably transfected with the mouse Per2:luc reporter. We found that both duper and WT fibroblasts oscillated robustly with similar, but again short, circadian periods. These data support the conclusion from modeling that duper alters pacemaker properties rather than the TTFL common to the central and peripheral oscillators. We are currently investigating determinants of the period of hamster cells, and exploring the use of the piggyBac system to make transgenic hamster strains for circadian studies.

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T74

Aging of the Circadian System in Short and Long Circadian Period Mutant Mice

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Abstract: The circadian system allows mammals to adjust physiological and behavioral processes to the momentum of the day, and as other biological processes, circadian rhythms deteriorate with age. To characterize circadian aging in morning- and evening-like phenotype mice, we performed longitudinal study of short-period (Cry1-/- and Ck1ετau/+ with circadian period of about 22h), and long-period (Cry2-/- with circadian period longer than 24h) mutant mice of both sexes. Mice at four different ages (100, 300, 500, and 700 days) were behaviorally screened in running-wheel cages to assess the effect of age on the level of activity, the phase angle of entrainment to 12h light and 12h dark cycle (LD), changes in free running period in constant dark (DD) and light (LL), as well as the effect of age on re-entrainment to 6h phase advance and 6h phase delay. Aged short-period mutant mice (both Cry1-/- and Cki tau/+) showed a decreased precision of entrainment to the LD cycle and aging-independent adjustment to a 6h phase advanced LD cycle as compared to aged wild type and long-period mutant mice (Cry2-/-). Furthermore, we compared expression of clock and clock-controlled circadian genes in the SCN and peripheral tissues (liver and kidney) in aged mutant mice. As we reported previously (Destici et al. 2013), expression of circadian genes in peripheral tissues in young Cry1-/- and Cry2-/- mice was shown to be markedly phase-advanced or phase-delayed, respectively, compared to wild type mice. Aged short- and long-period mutant mice show a further increase in alteration of clock gene expression in peripheral tissues and dampening of clock genes oscillations in the SCN.

We additionally investigated the levels of age-related markers in the central nervous system and the peripheral tissues to understand whether circadian phenotype (morningness vs. eveningness) and/or type of mutations contribute to organismal dysfunction during aging.

Research Funding: Personal grant from Technology Foundation STW (De Stichting voor de Technische Wetenschappen).

T75

Mice are Able to Acquire Multiple Independent Time Memories

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Abstract: Mice anticipate the time of day that important conditions or events are likely to occur, based on their previous experiences with those conditions. When events occur only once, or only at one time of day, the existence of a time memory is implicit in the anticipatory behavior of animals near the same time of day on the days following conditioning. This suggests that a condition-entrainable oscillator (CEO) that is set at the time of an experience is registered as one of the context features, so that similar conditions are expected to recur later at 24 hr intervals. However, timing can be reset subsequent to training, that is to say, time memory may be perturbed without affecting memory for other context features. We have hypothesized that the representation of time of day is a property of the same cells that participate in the encoding of other features of the event. We now show that mice

are able to acquire multiple independent time memories. Animals that are conditioned to avoid a mild foot shock (place avoidance conditioning, CPA) at one time of day, and to anticipate the location of food (conditioned place preference, CPP) at another time, are capable of retaining the "what, when, where" attributes of the events in long term memory without interference. But this occurs only when the CPA and CPP contexts are different. Interference in time memory (generalization) occurs when the contexts are similar. The results support the idea that time memory is a property of the cells that are involved in specific event-related semantic memory formation.

Research Funding: Supported by a grant from the Natural Sciences and Engineering Research Council of Canada to MRR.

T76

RAS2 is a Regulator of the Circadian Clock in Neurospora Crassa

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Abstract: Both experimental and clinical investigations indicate a tight interaction between metabolism and circadian timekeeping; however, characterization of the underlying mechanisms is still incomplete. Metabolic compensation allows circadian oscillators to run with a constant speed at different substrate levels and therefore is a substantial criterion of a robust rhythm in changing environments. Based on data suggesting a central role of RAS2-mediated signaling in the adaptation of yeast to different nutritional environments, we aimed to examine the involvement of RAS2 in the metabolic regulation of the clock in the circadian model organism Neurospora crassa. We show that in the strain expressing no ras2 the period is longer than in the control. Moreover, operation of the circadian clock is dependent on glucose in the mutant; i.e. compared to starvation conditions, in the presence of glucose the period is longer and the rhythm of frequency (frq) expression is dampened. At protein level the delayed phosphorylation of FRQ in constant darkness and the parallel shift towards the hyperphosphorylated forms of White Collar-1 are in accordance with the longer period of the knock-out strain. Addition of glucose induced the accumulation of nuclear FRQ in the mutant, whereas no significant change in the subcellular distribution of the protein was observed in wt. RAS2 interacted with the RAS-binding domain of the adenylate cyclase in vitro, and the cAMP analogue 8-Br-cAMP partially rescued the circadian phenotype of the mutant in vivo. We propose that RAS2 acting via a cAMP-dependent pathway is involved in the metabolic control of the Neurospora clock.

Research Funding: This project was supported by an EMBO-Howard Hughes Medical Institute startup grant and by the National Research, Development and Innovation Office - NKFIH (K115953).

T77

Transcriptional Regulatory Logic of the Diurnal Cycle in the Mouse Liver

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Abstract: Most forms of life exhibit temporal rhythms in gene expression that propel diurnal cycles in physiology. These rhythms are controlled by transcription-translation feedback loops of the core circadian clock and modulated by feeding-fasting rhythms. To better understand the regulatory interplay between the circadian clock and feeding-rhythmic metabolism, we examined DNase I hypersensitive sites (DHSs) in mouse liver during a diurnal cycle. DNase I signals cycled at a substantial fraction of all DHSs, suggesting that DHSs harbor regulatory elements controlling rhythmic transcription. Using ChIP-seq, we found that hypersensitivity cycled in phase with RNA polymerase II (Pol II) loading and H3K27ac. We exploited the DHSs to designed a penalized linear regression model to infer the activity of transcription regulators using Pol II loadings in WT and in Bmal1-/- mice. While our model identified most known circadian regulators, we found several motifs related to metabolism, such as CREB, SREBP, FOX and GR, showing circadian activity in WT, and that may compensate for the impairment of the circadian clock in Bmal1-/-. These transcription factors regulate genes that display circadian oscillations due to food entrainment under night restricted feeding. In arrhythmic Bmal1-/- mice, hypersensitivity was mildly affected genome-wide, though we observed a strong reduction at BMAL1 binding sites. Nucleotide resolution DNase I footprints at locations harboring BMAL1 bound tandem E-box motifs changed in shape over the diurnal cycle, suggesting a transient hetero-tetramer binding configuration at those loci between ZT6 and ZT10. Overall, DNase I mapping provided significant additional insights into the mechanisms of diurnal transcription regulation in mouse liver.

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T78

Comparison of the Circadian Clock of Social and Solitary Bees

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Abstract: The seasonal and daily changes in floral resources are a great challenge for bees. To predict the regular oscillations in the environment bees have evolved a circadian clock, which is involved in timing daily activities, the bees' time memory and their sun-compass orientation.

So far studies focus on the circadian clock of the honey bee (Apis mellifera), which lives in a complex social hive organization. This life style influences circadian rhythmicity whereby nurse bees exhibit no circadian locomotor activity, but forager bees show strong circadian activity rhythms. The honey bee molecular clock is based on transcriptional/translational negative feedback loops in which at least four clock genes - period (per), cryptochrome-m (cry-m), cycle (cyc) and clock (clk) - are involved. But also other factors like the neuropeptide PDF (Pigment Dispersing Factor), identified as part of the circadian clock in different insects, seem to be important for circadian timekeeping.

Much less is known about the circadian clock of another bee species: The red mason bee (Osmia bicornis). These solitary living bees do not have the social context of a hive community, but face the same oscillating changes in environment. We have started to investigate putative clock genes of the red mason bee on the transcriptional level, while on the anatomical level we have compared the expression of PDF and period in brains of red mason bees and honey bees by immunocytochemistry. Additionally we examine locomotor activity to identify possible differences in the output of the clock, wherefore we developed a set-up to monitor individual bees either solitary or in social context of a mini colony.

Our aim is to gain new insights into "social" and "nonsocial" clocks by comparing the circadian clocks of these two bee species.

Research Funding: German research foundation (DFG), collaborative research center SFB 1047 "Insect timing"

T79

Entrainment Maps: A New Tool for Understanding Properties of Circadian Oscillator Models

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Abstract: Entrainment implies that an endogenous oscillator has matched its period to that of an external periodic forcing and has established a stable phase relationship with the forcing signal. The process of circadian entrainment has been studied extensively using tools from oscillator theory, in particular phase response curves (PRCs) that measure the change in the phase of an endogenous limit cycle oscillation (typically in constant darkness or DD) induced by a perturbation (typically a light pulse) as a function of the phase at which the perturbation is applied. Alternatively, one could perturb an oscillator in constant light (LL) with dark pulses. Such PRCs can be constructed for light or dark pulses of arbitrary strength and duration. However, for a PRC to accurately predict properties of entrainment to periodic light or dark pulses, the perturbations must be weak or brief enough that the oscillator would relax back to the DD or LL limit cycle attractor before the next pulse arrives. We show that PRCs do not accurately predict the phase of entrainment in a mathematical model of the Drosophila circadian clock subjected to photoperiods with substantial amounts of both light and dark, such as 12:12, 16:8, or 8:16 light:dark (LD) cycles. For these LD cycles, the entrained solution is best thought of as a combination of two limit cycle attractors, i.e. the DD and LL limit cycles, rather than as a perturbation of a single limit cycle attractor. Here we introduce a new tool, the entrainment map, which is not based on perturbing DD or LL oscillators and thus is able to accurately predict the phase of entrainment for any photoperiod.

