XVII

EUROPEAN BIOLOGICAL RHYTHMS SOCIETY

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ABSTRACTS

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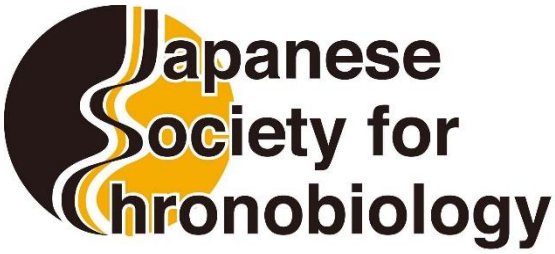
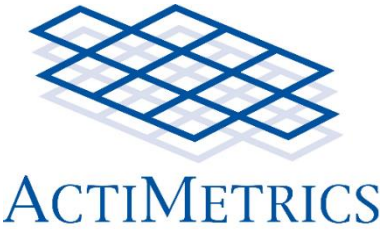
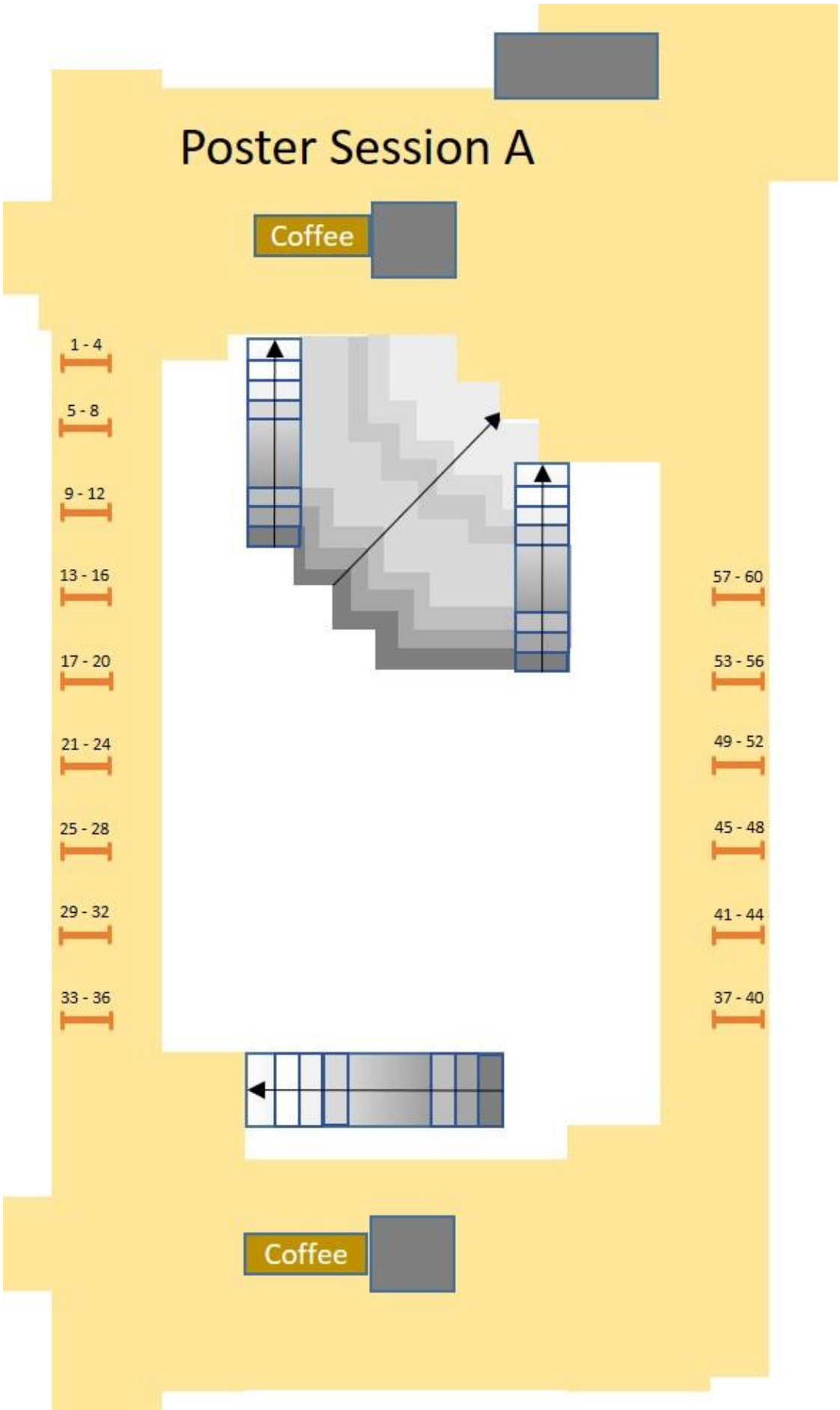