Research Funding: NSF DMS 1412877

T80

Genome-Wide Profiling of Diurnal Rhythmic Gene Expression in the Water Flea Daphnia Pulex

Samuel Rund[2], Boyoung Yoo[1], Camille Alam[1], Taryn Green[1], Melissa Stephens[1], Erliang Zeng[1], Gary George[1], Aaron Sheppard[1], Giles Duffield[1], Tijana Milenkovic[1], Michael Pfrender[1]

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Abstract: Marine and freshwater zooplankton exhibit daily rhythmic patterns of physiology and behavior which may be regulated by the LD cycle and/or endogenous circadian clock. One of the best-studied zooplankton taxa, the freshwater crustacean Daphnia, has a 24-hr diel vertical migration (DVM) whereby the organism travels up and down through the water column daily. DVM plays a critical role in resource tracking and the avoidance of predators and ultraviolet radiation. However, there is little information at the transcriptional level linking the expression patterns of genes to the rhythmic physiology/behavior of Daphnia. We therefore sought to perform a genome-wide temporal transcriptional analysis on D. pulex. Whole bodies were collected every 4 hr over 44 hr under LD cycle diel/diurnal conditions, RNA hybridized to microarrays and data analyzed using the JTK_CYCLE algorithm. From the 21,002 genes with expression levels above background, we identified 1,661 rhythmically expressed genes using a conservative q-value ($q < 0.1$; $p < 0.03$) and 22-26-hr period length cutoff criteria. These genes represent 5.7% of the total D. pulex gene-set, and 7.9% of genes considered expressed. Using a comprehensive network modeling and analysis approach, we identified an additional 133 co-regulated rhythmic genes that have similar network topological properties as those identified by JTK_CYCLE. This network approach also provided novel functional annotations for 374 genes. The rhythmically expressed genes possess diverse biological functions including immunity, oxidative detoxification, and sensory processes. We identified differences in the chronobiology of D. pulex as compared to other well-characterized terrestrial arthropods, including an apparent lack of rhythmicity in several of the expected-rhythmic gene families, e.g. in metabolic pathways and visual transduction. Furthermore, while D. pulex possess a full complement of the canonical clock genes found in other animals, the expected synchronized, rhythmic expression of these genes was not apparent. Our results reveal that Daphnia exhibits an abundance of rhythmic gene expression, and adds to a growing body of literature suggesting that the genetic mechanisms governing rhythmicity in crustaceans may be divergent from other arthropod lineages, including insects.

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T81 Open Board

T82

Using Signal Processing to Explore Diversity: Analyses of Locomotor Activity and Core Body Temperature Reveal Sex Differences in Mice

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Abstract: Females are historically understudied, partially because controlling for variation in hormonal and behavioral state across the estrous cycle is assumed to substantially increase variance. This has had unfortunate consequences, including unexpected effects of pharmaceuticals tested in men and then used on women (e.g., Ambien). However, Prendergast & Zucker (2014) recently reported that males and females exhibit comparable variance across many physiological traits. Because ovulatory cycles are a potential source of variance in only females, this suggests that unrecognized sources of variance exist between the sexes. Understanding the different patterns underlying overall variance would help determine the contribution of sex to observed variance in specific parameters under study. Biological rhythms are useful in capturing variance across time frequencies, from ovulatory cycles to changes across a day. In particular core body temperature (CBT) and locomotor activity (LA) show biorhythmic structure and carry information relevant to many physiological processes.

Here, we report analyses of 1-minute resolution CBT and LA data from 12 male and 12 female BALB/c mice. These data allow us to stage estrus in females, and reveal that males exhibit higher inter-individual variance than females across the day. To identify rhythms contributing to sex differences, we employed wavelet transforms (WTs) to analyze changes in power across a range of periodicities (1-24 hours). Our analyses pinpoint a sex difference in ultradian rhythms (URs) in which males show greater power than females. These URs are likely correlated with physiological systems (i.e., corticosterone pulses), suggesting targets for the future investigation of variables that contribute to sex differences, as in response to drugs. This work corroborates that female variance is not greater than that of males, and that time-series analysis allows more precise identification of the sources of variance in both sexes.

Research Funding: Supported by NRSA: F32HD081957 from NIH NICHD and NIH Grant HD-050470.

T83

TNF Signaling Regulates the Circadian Rhythm of Myogenic Responsiveness and Systemic Blood Pressure

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Abstract: Mean arterial pressure (MAP) exhibits only moderate circadian fluctuations because its two determinants, cardiac output (CO) and total peripheral resistance (TPR) oscillate in a phase shifted pattern: during the phases of low CO, TPR is high and vice versa. While mechanisms clocking CO are well studied, those driving the circadian rhythm of TPR remain enigmatic. We hypothesize that the circadian rhythm of TPR roots in molecular signals governing myogenic responsiveness (MR), defined as the ability of arteries to match resistance to pressure.

Mouse skeletal muscle resistance artery MR was assessed using pressure myography at specific times. MR at physiological pressures was maximal in the sleep phase (ZT07) and was minimal in wake (ZT23). General vasoconstriction (phenylephrine, KCl) displayed no rhythmicity. Sleep phase MR was blunted in mice with a mutated circadian molecular clock (ClockΔ19/Δ19 mice). Genetic deletion of smooth muscle-derived tumor necrosis factor (TNF), a key initiator of the MR, was most effective in reducing MR and MAP during the sleep phase.

These data support the concept that MR is a clocked parameter that (i) maintains MAP during sleep, (ii) offloads cardiac workload in sleep, and (iii) supports blood flow conductance in the wake phase. We speculate that rhythmic TNF signaling clocks MR; pathological elevations in TNF could desynchronize MR and contribute to cardiovascular dysfunction.

Research Funding: Canadian Institutes of Health Research Sleep and Biological Rhythms Fellowship

T84

Phase of Circadian Entrainment: A Simple Theory behind Complex Data?

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Abstract: When a circadian system is entrained by environmental cues, the phase difference between the circadian rhythmicity and the Zeitgeber assumes a stationary value. In humans, for example, this phase difference appears as a chronotype, separating night owls from early risers. Here, we aim at understanding the dependence of the phase of entrainment on the

properties of the circadian oscillator and the Zeitgeber. Motivated by results from the theory of nonlinear oscillators, we propose a so-called 12-hours rule for phase of entrainment. Comparing data from various sources such as human chronotypes, entrainment experiments with N. crassa and mammalian SCN slices, we point out the achievements and failures of our theory.

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T85

Using MRI to Observe Migratory Related Neurophysiological Changes

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Abstract: Most songbirds are nocturnal migrants, foraging during the day, and traveling most of the night during migratory periods. No apparent cognitive decline accompanies this chronic sleep deficit, in contrast with a clear loss of mental acuity during sleep deprivation when not migrating. In captivity, migrant birds will go through seasonal periods of nocturnal wakefulness and wing

whirring, which correspond to times of migration, and the associated cognitive protection is observed. Seasonal changes in neurophysiology have been observed in songbirds, particularly in the song system, but the areas and mechanisms contributing to cognitive protection are still unknown. We use a 7T Bruker MRI system to acquire images in vivo of wild-caught white-throated sparrows during different migratory states. A fast spin echo sequence (TE=6.6ms, TR=4s, resolution=78x78x500µm2, averages=8, RARE factor=8, scan time=10m40s) is used to acquire high-resolution T2-weighted coronal images of the whole brain. A diffusion tensor imaging (DTI; TE=20.1ms, TR=1s, Segments=4, resolution=230x340x340µm2, averages=1, directions=20, blips=2, b value=1000, scan time=3h6m) sequence is used to determine neural fiber directionality and connectivity. DTI images are masked and registered to the structural image. Magnetic field distortions are calculated and corrected by acquiring two complete DTI volume sets, each using oppositely polarized gradient blips. The corrected volumes are used to determine fiber tracts and fractional anisotropy. This enables us to compare structure and connectivity changes in birds when exhibiting migratory behavior and when migratory behavior is absent. Relevant brain structures will be identified for further study of cognitive protection.

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T86

Testing Novel Objective Parameters for Alertness

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Abstract: The Pupillary Unrest Index (PUI) was discovered as an objective parameter for alertness, when it was noticed that alert individuals were able to maintain a stable pupil size for ten minutes when situated in a dark and quiet room, while drowsy individuals could not (Lowenstein et al 1963). In alert individuals, excitatory impulses from the cerebral cortex inhibit the Edinger-Westphal nucleus and parasympathetic constrictor activity, resulting in a stable dilated pupil.