POSTER SESSIONS

Poster Session A – Monday, 25.07.2022



Abstracts to poster session A on Monday 25.07.2022

1. Circadian regulation of the transcriptome in a complex polyploid crop

Main author: Hannah Rees
Affiliation: Earlham Institute
Co-authors: Hannah Rees[1], Rachel Rusholme-Pilcher[1], Paul Bailey[2], Joshua Colmer[1], Benjamen White[1], Connor Reynolds[1], Sabrina Jaye Ward[1], Calum A. Graham[3,4], Luíza Lane de Barros Dantas[3], Antony N. Dodd[3], Anthony Hall[1]

Keywords: -

The circadian clock is a finely balanced time-keeping mechanism that coordinates programmes of gene expression. It is currently unknown how the clock regulates expression of homoeologous genes in polyploids. Here, we generate a high-resolution time-course dataset to investigate the circadian balance between sets of three homoeologous genes (triads) from hexaploid bread wheat. We find a large proportion of circadian triads exhibit imbalanced rhythmic expression patterns, with no specific sub-genome favoured. In wheat, period lengths of rhythmic transcripts are found to be longer and have a higher level of variance than in other plant species. Expression of transcripts associated with circadian controlled biological processes are largely conserved between wheat and Arabidopsis, however striking differences are seen in agriculturally critical processes such as starch metabolism. Together, this work highlights the ongoing selection for balance versus diversification in circadian homoeologs, and identifies clock-controlled pathways that might provide important targets for future wheat breeding.

2. A single atypical phosphoswitch in specific SCN neurons gates winter seasonality in mice

Main author: Sara Pierre-Ferrer
Affiliation: University of Zurich
Co-authors: Ben Collins, David Lukacsovich, Shao'Ang Wen, Yuchen Chai, Jochen Winterer, Benjamin Auerbach, Mingyao Li, Jun Yan, Lene Pedersen, Csaba Földy and Steven A. Brown

Keywords: Suprachiasmatic Nucleus (SCN), Neuronal firing, Single cell transcriptomics, Seasonality, Winter, VIP neurons

Background/Objectives:Circadian rhythms in behavior and physiology can adapt to different daylengths (photoperiods) in different seasons. This is accompanied by a change in the daily firing window of SCN neurons, generally more active during the day and more silent at night. However, it has been shown that a subpopulation of VIP+ SCN night-active neurons also controls the daily nighttime siesta. Here, we aimed at understanding what makes these neurons become active at night, and in doing so, also discovered a novel molecular mechanism by which the SCN adapts to daylength. Methods/Results: To explore the cellular identities and gene expression patterns of neurons that are night-active in SCN, we used the patch-seq technique, which consists of patch-clamp recordings followed by single-cell sequencing from ex-vivo slices. Differential transcriptomics identified the gene SLC20A2/PiT2, which encodes a sodium-dependent inorganic phosphate transporter, as the most differentially expressed gene. PiT2 shows a constant expression pattern in global SCN, but is more expressed in VIP+ night- active neurons compared to VIP+ day-active neurons, where it is basically absent. Long term multi-electrode array recordings from wildtype and PiT2-deleted mice show the same number of active units overall across the day in PiT2-/- SCN slices as in wildtype SCN. However, units from PiT2-/- slices were firing at a higher firing rate, specifically at subjective dawn and dusk.

Confirming the importance of this mechanism in vivo, although PiT2-/- mice show normal circadian period length and phase-shifting behavior, they show a shorter length of active phase in constant conditions and a complete failure to expand their active period during a winter daylength.
Conclusions: We were able to identify a phosphate transporter that is expressed in circadian fashion in VIP+ SCN neurons. Normally, switching on the expression of this transporter during nighttime has a repressive effect on overall SCN firing, thereby apportioning mouse behavioral activity to specific nighttime windows. When this switch is defective, mice are then unable to expand their window of activity during longer winter nights. In this way, a single circadian switch enabling an atypical phosphate current gates winter seasonal behavior in mice.

3. Food-log app-based chrono-nutritional analysis reveals an association between low carbohydrate at dinner and weight loss

Main author: Yu Tahara
Affiliation: Hiroshima University
Co-authors: Yu Tahara, Mai Kuwahara, Saneyuki Makino, Farnaz Roshanmehr, Takae Shinto, Gentaro Yokoyama, Shion Hosoda, Tsukasa Fukunaga, Ayako Tada, Nanako Abe, Mikiko Michie, Hyeon-Ki Kim, Masaki Takahashi, Michiaki Hamada, Shigenobu Shibata

Keywords: chrononutrition, food timing, phone app, weight management

Prevention of obesity and type 2 diabetes is an essential research issue worldwide. However, effective strategies for weight loss or prevention of obesity are constantly under debate. Chrono-nutrition-based optimal weight-loss strategies have not been well-studied, and the current study tried to find it using a diet-tracking app. In a retrospective cohort study (experiment 1), we explored the weight-loss parameters from six months of dietary data from 10,000 individuals (20–69-year-old men and women) recorded in a diet-tracking app. The results showed that carbohydrate intake contribution was significant along with age, sex, and app use duration. Particularly, carbohydrate intake correlated with weight loss in the order, dinner, lunch, and breakfast. Next, we conducted a case-control study (experiment 2), in which one-month app use and online surveys with/without chrono-nutritional suggestions were asked. Based on the previous and current chrono-nutritional evidence, 12-h time-restricted eating (TRE), increasing breakfast and decreasing dinner, decreasing carbohydrate content at dinner, and frequently logging the food intake and body weight were suggested. Improved sleep, well-being, and daytime physical fitness were seen only in the participants with chrono-nutritional suggestions, but the weight loss was identical between groups. Cluster and multiple regression analyses revealed that the subjective achievement of decreasing carbohydrate at dinner positively correlated with weight change. Thus, although the low carbohydrate diet or the low energy intake at dinner have been recognized for weight loss, the current data-driven analysis confirmed the importance of the timing of carbohydrate intake on the weight loss. In addition to the above topic, we would like to share our chrono-nutritional research, including investigation of the functional nutrients and the personalized chrono-nutrition.

4. Clock proteins and training modify exercise capacity in a daytime-dependent manner

Main author: Vaishnavi Dandavate
Affiliation: Weizmann Institute of Science, Israel
Co-authors: Vaishnavi Dandavate, Yaarit Adamovicha, Saar Ezagouri, Gal Manella, Ziv Zwighaft, Jonathan Sobel, Yael Kuperman, Marina Golik, Asher Auerbach, Maxim Itkin, Sergey Malitsky, and Gad Asher
Keywords: Circadian rhythms, exercise, training, glycogen, metabolism.

Exercise and circadian biology are closely intertwined with physiology and metabolism, yet the functional interaction between circadian clocks and exercise capacity is only partially characterized. Here, we tested different clock mutant mouse models to examine the effect of the circadian clock and clock proteins, namely PERIODs and BMAL1, on exercise capacity. We found that daytime variance in endurance exercise capacity is circadian clock controlled. Unlike wild-type mice, which outperform in the late compared with the early part of their active phase, PERIODs- and BMAL1-null mice do not show daytime variance in exercise capacity. It appears that BMAL1 impairs and PERIODs enhance exercise capacity in a daytime-dependent manner. An analysis of liver and muscle glycogen stores as well as muscle lipid utilization suggested that these daytime effects mostly relate to liver glycogen levels and correspond to the animals' feeding behavior. Furthermore, given that exercise capacity responds to training, we tested the effect of training at different times of the day and found that training in the late compared with the early part of the active phase improves exercise performance. Overall, our findings suggest that clock proteins shape exercise capacity in a daytime dependent manner through changes in liver glycogen levels, likely due to their effect on animals' feeding behavior.