When drowsiness increases, the opposite mechanism occurs, resulting in a decrease in pupil size and frequent, slow pupillary oscillations (lasting from 4 till 40 s). This is defined as pupillary unrest and has only been measured in darkness.

The objective of this (pilot) study was to investigate whether the PUI could also be used as a parameter for alertness during exposure to light. In addition, blink frequency and duration were investigated as alternative parameters of alertness. The sensitivities of these parameters were also taken into account.

6 subjects (3 males, 3 females) with a mean age of 24.3 years (±2.5) were subjected to a paradigm lasting from 8 pm till 3 am. They were asked to complete the Karolinska Sleepiness Scale hourly. Subsequently, the PUI was measured using an eye-tracking device (sampling frequency=30 HZ). The PUI was calculated by adding up the absolute differences between the average pupil size of subsequent 30 data points (=1 sec) (Lüdtke et al 1998). The total PUI per measurement was calculated as the sum of all the differences. An eye blink was defined as a diameter of 0 pixels for <1 second and the duration was calculated. An outlier was defined as missing data points for more than 500 ms.

Sleepiness increased significantly over time ($p<0.0001$), however there was no significant increase in the PUI over time ($p=0.079$). Results showed that there was a significant increase in both blink frequency ($p<0.05$, $R2=0.54$), -duration ($p<0.001$, $R2=0.67$) and the product of the both ($p<0.001$, $R2=0.22$).

This pilot suggests that the PUI might not be a representative parameter for alertness during light, however other objective parameters such as eye blink duration and –frequency could.

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T87

Bmal1 Deletion in Adulthood Facilitates Adaptation to Disrupted Light/Dark Schedules in Mice

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Abstract: Recently, by utilizing both conventional and tamoxifen inducible Bmal1 knockout mice (cKO and iKO, respectively), we found that the loss of circadian rhythm in adulthood has an attenuated impact on general integrity and survival than expected at least under 12h light/12h dark condition (LD). To understand further the contribution of Bmal1 in post-natal life under conditions of circadian disruption, we subjected 4-5m old iKO mice and their littermate controls (Ctrls) to forced desynchrony protocols and monitored their locomotor activity using radiotelemetry. Mice were kept under constant darkness for 2 weeks followed by 10 cycles of 10h light/10h dark, 8 cycles of 8h light/8h dark, and 14 cycles of randomized light/dark regimes with ad libitum access to food and water. Under these conditions, control mice cannot be entrained, reflected by half cycles showing higher activity levels during light than dark phases. By contrast, iKO mice displayed higher activity levels in the dark phases of all cycles. Similarly, iKOs adjusted to jet lag (an 8h delay shift) more quickly than Ctrls. Under 12 cycles of 3h light/3h dark regime, however, Ctrls displayed higher activity levels in the dark phases of all cycles although there were still obvious circadian rhythms, suggesting that an ultradian mechanism is also involved. Insulin sensitivity was markedly reduced by disrupted light schedules as expected in Ctrls, but not in the iKOs. Thus, Bmal1 deletion in adult mice facilitates adaptation to new light/dark schedules and protects from the circadian disruption induced insulin resistance.

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T88

Translation Across Time and Space

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Abstract: Oscillatory gene expression underlies circadian rhythms in physiology and behaviour. In this study we addressed two poorly understood aspects of circadian gene expression, namely, its tissue specificity and the contribution of translation efficiency to daily changes in protein synthesis.

To this end, we performed ribosome profiling (RPF-seq) and parallel RNA-seq in mouse liver [Janich et al, Genome Res.,2015] and kidney and generated data of good quality and codon-resolution.

We noted that rhythmic expression is globally less widespread in kidney than in liver and of lower amplitude. Similar to the reported poor overlap in rhythmic transcriptomes between organs, we observed a prevalent tissue-specificity in translational (RPF-seq) rhythms. Moreover rhythmic translation of constitutively expressed mRNAs was detected in less than 100 genes in kidney (vs. 150 genes in liver), and this set did not contain ribosomal protein genes, a known example of rhythmic translational regulation in liver. The translation of these genes is thought to be directed by mTor signalling via 5'TOP motifs located on the 5'UTR, and our results would indicate that kidney is less responsive to the feeding cues triggering this translational regulation.

Beyond the circadian field, we investigated the extent to which translation efficiency (TE) determines differences in gene expression across tissues.

Protein synthesis is determined by transcript availability and translation efficiency, but their relative contribution is still a matter of debate. By comparing the differential mRNA abundance to the differential TE between liver and kidney, we observed that the former spans a more than 100 fold range, whereas the latter shows less than 3-fold variation, suggesting that divergent protein output is largely determined by transcript abundance differences. Nevertheless we noted that footprint abundance correlated significantly better than mRNA abundance between organs, indicating that differences in transcript availability are partly compensated by TE modulation, leading to a greater similarity in protein biosynthesis between organs.

In summary, our study reports on the contribution of translational regulation to tissue-specific rhythmic and non-rhythmic gene expression.

Research Funding: National Center for Competence in Research (NCCR - RNA and Disease) funding from the Swiss National Science Foundation (SNSF).

T89

Mitochondrial Network Morphology Changes with a Circadian Rhythm in Cell Lines

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Abstract: Mitochondria are often referred as "the powerhouse of the cell" because they are the main suppliers for ATP. In cells, mitochondria form highly dynamic networks, which are reshaped according to cellular needs. A strongly interconnected network (fused mitochondria) is associated with high-energy demand. It is thought that mixing of the mitochondrial content, i.e. proteins involved in ATP production, lipids, metabolites and mitochondrial DNA allows the mitochondria to work most efficiently. Fragmented mitochondria are frequently found in resting cells and allow for degradation of damaged mitochondria. Mice with a disrupted fusion machinery die in mid-gestation, and disturbed mitochondrial dynamic is associated with a variety of diseases, including Alzheimer's, Parkinson's and Huntington's disease.

In this light of these observations, we hypothesized that the basal state of mitochondrial network dynamics is regulated by the circadian clock. This basal level would then be prone to further changes by metabolic signals. In this way, mitochondria would be fused and provide high-energy production during food intake and organismal activity and undergo fragmentation during resting state. This would allow for a regular clearance of damaged mitochondria. In order to investigate this idea we characterized mitochondria in cell lines, a model which excludes influence of food intake and organismal activity on mitochondria. We characterized three cell lines, U2OS, MEF and HEK293A.

The cells were synchronized with a serum shock and harvested at 4 time points. Mitochondria were labelled with a fluorescent marker and imaged with a confocal microscope. The mitochondrial network of each imaged cell was evaluated with an automated image analysis algorithm. We found that in U2OS and MEF cells, both known to have a functioning circadian clock, the mitochondrial network morphology changes with a circadian rhythm. However, in HEK293A cells the mitochondrial network did not show this rhythmicity. This is in line with our hypothesis, since HEK293A cells do not have a functioning circadian clock. Furthermore, we used publicly available data sets characterizing the circadian transcriptome of the investigated cell lines to identify putative key factors of the core fusion and fission machinery with circadian abundance.

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T90

Circadian Rhythms in Neurospora Crassa are Regulated by a Component of a Conserved Nutrient-Sensing Pathway

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Abstract: Circadian (daily) rhythms are found in all domains of life and control many physiological processes. The oscillators that drive these rhythms have been studied in a few model organisms, including the fungus Neurospora crassa. Rhythmic transcription/translation feedback loops (TTFLs) are important mechanisms in circadian systems, but there are many reports of rhythms that persist when TTFLs are not functional and the oscillators that drive these rhythms are unknown in eukaryotes. In N. crassa, a TTFL oscillator including frequency (FRQ) and white collar complex (WCC) has been well-studied but many rhythms can still be seen in FRQ or WCC null mutants, and these are said to be driven by one or more FRQ-less oscillators (FLOs). We have used a genetic approach to identify components of FLO(s). We have previously found three mutations, prd-1, prd-2 and UV90, that abolish rhythms in FRQ-less conditions and also affect rhythmicity when FRQ is functional, demonstrating the close integration between the TTFL and FLO(s). We now report the identification of UV90 as a homologue of a component of a nutrient-sensing pathway conserved in eukaryotes. A knockout mutant is defective in its growth response to amino acids confirming its function in nutritional sensing. These results point towards a network integrating metabolism and TTFLs in the complete circadian system.

Research Funding: NSERC

T91

Differences in the Circadian Phenotype among Substrains of CBA Mice

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Abstract: Six substrains of the inbred CBA mouse strain are currently available commercially. However, to the best of our knowledge, the differences in the circadian phenotype between the substrains have not been studied to date. Here, we have determined the characteristics of circadian behavioral rhythms in CBA/N and CBA/J substrains. Each eight-week-old male mouse was housed in a cage with a wheel and maintained under a 12:12 h light-dark (LD) cycle for at least 2 weeks. The wheel running activities of these mice were recorded under LD and constant dark (DD) conditions. These mice were then re-entrained to the LD cycle and their activities were recorded under constant light (LL) conditions. Furthermore, mice were housed in DD conditions for 3 days and sacrificed every 4 h for 24 h. The pituitary, kidney, lung, and adrenal gland were harvested to examine circadian profiles of Per2 and Bmal1 mRNA expression levels using real-time PCR.

Under DD conditions, CBA/N mice showed a significantly shorter free-run period than CBA/J mice (CBA/N: 22.59 ± 0.18 h; CBA/J: 23.12 ± 0.28 h) and the total activity of CBA/N mice (5.32 ± 0.73 x 104 revolutions/cycle) was significantly higher than that of CBA/J mice (3.59 ± 1.16 x 104 revolutions/cycle). Under LL conditions, CBA/N mice showed a significantly longer free-run period than CBA/J mice (CBA/N: 25.62 ± 0.19 h; CBA/J: 24.88 ± 0.42 h). The expression levels of Per2 and Bmal1 mRNAs in sampled tissues showed circadian rhythms reflecting their circadian periods. However, no statistically significant difference was found between the expression profiles of CBA/N and CBA/J mice.

It is well known that the CBA/J substrain has a Pde6brd1 mutation that causes blindness. We crossed CBA/N and CBA/J mice to investigate the correlation between this mutation and phenotypes observed in the F2 generation. We observed a correlation between the genotype and the free-run period in LL condition, but not between the free-run period and activity in DD conditions. These results suggest the existence of unknown polymorphisms that influence circadian rhythms. We are currently performing QTL analyses to identify the gene(s).

Research Funding: None

T92

Understanding Timekeeping in an Intertidal Crustacean Eurydice Pulchra

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Abstract: Unlike terrestrial organisms where the circadian 24 h solar cycle dominates, organisms that live in the intertidal habitats are also exposed to a prominent 12.4 h tidal rhythm as well as semi-lunar (~15 day) and lunar (29 day) cycles. Whereas our knowledge of the molecular basis of circadian timekeeping in terrestrial organisms is extensive, that underlying tidal cycle is entirely unknown. We have studied an intertidal crustacean, Eurydice pulchra, which shows robust self-sustained

tidal swimming activity as well as circadian physiological and molecular cycles. The Eurydice tidal rhythm is temperature compensated and can be entrained by a vibrational stimulus. In addition, the circadian rhythm of chromatophore-pigment dispersion can be entrained with light/dark cycles. Using inhibitors of casein kinase 1 (CK1 ε/Δ), a cardinal component of the circadian clock in vertebrates and invertebrates, revealed that both chromatophore and tidal period are significantly lengthened (1) Phosphorylation of Eurydice's CLK and BMAL1, the two cardinal circadian transcription factors, was also disrupted by the inhibitor in Drosophila S2 cells leading to a reduction of their transactivating potential. Knockdown of the Eurydice period negative autoregulator affects circadian but not tidal cycles (1). Here, we show the results of recent experiments in which we have knocked down the positive regulators.

Reference:

1. Lin Zhang, Michael H. Hastings, Edward W. Green, Eran Tauber, Martin Sladek, Simon G. Webster, Charalambos P. Kyriacou, and David C. Wilcockson. (2013). Dissociation of Circadian and Circatidal Timekeeping in the Marine Crustacean Eurydice pulchra. Current Biology 23(19), 1863-73.

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T93

Hepatic miRNA Loss Resulted in Altered Adaptation to Food Restriction in Mice

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Abstract: Recently, we have reported a comprehensive profiling of the rhythmic transcriptome in mouse livers depleted of miRNAs (hepatocyte-specific Dicer knockout) and proposed roles for miRNAs in modulating rhythmic gene expression[1]. In brief, we found that miRNA-mediated regulation affected as much as 30% of the rhythmic transcriptome and influenced the phase and amplitude relationships between rhythmic transcription and rhythmic mRNA accumulation. In contrast, the core clock was surprisingly resilient to miRNA loss with the exception of mild posttranscriptional upregulation in Per2, Per1 and Cry2 mRNAs. At the protein level, however, only PER2 was significantly increased. Moreover, free-running PER2::LUC rhythms measured in liver explants showed long periods in the absence of miRNAs. We concluded that the liver clock is fully functional upon miRNA loss, but that a core clock period length phenotype is masked in the entrained animal. This hypothesis prompted us to explore the role of miRNAs in the hepatic core clock under conditions when the relationship between the master clock in the SCN and the peripheral liver clocks is brought out of equilibrium such as when food is restricted to the light phase of the day.

To this end, we have now measured hepatic PER2::LUC rhythms in freely moving Dicer knockout and control animals upon restriction of feeding time. Mice were fed ad libitum before switching to 6-h food restriction between ZT2-ZT8, and PER2::LUC rhythms were recorded using the RT-Biolumicorder device[2]. Our experiments suggest advanced kinetics of phase-shifting in Dicer knockouts compared to controls. At the behavioural level, Dicer knockout mice lost food-anticipatory-like activities that were observed before meal in control mice. At present, we are increasing the number of analysed animals in order to precisely quantify the effect and designing experiments to pinpoint the responsible miRNA(s). Altogether, our data point to an involvement of miRNAs in regulating flexibility of the clock towards changes in entrainment conditions.

1. Du NH.*, Arpat B.*, et al., eLife, 3:e02510, 2014. (* co-author).

2. Saini et al., Genes Dev., 2013, 27:1526.

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T94

Secreted Proteins Exhibit Diurnal Profiles in Human Plasma

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Abstract: The circadian clock as an autonomous and organ specific oscillator coordinates various physiological processes in humans. Shift-workers experience a chronic misalignment of their peripheral organ clocks which is associated with the development of depression, diabetes, obesity, or cancer. However, a blood-based test to assess such misalignments is currently lacking. Tissue-specific secreted proteins can be key to monitor and identify health risks of circadian misalignment. Indeed, the work of our team and others indicate that the secretion of hepatic proteins into the plasma is controlled in a rhythmic fashion. Our proteomics approach of mouse liver revealed that non-rhythmic mRNA encodes the major part of rhythmic proteins. Interestingly, secreted proteins are significantly overrepresented within this group. In line with this observation, proteins such as ALBUMIN, APOA4, and SERPINA1D are reported to be rhythmic in human plasma. However, to date, a systematic assessment of rhythmicity of plasma proteins has yet to be conducted. In order to obtain a comprehensive view of rhythmically secreted proteins, we performed an aptamer-based proteomic profiling approach on human plasma samples. In total 17 human subjects participated in this study. Plasma samples were collected every 4 hours for a period of 24 hours. During the experiment each subject was served three standardized meals (breakfast, lunch and dinner). In total, 43.2% (488 out of 1122) of the proteins assessed in human plasma showed a rhythmic pattern. In order to decipher the contribution of timed food intake in this study, we also measured plasma proteins in a modified study design. This time, 10 human subjects had a continuous intake of a nutritionally balanced solution at 1-hour intervals during their waking hours (0700h-2200h). The majority of human plasma proteins follow a similar temporal profile with a peak at 1800h. A subset of plasma proteins is dependent on timed food ingestion and a loss of rhythmic properties in a setting of continuous calorie intake. We also identified a set of tissue-enriched secreted plasma proteins that potentially can be used to assess the circadian phase of different organs.

Research Funding:

T95

Circadian Control of Global Proteomic Output in Neurospora Crassa.

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Abstract: Eukaryotic circadian clocks involve transcriptional/translational feedback loops that drive 24hr rhythms in transcription. It is hypothesized that these transcriptional rhythms underlie oscillations of protein abundance, thereby mediating circadian rhythms of behavior, physiology, and metabolism. Research in Neurospora crassa pioneered the isolation of clock-controlled genes (ccgs) through the use of subtractive hybridization, microarrays and RNA-seq, as well as a host of other techniques. While these transcriptomic methods have been the traditional way to identify clock-regulated elements, new data show that concordance between mRNA and protein levels, including the relationship between circadianly oscillating mRNAs and proteins, has consistently been low. In order to better understand the correlation between circadian output at the level of expression, as compared to the level of protein, we used Tandem Mass Tag Mass Spectrometry to identify global protein levels over a period of 48 hours with a resolution of 2 hours. We then subjected these rhythmic proteins to categorization using Gene Ontology (GO), demonstrating enrichment in specific ontological classifications. Finally, we matched this data to previously published analyses of rhythmic levels of mRNA determined via RNA-seq. This analysis showed that while there are many rhythmic proteins correlated with oscillating mRNAs, the association of expression levels with proteomic levels was less than expected, suggesting that while circadian expression is important to clock-regulated element rhythmicity, there are other factors that determine rhythmicity at the protein level. Furthermore, it insinuates that in order to have a complete picture of what the clock regulates, one must investigate all levels of cellular output.

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T96

A Systems-Driven Experimental Approach Reveals the Complex Regulatory Distribution of p53 by Circadian Factors

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Abstract: Unlike single-cell organisms with self-contained timekeeping systems, multicellular organisms partition their oscillators among different cell types and depend on more complex molecular networks to sense signals and coordinate effective responses. We found that the core circadian clock protein Period 2 (Per2) directly interacts with the checkpoint regulatory component p53, promoting its stabilization and controlling p53 transcriptional activity. Remarkably, circadian phases of Per2 and p53 are anti-phase in the cytoplasm and in-phase in the nucleus, posing new questions about the extent to which Per2 association modulates p53 distribution. Therefore, we focused our efforts on investigating what simulated conditions better relate to the experimental data using mathematical models. Specifically, the model predicted that the phase of the Per2:p53 interaction strongly depends

on the binding mechanisms between Per2 and p53 mediated by ubiquitin, as determined by evolving the interaction types between Per2 and p53 in the model during the fitting process. As a result, the ubiquitilation state of p53 impacts Per2 binding and subcellular distribution. All predictions were confirmed experimentally.