5. Metabolic disorders and circadian dysfunction are intertwined: metabolic disorders disrupt circadian function and disrupted circadian systems in turn worsen metabolic disorders.

Main author: Ngoc-Hien Du
Affiliation: Institute of Pharmacology and Toxicology
Co-authors: Flore Sinturel, Nora Nowak, Pauline Gosselin, Camille Saini, Idris Guessous, François R. Jornayvaz, Jacques Philippe, Guillaume Rey, Emmanouil T. Dermitzakis, Renato Zenobi, Charna Dibner, and Steven A. Brown
Keywords: -

However, how the two systems affect each other is not well understood, nor are the genetic factors that might exacerbate this pathological interaction. Blood chemistry is profoundly changed in metabolic disorder, and we have previously shown that serum factors change cellular clock properties. We hypothesized that altered circulating factors in metabolic disorders such as diabetes and obesity have circadian modifying effects that are associated with disease state, and further that common genetic variation influencing both could be traced directly from subject serum. To test this hypothesis, we examined changes in cellular circadian clock properties within a common cell line in the presence of serum collected from diabetic, obese, and control subjects. In general, circadian period lengthening was associated with outcome blood chemistry that is characteristic of insulin resistance. Characterizing the genetic variants by genome-wide association analysis that altered circadian period length in cultured cells, we found that one of the top variants mapped to the E3 ubiquitin ligase MARCH1 that involves in insulin sensitivity. Confirming our data, the serum circadian modifying variants we found are also enriched in type 2 diabetes and chronotype variants identified in the UK Biobank cohort. Finally, to examine possible serum factors that might be involved, we performed detailed metabolomics upon our subjects' serum, and found that circadian modifying variants are

particularly associated with branched chain amino acids (BCAAs), whose levels are well-established to be correlated with diabetes and insulin resistance. Our multi-omics data showed comprehensively that systemic factors serve as a path through which metabolic disorders influence circadian systems, and these can be examined in human populations directly by simple cellular assays in common cultured cells.

6. Synchronizing family rhythms, work rhythms and biological rhythms

Main author: Camilla Kring
Affiliation: Center for Applied Chronobiology
Co-authors: -

Keywords: Sustainable life, family rhythms, work rhythms, biological rhythms, applied chronobiology, productivity, life quality

Rhythm is something close to the most basic in a person's life. As long as there is a rhythm, a pulse, a heartbeat, a beautiful melody, just as long there is life, and just as long it is worth living.
My goal with this paper is to awaken your sense of rhythm and give you a new understanding of who you are and how finding and following your rhythm can change your life. Through my work over the last 17 years, it has occurred to me more and more that we experience happiness when we can synchronize the rhythms of our lives; your circadian rhythm, your work rhythms and family rhythms, and how you can organize your life in a way that you experience synchronicity. It is in the synchronization of the rhythms of life that we unite quality of life and productivity.
But in order for you to hear, find, feel and live according to your own rhythm at all, you also need to understand all the traditional, mechanical rhythms in society that we as individuals are up against. The cockroaches of the agricultural society and the rhythmic assembly lines of the industrial society have, through generations and centuries, imprinted their rhythms on our way of thinking. This paper is a showdown with the industrial society's "one size fits all", and you will be presented with a completely different mindset: "One size fits one." I believe that we must create a society that gives the individual a greater freedom to organize the hours of the day in a way that supports the circadian rhythm we are each born with, but also the different work and family rhythms we have. Because when you find your rhythm, you get a better and more sustainable life.

7. The clock is ticking for HIV-1

Main author: Helene Borrmann
Affiliation: Nuffield Department of Clinical Medicine, University of Oxford, Oxford, UK
Co-authors: Helene Borrmann (presenter), Mirjam Schilling, Dini Ismed, Anna Kliszczak, Sridhar Vasudevan, Persephone Borrow, Xiaodong Zhuang and Jane A McKeating

Keywords: Virus-Clock, HIV-1, Antiviral Drugs, Infectious Disease

Human immunodeficiency virus 1 (HIV-1) is a life-threatening pathogen that still lacks curative therapies or vaccine. Despite the reduction in AIDS-related deaths achieved by antiretroviral therapies, drug resistance and failure to eradicate infection highlight the need to understand host pathways that define HIV-1 replication to uncover new therapeutic targets.
Circadian rhythms are endogenous 24-h oscillations which regulate rhythmic gene expression and physiological processes including host immune responses to infection. As obligate parasites, viruses are reliant on their hosts to replicate and disseminate and recent reports have identified an interplay between the circadian clock and viruses. Clinical studies observed circadian cycling of HIV-1 RNA in males receiving antiretroviral therapy, which was associated with changes in transcript levels of the

circadian factor BMAL1. These reports demonstrate the clinical importance of the HIV-circadian interplay, while the underlying mechanisms remain to be elucidated.
Pharmacological activation of the circadian repressor REV-ERB in cell lines, primary human T cells and macrophages reduced HIV-1 replication, suggesting a role for circadian signalling in HIV. We show a role for REV-ERB agonists to repress HIV-1 promoter activity, whilst antagonism or genetic disruption of REV-ERB activated viral transcription. The HIV-1 promotor contains conserved circadian motifs and we provide the first evidence for REV-ERB binding the HIV-1 promoter. Furthermore, infection of circadian synchronised target cells shows that HIV transcription is rhythmic. Our study shows a role for REV-ERB to regulate HIV-1 transcription by direct binding to the viral genome, providing a rationale for future work to identify circadian modulators with antiviral properties that may augment existing treatment regimens. In a wider context, the need for multidisciplinary approaches involving virology, circadian biology, immunology, and pharmacology is increasingly important in an era of shift work–related sleep disorders and global viral pandemics.

8. The circadian clock component BMAL1 regulates SARS-CoV-2 entry and replication

Main author: Alan Xiaodong Zhuang
Affiliation: University of Oxford
Co-authors: Xiaodong Zhuang (Presenter), Senko Tsukuda, Florian Wrensch, Peter Ac Wing, Mirjam Schilling, James M Harris, Helene Borrmann, Sophie B Morgan, Jennifer L Cane, Laurent Mailly, Nazia Thakur, Carina Conceicao, Harshmeena Sanghani, Laura Heydmann, Charlotte Bach, Anna Ashton, Steven Walsh, Tiong Kit Tan, Lisa Schimanski, Kuan-Ying A Huang, Catherine Schuster, Koichi Watashi, Timothy Sc Hinks, Aarti Jagannath, Sridhar R Vausdevan, Dalan Bailey, Thomas F Baumert, Jane A McKeating
Keywords: Circadian rhythm; COVID-19; Interferon-Stimulated Genes

The coronavirus disease 2019 pandemic, caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) coronavirus, is a global health issue with unprecedented challenges for public health. SARS-CoV-2 primarily infects cells of the respiratory tract via spike glycoprotein binding to angiotensin-converting enzyme (ACE2). Circadian rhythms coordinate an organism's response to its environment and can regulate host susceptibility to virus infection. We demonstrate that silencing the circadian regulator Bmal1 or treating lung epithelial cells with the REV-ERB agonist SR9009 reduces ACE2 expression and inhibits SARS-CoV-2 entry and replication. Importantly, treating infected cells with SR9009 limits SARS-CoV-2 replication and secretion of infectious particles, showing that post-entry steps in the viral life cycle are influenced by the circadian system. Transcriptome analysis revealed that Bmal1 silencing induced interferon-stimulated gene transcripts in Calu-3 lung epithelial cells, providing a mechanism for the circadian pathway to limit SARS-CoV-2 infection. Our study highlights alternative approaches to understand and improve therapeutic targeting of SARS-CoV-2.