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T97

Entrainment Ability of the Peripheral Circadian Clocks by Light, Food, Stress, and Exercise in Aged Mice

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Abstract: Aging has been well recognized to be associated with a decline in day-night differences in the sleep-wake pattern, body temperature, or hormonal secretions in humans and rodents. Although several studies have investigated the attenuated central clock functions in the suprachiasmatic nucleus (SCN), the functions of peripheral clocks have not been well understood. In the present study, we examined the entrainment ability of aged peripheral clocks to well-known entrainment cues such as light, food, stress, and exercise.

By using the in vivo whole-body imaging method, we obtained the peripheral PER2::LUC rhythms of the liver, kidney, and submandibular gland in a living mouse. Based on these rhythms, we found that the aged peripheral clocks (>18 months old) oscillated normally in the regular condition when compared with the young peripheral clocks (3–6 months old). However, altered phase shifts were shown in the aged peripheral clocks compared with the young mice at 5 or 9 days after the 8-h phase advance of the light-dark cycle, along with an attenuated re-entrainment of the locomotor activity rhythm. In addition, the phase shifts induced by restraint stress or treadmill exercise performed at ZT4-4.5 for 3 consecutive days were attenuated in the aged mice, especially in the salivary gland. This is due to an age-related reduction in stress/exercise-induced sympathetic nervous and glucocorticoid activations, and adrenergic receptor expression levels in the salivary gland. Because food-induced entrainment is negatively associated with SCN-regulated sympathetic and glucocorticoid pathways, we further detected greater harmonized entrainment among tissues to the daytime refeeding paradigm in the aged mice than in the young mice.

Overall, our findings suggest that age-related decline in the connection between the central and peripheral clocks causes attenuated re-entrainment to light, stress, and exercise, but causes enhanced re-entrainment to food in aged peripheral clocks.

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T98

Sleep and Circadian Regulation of Metabolic Rate in Drosophila

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Abstract: Dysregulation of sleep and circadian rhythms is linked to deficiencies in metabolic function, yet the mechanisms that regulate the reciprocal interactions between these processes are unclear. We have established a single-fly respirometry system to simultaneously measure activity and metabolic rate for periods as long as two days. Metabolic rate is robustly influenced by the circadian clock, with a lower metabolic rate during the night that persists in constant darkness and is absent in Clock mutant flies. Metabolic rate also decreases when flies sleep and within individual sleep bouts, suggesting the presence of light and deep sleep states in Drosophila. The reduction in metabolic rate during sleep is greater during the night than during the day, and metabolic rate is lower during sleep rebound following sleep deprivation. This fortifies the notion that reduced metabolic rate is a marker of sleep intensity. Taken together, these findings reveal that metabolic rate is influenced by sleep and circadian processes, and they provide a system to investigate the genetic basis for the integration of sleep and metabolic state.

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T99

Amplitude Response of Circadian Clock System to External Stimuli

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Abstract: In mammals, many physiological processes and behaviors are orchestrated via circadian clock. Attenuated circadian amplitudes are usually related with many diseases. Both the strengths and phases of the external stimuli can affect the amplitude of circadian clock. However, due to numerous combinations of phases and strengths, it is difficult to study the amplitude response systematically. In this study, we used a previous mathematical model and a simple limit cycle model to mimic the amplitude responses of the circadian clock in the following 24 hours upon the stimulations with different phases and strengths. Regarding to the stimuli, previous studies showed that light can induce the expression of the Per gene. Therefore, the effect of stimuli is incorporated into the models by the rate of Per1/2 transcription. The silicon simulations suggest the following conclusions: 1) modern life style with long time of light exposure may not reduce the amplitude of circadian and impair fitness; 2) there are stimulus phases which are the most or the least in favor of health in 24 hours 3) when a set of cellular circadian clocks are stimulated at certain phase and strength, singularity behavior can be observed. We further found that at multi-cell level, both amplitude change of single cell and synchronization of cell population can contribute to the average amplitude response of cells. Finally, we explained the mechanism of above conclusions by the limit cycle model. This study systematically investigated the amplitude response of the circadian clock to external stimuli, which provides strategy for optimizing the amplitude response in circadian clock.

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T100

Neuronal Activity Induced Changes of Energy Metabolites in the Mouse Suprachiasmatic Nucleus

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Abstract: The suprachiasmatic nucleus (SCN), located in the ventral hypothalamus, is considered to be the central regulator of daily rhythms in physiological and metabolic processes throughout the brain and body. The SCN expresses an intrinsic rhythm - which is similar in nocturnal and diurnal animals - with neurons mainly active during the day. Generation and regulation of cellular circadian rhythms is achieved by the interaction of molecular and cytosolic clocks, which involve metabolic pathways. Metabolites like cAMP and NAD+ are found to be important for proper clock functioning, but detailed knowledge on metabolite cycling and their functional importance in the SCN is lacking. In this study we aimed at testing the feasibility of metabolic profiling of the SCN and assess the influence of neuronal activity on metabolite levels.

We used Zwitter Ionic Hydrophilic Interaction Liquid Chromatography - Mass Spectrometry (ZIC-cHILIC-MS) for metabolic profiling of the mouse SCN. We identified and semi-quantified a subset of energy metabolites. To test the influence of neuronal activity on the level of metabolites in the SCN cells, tissue samples were taken at midday and midnight, and either incubated in 1) normal artificial cerebrospinal fluid, (ACSF), in 2) ACSF with tetrodotoxin (0.5 uM TTX; at midday), or 3) ACSF with higher K+ concentration (15 mM; at midnight). The SCN tissue was isolated by bilateral punches with a Ø 500 µm from two consecutive 250 µm thick coronal slices and metabolites were extracted by sonication.

Of the 58 metabolites that could be detected in the samples, two were significantly higher at midday, compared to midnight. Exposure to TTX had little effect on metabolite levels. Interestingly, the incubation with higher K+ concentration significantly upregulated only 11 of the 58 measured metabolites. Among the upregulated metabolites, five were intermediates of the tricarboxylic acid (TCA) cycle (six of the ten intermediates could be measured). Exposing the SCN slices to higher K+ concentrations depolarize the neurons, inducing action potentials. Facilitating this neuronal activity is known to be highly energy-consuming, explaining the upregulation in the ATP producing TCA cycle. Overall, the results of this study demonstrate the feasibility of metabolic profiling of the SCN.

Research Funding: Foundation of Technology STW, Leids Universiteits Fonds LUF

T101

SCN Phosphoproteomic Analysis Reveals GRK2 as an Important Modulator of Neuronal Structure and Cytoskeleton Organization

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Abstract: Suprachiasmatic nucleus (SCN) serves as the master circadian pacemaker to generate self-sustained ~24h rhythms to the external environment by daily resetting in response to light. The clock neurons in the SCN communicate with each other via the activation of G protein-coupled receptors (GPCRs) by their extracellular ligands. In our previous study, GRK2, unlike other GPCR kinases, has been shown to possess a non-canonical function whereby it physically associates with PER proteins and promotes the phosphorylation of PER2. However, the global proteomic effects of GRK2 modulation on the murine SCN in the response to light are not completely understood. In this study, we performed a SILAC-based phosphoproteomic approach to analyze the SCN phosphoproteome of wild-type and conditional knock-out of grk2 (grk2 cKO) mice in the presence and absence of light stimulation (15-min light pulse at CT 15). Of the 1287 accurately quantified phosphorylation sites corresponding to 599 proteins, 133 phosphorylation sites were significantly altered between grk2 cKO and wild-type SCN phosphoproteome under light stimulation, compared to 132 phosphorylation sites altered in the absence of light stimulation. Bioinformatics analysis of the light-inducible phopshoproteome reveals their diverse distribution in different canonical pathways; most notably, neuron projection development, and regulation of cytoskeleton organization. Additionally, 30 light-stimulated SCN phosphopeptides contained the GRK2 consensus substrate motifs. This finding will facilitate a more integrative understanding of GRK2 function on the SCN clock.

Research Funding: Canadian Institute of Health Research (CIHR) and the National Sciences and Engineering Research Council (NSERC) of Canada.

T102

Ontogeny of Circadian Synchrony in the Suprachiasmatic Nucleus

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Abstract: The hypothalamic suprachiasmatic nucleus (SCN) coordinates daily rhythms including sleep-wake behavior, hormone release and gene expression. Although previous reports revealed in utero and in vitro daily rhythms in physiology and metabolism in the embryonic SCN, little is known about the development of cellular circadian rhythms in the SCN.