9. Circadian control of hepatitis B virus replication

Main author: Alan Xiaodong Zhuang
Affiliation: University of Oxford
Co-authors: Xiaodong Zhuang (Presenter), Donall Forde, Senko Tsukuda, Valentina D'Arienzo, Laurent Mailly, James M. Harris, Peter A. C. Wing, Helene Borrmann, Mirjam Schilling, Andrea Magri, Claudia Orbegozo Rubio, Robert J. Maidstone, Mudassar Iqbal, Miguel Garzon, Rosalba Minisini, Mario Pirisi, Sam Butterworth, Peter Balfe, David W. Ray, Koichi Watashi, Thomas F. Baumert, Jane A. McKeating
Keywords: Circadian rhythm; clock; HBV; viral hepatitis

Chronic hepatitis B virus (HBV) infection is a major cause of liver disease and cancer worldwide for which there are no curative therapies. The major challenge in curing infection is eradicating or silencing the covalent closed circular DNA (cccDNA) form of the viral genome. The circadian factors BMAL1/CLOCK and REV-ERB are master regulators of the liver transcriptome and yet their role in HBV replication is unknown. We establish a circadian cycling liver cell-model and demonstrate that REV-ERB directly regulates NTCP-dependent hepatitis B and delta virus particle entry. Importantly, we show that pharmacological activation of REV-ERB inhibits HBV infection in vitro and in human liver chimeric mice. We uncover a role for BMAL1 to bind HBV genomes and increase viral promoter activity. Pharmacological inhibition of BMAL1 through REV-ERB ligands reduces pre-genomic RNA and de novo particle secretion. The presence of conserved E-box motifs among members of the Hepadnaviridae family highlight an evolutionarily conserved role for BMAL1 in regulating this family of small DNA viruses.

10. The regulation of circadian rhythms by the natural light environment

Main author: Laura Steel
Affiliation: Sleep and Circadian Neuroscience Institute, Nuffield Department of Clinical Neuroscience, Oxford University
Co-authors: (Supervisors: Stuart Peirson and Russell Foster)

Keywords: Natural light environment, Circadian Ecology, Circadian photoentrainment, Light sampling behaviour

The natural light environment is a complex and dynamic signal, varying in intensity and spectrum across the 24hr period. The light available to an organism is further influenced by the specific timing of behaviour, since this creates a distinct pattern of light sampling and is therefore a critical, and frequently overlooked, aspect of circadian photoentrainment.

However, the light environment, as well as the way organisms sample it, has become extensively simplified within the laboratory setting. Model organisms are maintained under 12hr:12hr light dark cycles - with no spectral changes or gradual transitions in intensity, and no opportunity for nocturnal den-dwelling rodents to exhibit natural light sampling behaviour.

Since biological systems can only be fully understood when studied in the context of the ecological pressures under which they evolved, it becomes difficult to disentangle whether the data collected under these simplified conditions reflect underlying mechanisms or laboratory artefacts. Therefore, this project aims to combine visual ecology with circadian neuroscience to further our understanding of how features of the natural light environment, including the behavioural sampling of it, regulate circadian entrainment in Mus musculus.

Here we use hyperspectral imaging to characterise a natural woodland light environment in a highly spatially and spectrally resolved way, and now aim to recreate the key features of this light environment in the laboratory. This includes introducing ramped light cycles to simulate twilight transitions, as well as appropriate spectral changes using multichannel LEDs. In parallel, light sampling behaviour is quantified using specially developed darkened nest-boxes, fitted with passive infrared sensors, to allow mice the choice over their light environment. In the future we plan to use transgenic mouse models to determine the role of different photoreceptors in regulating light sampling behaviour and circadian photoentrainment under this naturalistic light environment.