We hypothesized that synchrony among circadian cells in the SCN occurs prior to birth when vasoactive intestinal polypeptide (VIP) is first expressed. We recorded bioluminescence from 300 um coronal SCN slices from fetal (E13.5-E15.5 and E17.5, we confirmed the overnight matings by the presence of vaginal plugs and designated as embryonic day 0.5) and postnatal (P2, the day of born was designated like postnatal age 0) homozygous Per2Luc mice with a cooled CCD camera. We analyzed the bioluminescence of the developing SCN. Briefly, we identified circadian pixels (37 x 37

um) with dominant periods between 18-32h by Lomb-Scargle periodogram analysis and measured their synchronization index. We measured the expression of VIP and VPAC2R, by immunochemistry and quantified the effect of the antagonist of VPAC2R on the synchronization during embryonic development.

The SCN explants isolated on E13.5did not express circadian rhythms in vitro. We found a suddenly increase in the fraction of circadian oscillators between E14.5 (0.10+0.07 fraction of pixels, mean+SEM; n=6/11) and E15.5 (0.72+0.11 fraction of pixels, mean+SEM; n=9). SCN oscillators shown an increase in the precision of period after E14.5 (4.14+0.45 h, mean+SEM; n=11 SCN), compared to E15.5 (1.80+0.29 h, mean+SEM; n=9). By E17.5, all recorded SCN were circadian with a narrow distribution of periods (24-25.5 h) and low cycle to cycle variability (2.04+0.29 h, mean+SEM; n=4). Synchrony among circadian cells started as early as E15.5 (SI =0.87±0.17, n=9 SCN) before the expression of VIP or VPAC2R, which were detectable by E17.5, at this age the synchrony reaching a maximum (SI=0.97±0.004, n=4). Preliminary result shown that synchrony between cells is not affect by the antagonist of VPACR2 in the early development stages (E15.5).

We conclude that SCN cells become circadian around E14, synchronize to each other around E15 independent of VIP and then use VIP to establish the mature pattern of PER2 circadian rhythms after E17.

Research Funding: The March of Dimes and NIGMS

T103

Lhx1-Regulated Transcriptional Networks Control Sleep/Wake Coupling and Thermal Resistance of the SCN Clockworks

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Abstract: In this study, we find that Vip regulates expression of a large suite of mostly circadian genes in a manner not fully explainable simply by desynchrony of cellular oscillators. In addition to regulating Vip and Vip-regulated genes, Lhx1 controls expression of a distinct second network of genes that are both SCN-enriched and less dependent on the clock. This Vip-independent pool of Lhx1-regulated genes is essential for both light and circadian control of sleep rhythms, as well as thermal resistance of the SCN clock. Specifically, we find that both photoentrainment and circadian rhythms of sleep are largely eliminated by Lhx1 deletion, in sharp contrast to more modest defects in sleep rhythms previously observed in Vip-null animals under a light:dark cycle. Furthermore, resistance to temperature entrainment and resetting of circadian behavior in intact animals by fever is largely abolished by Lhx1 but not Vip deficiency, a result supported by entrainment and resetting of the Lhx1-deficient SCN by direct temperature manipulations in culture. In sum, these results identify functionally distinct Vip-dependent and Vip-independent Lhx1-regulated transcriptional networks. Together, these bipartite networks contribute to many features critical for the SCN's role as the central circadian pacemaker, including its robust and well-synchronized cellular clockworks, ability to entrain circadian behaviors to light, and resilience against non-photic zeitgebers such as temperature.

Research Funding: This project was funded by the Brain Science Institute of Johns Hopkins. JLB was supported by Visual Neuroscience Training Program and NSF Graduate Research Fellowships.

T104

Isoflurane Anaesthesia Phase Shifts the SCN: Recordings from PER2::LUC Mice

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Abstract: Emerging evidence suggests that general anaesthesia may cause post-operative sleep disruption. Robust data from the honey bee has revealed that the general anaesthetic isoflurane causes phase delays of the circadian clock as a result of a shift of the expression of core circadian clock genes (crytochrome and period). In order to determine whether isoflurane causes robust and reproducible phase shifts in mammals, and whether these effects result from isoflurane acting directly on the SCN or not, we have been examining the effect of isoflurane on Per2 expression in SCN slices using PER2::LUC mice.

PER2::LUC SCN tissue (n=32) was cultured over eight days and exposed to a six hour isoflurane anaesthetic (or air control) after four cycles. Phase shifts and period changes were analysed using the peak expression time (acrophase) on each of the days following anaesthesia/air compared to the days prior.

Administration of a six hour of isoflurane anaesthetic to SCNs between CT12 and CT18 resulted in an average phase delay of -7.7 h [±2.5 SEM],) compared to air administration which resulted in an average phase delay of 1.83 h (±3.9 SEM), (p=0.06). Neither isoflurane nor air administered at other CTs elicited significant phase shifts.

There was no significant difference change in the mean tau following isoflurane administration (25.37 [±0.22 SEM], 26.87 [±0.94 SEM]), (p=0.06) or of air (25.50 [±0.32 SEM], 25.93 [±0.55 SEM]) (p=0.19). There were however specific examples of extreme period lengthening (30+h) following isoflurane exposure.

While air appears to cause moderate shifts of the SCN between CT12 and CT18, the isoflurane induced shifts are substantially larger. This is in line with previous findings in invertebrates where the largest phase shifts were detected around CT12.

These findings are important for two reasons 1) the phase shifting effect we see in insects also occurs in mammals (and presumably in humans) and 2) the effect is attributable at least in part to isoflurane acting directly on the SCN (thus opening up the potential for treatment strategies). This work has paved the way to a clinical trial we are currently conducting to examine the efficacy of light administration in reducing anaesthesia-induced postoperative circadian and sleep disruption.

Research Funding: This research was funded by a grant from the Office of Naval Research, USA (N000141310879) awarded to GRW and JFC and a University of Auckland Doctoral Scholarship awarded to NML.

T105

CRTC1-SIK1 Pathway is Significant to Light Adaptation Capability

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Abstract: In answer to light stimulus, mammalian circadian clock first responses sensitively, then it returns to original state even when external light persists. Recent experimental results showed that this adaptation capability is due to the CRTC1-SIK1 pathway which includes a negative feedback loop; however, the implications of this pathway for light adaptation dynamics remains to be further elucidated. To investigate how critical the light adaptation property is to the robustness of the circadian clocks, we developed a light-dependent mathematical model combining this CRTC1-SIK1 module with existing core circadian module. Our findings show that the light adaptation due to CRTC1-SIK1 pathway helps the core clock to resist fluctuations of the environment. Under sustained light stimulus, it provides the phase robustness and prevents the singularity behavior, which is the total abolishment of circadian rhythms. It also generates the refractory effect under frequent light stimulus. Numerical simulations and theoretical analysis reveal the underlying mechanisms of environmental adaption: CRTC1-SIK1 negative feedback loop reduces the lasting synthesis of per mRNA to maintain its homeostasis in the presence of external stimuli.

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T106

Cannabinoid Signaling Alters Clock Phase and GABAergic Neurotransmission within the SCN

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Abstract: The major psychoactive ingredient in marijuana, (–)-trans-Δ9-tetrahydrocannabinol, is an agonist for the CB1R and CB2R cannabinoid receptors. CB1/2 receptor (CB1/2R) agonists can block the phase shifting effects of light and inhibit GABAergic neurotransmission in the suprachiasmatic nucleus (SCN), but little research has been done to study the phase shifting effects of CB1/2R agonists themselves. Also, the mechanism behind the inhibition of GABAergic neurotransmission remains unknown. In other areas of the brain, cannabinoid signaling, and alterations in neurotransmission are mediated by astrocytes. Here, we test the hypothesis that CB1/2R activation acts as a nonphotic cue in an astrocytes-dependent manner. We found that activation of cannabinoid signaling using the CB1/2R agonist WIN 55,212-2 (3 μM) phase advanced Per2 bioluminescence rhythms. Application of WIN (3 μM) decreases the frequency of GABA(A) receptor-mediated mIPSCs, decreasing GABAergic neurotransmission. Fluorocitrate (50 μM), a selective metabolic inhibitor of astrocytes, attenuates the effects of WIN on mIPSCs. Furthermore, fluorocitrate (1 μM) also desynchronizes the circadian rhythms of SCN neurons, as measured by Per1:Venus fluorescence. This study may explain the evidence of circadian disruption seen in chronic marijuana users. Also, it demonstrates that astrocytes are an essential part of the circadian pacemaker.

Research Funding: This research is funded by NIH grants: T32DA007262 to Kim Neve and R01NS036607 to Charles Allen.

T107

GRK2 Regulates Nucleocytoplasmic Distribution of PERIOD1/2 and Major Ligand-GPCR Systems in Circadian Timekeeping

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Abstract: Most aspects of behaviour and physiology exhibit circadian rhythms that align with the day-night cycle. In mammals, these overt rhythms are controlled and coordinated by a central circadian pacemaker in the hypothalamus known as the suprachiasmatic nucleus (SCN). SCN neurons express a rich array of G protein-coupled receptors (GPCRs) that allows them to respond to environmental time cues and to communicate with one another in a neuronal network. The activity of GPCRs is regulated by G protein-coupled receptor kinases (GRKs), a family of serine/theorine kinases that directly phosphorylate intracellular domains of agonist-bound GPCRs and attenuate G protein-dependent signaling via receptor internalization. In addition to their canonical function as GPCR kinases, GRKs can also regulate non-GPCR receptors and other signaling proteins in both kinase-dependent and kinase-independent manner. In a recent study, we have demonstrated the importance of a ubiquitously expressed GRKs, GRK2, in mediating the desensitization of major ligand-GPCR systems in the SCN and nuclear trafficking of PERIOD1/2 proteins. Using GABAergic neuron-specific conditional grk2 knock-out mice and cell line models, we have established that GRK2 1) facilitates ligand-induced VPAC2 and PAC1 internalization, 2) post-transcriptionally regulates VPAC2 expression in the SCN, and 3) functionally converge with CK1Δ/ε and other prominent protein kinases to suppress nuclear trafficking of PERIOD protein, delaying their accumulation in the nucleus.

Research Funding: By grants to H.-Y.M.C. and D.F. from CIHR and NSERC. H.-Y.M.C. is a Tier II CRC in Mol. Genetics of Biological Clocks, and D.F. is a Tier I CRC in Proteomics and Systems Biology.

T108

Gpr176 is an SCN-specific Gz-coupled Orphan GPCR that Controls Circadian Behavior

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Abstract: The suprachiasmatic nucleus (SCN), the brain's circadian pacemaker, governs daily rhythms in behavior and physiology. G protein-coupled receptors (GPCRs) participate in a broad range of physiological functions. A priority for fundamental and clinical research, therefore, is to decipher the function of over 140 remaining orphan GPCRs. In the hope of identifying novel GPCRs that tune circadian timing, we initiated the SCN orphan GPCR project to (i) search for orphan GPCRs with enriched expression in the SCN, (ii) generate mice deficient in candidate GPCRs, and (iii) analyze the impact on circadian rhythms. We thereby identified Gpr176 as an SCN-enriched orphan GPCR that sets the pace of circadian behavior. Gpr176 is expressed in a circadian fashion by SCN neurons, and molecular characterization revealed that it represses cAMP signaling in an agonist-independent

manner. Gpr176 acts independently of, and in parallel to, the Vipr2 GPCR, not through the canonical Gi, but via the unique G-protein subclass Gz.

Research Funding: Supported in part by the Core Research for Evolutional Science and Technology in JST & the Ministry of Education, Culture, Sports, Science & Technology of Japan.

T109

Circadian Arrhythmia Disrupts Theta Oscillations in the EEG

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Abstract: This study investigated the role of the suprachiasmatic nucleus (SCN) in the expression of hippocampal theta rhythms by electroencephalographic (EEG) recordings in freely behaving Siberian hamsters (Phodopus sungorus). The SCN of these animals can be made circadian-arrhythmic by a disruptive phase shift (DPS) protocol that induces arrhythmia while leaving the animals neurologically and genetically intact. The DPS protocol consists of administering a phase-advancing light signal on one night and then a phase-delaying signal on the following night. Arrhythmia is manifested at the level of clock genes in the SCN, and these animals remain arrhythmic indefinitely despite the continued presence of the light-dark cycle. Hippocampal theta rhythms are necessary for encoding spatial information and provide spatiotemporal information to hippocampal place cell assemblies. Because DPS-arrhythmic Siberian hamsters exhibit substantial impairments in hippocampal-dependent memory tests, we recorded EEG signals during an object exploration task and investigated whether the expression of theta (5-9 Hz) was compromised in these animals.

Entrained (ENT) rhythmic hamsters (n=8) were given a novel object to explore in their home cages while the EEG was recorded for 5 min. These animals were then made arrhythmic (ARR; n=8) by the DPS protocol and, four weeks later, the object exploration protocol was repeated, thus, each animal served as its own control. Object exploration elicited robust increases in power within the theta band, and decreases in the delta band (1-4 Hz) in ENT hamsters. These responses were significantly attenuated once the animals were arrhythmic, even though the animals engaged in vigorous object exploration. The number of EEG epochs that were dominated by theta did not differ significantly between ENT and ARR conditions. The expression of theta was fragmented in ARR animals during exploration such that the duration of theta-dominated bouts in the EEG was reduced by ~50% after animals were made ARR. Overall, ARR animals exhibited less power in the theta band and could not sustain elevated power in theta oscillations during exploration.

Deficits in cognitive performance caused by disrupted circadian timing have become a growing concern among health care professionals. However, there are no mouse or rat models of adult-onset circadian dysfunction in genetically and neurologically intact animals living in standard laboratory conditions. The arrhythmic Siberian hamster model might provide mechanistic insights into the underlying causes of dementia among individuals with compromised circadian function. The mechanism by which arrhythmia within the SCN impairs hippocampal function is unknown. Given the critical involvement of septohippocampal circuits in generating theta oscillations, however, it is likely that a dysfunctional SCN impairs neurotransmission in those structures.

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T110

Integrative Analysis of Multiple Genomics Datasets Reveals Key Networks and Pathways Underlying the Circadian and Homeostatic Regulation of Sleep

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Abstract: The timing and propensity of sleep are regulated by two interactive processes: circadian rhythmicity and sleep homeostasis. It has been established that the two regulatory processes converge at molecular levels, as the circadian clock genes not only control circadian timing of sleep but also influence homeostasis as well as many other aspects of sleep. However, since the molecular pathways involved in sleep homeostasis are still elusive, it is unclear how the circadian and homeostatic signals integrate to regulate sleep beyond a handful of clock genes. Previously, our group has utilized systems approaches to understand molecular networks and pathways involved in sleep function and regulation by integrative analysis of sleep phenotypic, genotypic and gene expression datasets. These efforts include one recent study of sleep traits collected during baseline as well as after sleep deprivation and restraint stress in ~200 (C57BL/6J x 129S1/SvImJ) F2 mice. Using microarray data in four brain regions (frontal cortex, hippocampus, thalamus/midbrain, and hypothalamus), we uncovered brain-region-conserved, as well as brain-region-specific, gene networks that are important for sleep. Here, we report an integrative analysis utilizing gene networks uncovered in the (C57BL/6J x 129S1/SvImJ) F2 mice and publicly available genomics datasets regarding sleep homeostasis and circadian timing in order to understand converged networks and pathways that regulate sleep timing and homeostasis. Our preliminary analysis identifies gene networks that are enriched with genes perturbed by sleep loss as well as cycling genes peaking at particular times of the day. These networks can be associated with sleep phenotypes measured in (C57BL/6J x 129S1/SvImJ) F2 mice, and are functionally annotated with specific cellular processes and pathways, thus revealing novel insights into the converged pathways that integrate circadian rhythmicity and homeostatic signals to regulate sleep.

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T112

Recovery of Circadian Time-Place Learning in Rats with Hippocampal Lesions

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Abstract: Rats, mice and other species can anticipate a daily mealtime by entrainment of circadian oscillators that drive daily rhythms of foraging activity. At least some of these species can also discriminate time of day and link predictable feeding times with unique feeding places, an ability known as time-place learning (TPL). Neural substrates of TPL remain unclear. The circadian clock in the suprachiasmatic nucleus is not required for TPL in rats and mice (Mistlberger et al, 1996; Mulder et al 2014), but the role of circadian clocks elsewhere in the brain has not been evaluated. A candidate structure is the hippocampus (HPC) (Mulder et al, 2016), an area that expresses food-entrainable

clock gene rhythms (Loh et al 2016) and that is essential for allocentric spatial learning and memory (O'Keefe and Nadel, 1978). Using a novel group-training TPL paradigm, male Long-Evans rats were trained to locate a food reward twice daily (ZT13.5-15.5 and ZT20-22) in a complex, 4-level apparatus (5 x 4.75 x 2.8 ft), with correct location contingent on time of day. Skipped sessions were used to rule out an alternation strategy. After 5 weeks of group training, individual testing revealed task acquisition in 9 of 20 rats (5 failed to learn and 6 used an alternation strategy). The rats that acquired TPL then received either HPC (N=5) or SHAM (N=4) lesions. Following 2 weeks recovery, rats with HPC lesions were initially impaired on the task relative to SHAM rats, but with continued testing, performance recovered. Skip-session and DD tests confirmed that performance was not based on simple alternation or learning to associate feeding locations with the interval between mealtime and LD transitions. The initial impairment may be due to 1. a lesion-induced disruption of circadian timing (involving clocks elsewhere in the brain) that recovers with continued exposure to the LD and feeding schedules, and/or 2. adoption of an egocentric spatial navigation strategy to compensate for loss of HPC-dependent allocentric navigation (Mumby et al, 1999). In either case, the results demonstrate that the circadian timing and spatial navigation functions of the HPC are not required for circadian TPL in rats.

Research Funding: Supported by operating grants from NSERC Canada to RM and DM.

T113

Sex Differences in Circadian Food Entrainment Are Unrelated to Gonadal Sex Hormones

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Abstract: Circadian food entrainment studies have typically used male mice as experimental models. In recent years, studies have demonstrated that a sex difference exists in food entrainment where males develop food anticipation much quicker than females and illustrate higher amplitudes of activity. In this experiment we tested the difference in circadian food entrainment using 32 inbred C57BL/6J mice. We hypothesized that circulating gonadal-derived sex hormones may be responsible for this sex difference. Much to our surprise, our experiment provided no evidence to support that gonadal-derived sex hormones were at play in developing food anticipatory activity (FAA). We observed no significant difference ovariectomized and intact females. Similarly, castrated males showed a similar magnitude of FAA in comparison to intact males. In sum, our experiment suggests that gonadal-derived sex hormones are not responsible for the enhanced ability of male mice to show FAA. Also, studies on older mice (9 months) did not reveal a sex difference. Thus, sex differences in FAA are confined to younger mice and may be due to differences in adiposity, metabolism, or brain development.

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Notes

Index of Authors

Legend
SS = Slide Session
S = Sunday Poster
M = Monday Poster
T = Tuesday Poster
SP = Symposium
W = Workshop

Index of Keywords

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Notes

Innisbrook Resort Information

Travel:

- Innisbrook offers complimentary on property transportation. Please dial '0' to arrange a pickup.
- Innisbrook Resort guests benefit from car rental special rates provided by Enterprise Rent-A Car. To take advantage of the special rates, call 727-942-3155.
- Innisbrook offers courtesy transportation to and from nearby Honeymoon Island Beach and Caladesi Island ferry. Please dial '0' for the operator from any house phone to inquire about times and availability.

Resort Dining:

Innisbrook hosts six restaurants on property with a diverse range of cuisine. Hours of operation vary slightly throughout the year; please call to check availability.

- Turnberry Pub (Breakfast, Lunch)
- Market Salamander Grill (Breakfast, Lunch, Dinner)
- Packard's Steakhouse (Dinner, Evening Bar)
- Loch Ness Bar & Grill (Mid Day to Early Evening)
- Osprey Bar (Lunch, Dinner, Evening Bar)
- Room Service (Breakfast, Lunch, Dinner)

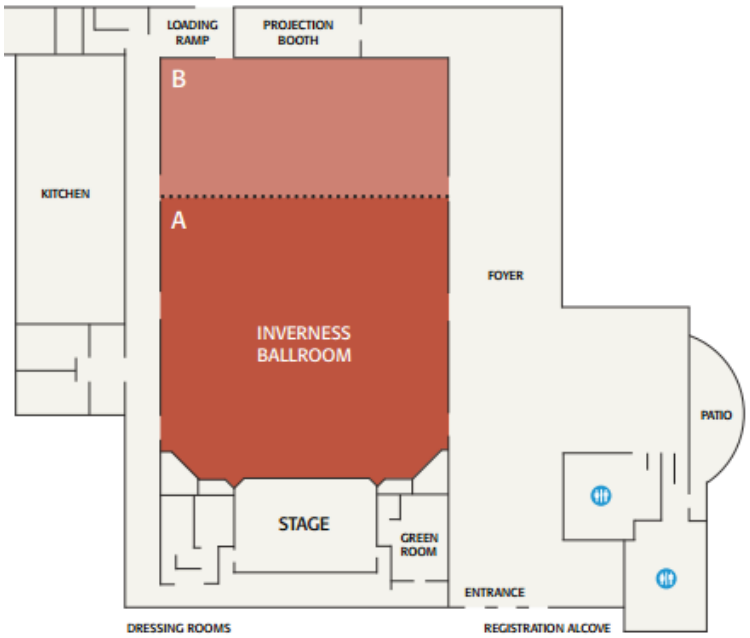
Resort Activities:

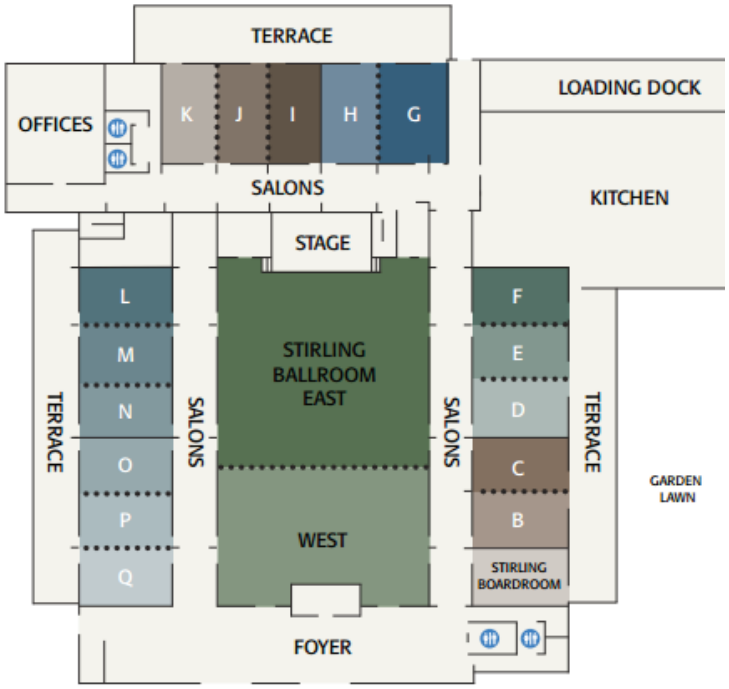
Please dial '0' from any house phone to inquire about availability and pricing.

- **Golf.** Innisbrook's four golf courses are considered some of the best in the world, including Copperhead Course, home to the PGA TOUR's Valspar Championship. Additional fees apply.
- **Retreat to Indaba Spa.** Innisbrook's new 18,000 square-foot spa, salon and fitness center. Reservations recommended.
- **Fitness Center.** The 4,800-square-foot facility features elliptical machines, exercise bikes, treadmills and free weight equipment and fitness classes. Access is complimentary for all guests.
- **Tennis.** Innisbrook's Tennis Center offers 11 Har-Tru® courts, with seven lighted for night play, a pro shop and 3 racquetball courts. Rental fees apply.
- **Water Activities.** The resort features six different pools and are temperature controlled. The Copperhead, Island and Loch Ness pools provide food and beverage service.
- **Resort's Natural Landscape.** 900 acres of undulating landscape, large expanses of open space and protected wetland areas. Take advantage of the nature trails and bike rental service.
- **Activities for Kids.**
  - Camp Nessie - offers a safe and enjoyable place for kids with great crafts, exciting games and lots of fun in the sun.
  - Camp Nessie Kids Night Out - Every Friday & Saturday night, 6pm - 10pm. $40 per child and includes dinner.
  - Enjoy other Family Activities including: basketball, volleyball, miniature golf, shuffle board, racquetball, bike rentals, fishing, nature trails and kid's camps.

Conference Center Floor Plans

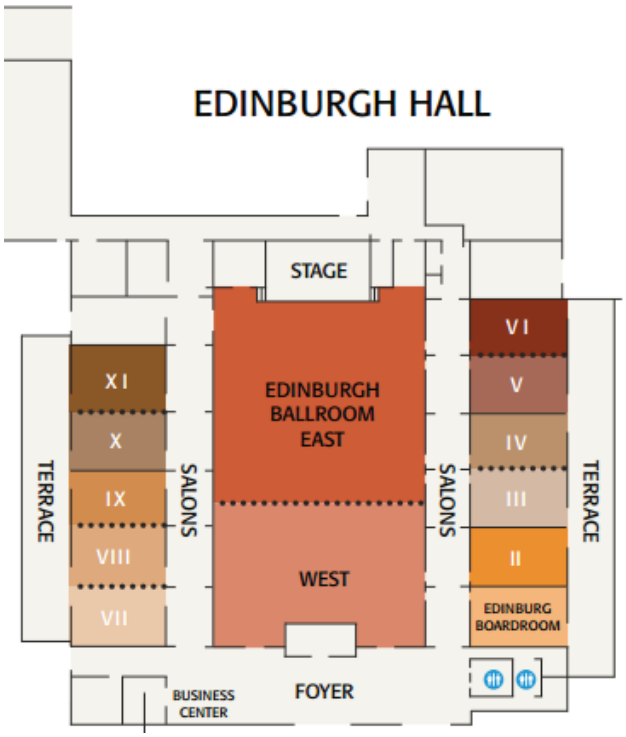
INVERNESS HALL

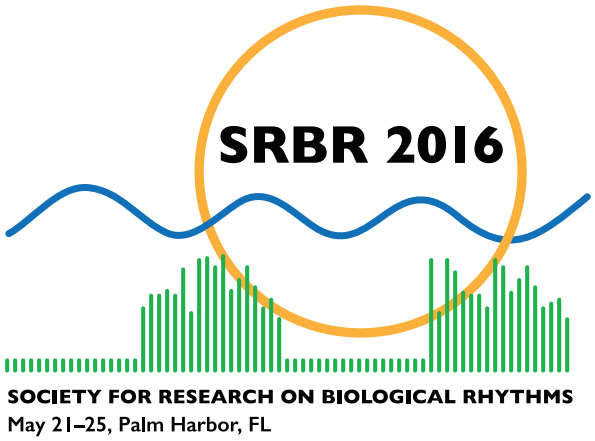




STIRLING HALL

EDINBURGH HALL





Logo Contest Winners

Winning Design (center)
Louise Hansen
Graduate Student
University of Edinburgh

1st Runner Up (left)
Alicia Michael
Graduate Student
University of California, Santa Cruz

2nd Runner Up (right)
Marie Pariollaud
Graduate Student
University of Manchester