11. Non-canonical circadian repressor CHRONO regulates TTFL components through multiple interactions'

Main author: Priya Crosby
Affiliation: University of California, Santa Cruz
Co-authors: Priya Crosby, Nicolette F. Goularte, Diksha Sharma, Effei Chen, Gian Carlo G. Parico, Jonathan M. Philpott, Carrie L. Partch
Keywords: CHRONO, transcriptional repression, protein, biochemistry

The mammalian protein CHRONO was previously identified via high-throughput screening to be a rhythmically expressed repressor of the circadian transcriptional activator complex CLOCK:BMAL1. Mice and cells lacking CHRONO display a lengthened circadian period and altered circadian gene expression. Currently, however, we lack specific mechanistic understanding of CHRONO's activity and function. Here we define an evolutionarily conserved minimal repressive domain (MRD) of CHRONO and demonstrate this domain's capacity to repress CLOCK:BMAL1 activity through interaction with the BMAL1 C-terminal transactivation domain (TAD). Notably, CHRONO binds to the TAD with an affinity several fold tighter than the canonical circadian repressor, CRY. Using NMR spectroscopy, we map the residues of the TAD that interact with CHRONO, identifying a two-point mutation in BMAL1 sufficient to disrupt this binding, and so CHRONO's regulation of CLOCK:BMAL1. This binding region overlaps with the binding site for CRY and coactivators CBP/p300, demonstrating that all three proteins target a hot spot for BMAL1 regulation. Moreover, CHRONO is capable of completely displacing CRY from the BMAL1 TAD, further highlighting its potential to regulate CLOCK:BMAL1 in tandem with this more established repressor.
Additionally, we investigate the previously unexplored interaction between CHRONO and another major circadian repressor, PER2. We show that CHRONO reduces PER2 stability through interaction between the CHRONO C-terminus and the casein kinase 1 (CK1)-binding domain of PER2. Preliminary data suggests likely competition between CHRONO and CK1 for binding at this site on PER2, adding another layer to our understanding of PERIOD protein regulation. Taken together, these data show a more substantive role for CHRONO within molecular circadian timekeeping than previously posited, and provide a platform for further investigation into CHRONO's role within the circadian repressive complex.

12. Distinct molecular clockworks underlying hierarchically organized pacemaker neurons

Main author: Jae Kyoung Kim
Affiliation: KAIST Mathematical Sciences & IBS Biomedical Mathematics Group
Co-authors: -
Keywords: circadian rhythms, CLOCK, dorsal neuron, lateral neuron, mathematical modeling

In metazoan organisms, circadian (~24 h) rhythms are regulated by pacemaker neurons organized in a master–slave hierarchy. Although it is widely accepted that master pacemakers and slave oscillators generate rhythms via an identical negative feedback loop of transcription factor CLOCK (CLK) and repressor PERIOD (PER), their different roles imply heterogeneity in their molecular clockworks. Indeed, in Drosophila, defective binding between CLK and PER disrupts molecular rhythms in the master pacemakers, small ventral lateral neurons (sLNvs), but not in the slave oscillator, posterior dorsal neuron 1s (DN1ps). In this talk, I will introduce our systematic and expandable approach that unbiasedly searches the source of the heterogeneity in molecular clockworks from time-series data. Then, I will describe how we use this approach in combination with in vivo experiments to identify hat sLNvs exhibit higher synthesis and turnover of PER and lower CLK levels than DN1ps.

Importantly, our light shift analysis reveals that due to such a distinct molecular clockwork, sLNvs can obtain paradoxical characteristics as the master pacemaker, generating strong rhythms that are also flexibly adjustable to environmental changes. Our results identify the different characteristics of molecular clockworks of pacemaker neurons that underlie hierarchical multi-oscillator structure to ensure the rhythmic fitness of the organism.

13. Light is a zeitgeber for the circadian clock of a non-photosynthetic prokaryote

Main author: Francesca Sartor
Affiliation: Institute of Medical Psychology, Medical Faculty, LMU Munich, 80336 Munich, Germany.
Co-authors: Francesca Sartor(1), Zheng Eelderink-Chen(1), Xinming Xu(2), Antony, Dodd(3), Àkos Kovács(2), Martha Merrow(1)
1) Institute of Medical Psychology, Medical Faculty, LMU Munich, 80336 Munich, Germany.
2) Bacterial Interactions and Evolution Group, DTU Bioengineering, Technical University of Denmark, 2800 Kongens Lyngby, DK
3) John Innes Centre, Norwich Research Park, Norwich NR4 7UH, UK

Keywords: circadian clock, non-photosynthetic bacteria, Bacillus subtilis, entrainment, light

The circadian clock in animals, plants, fungi and photosynthetic bacteria has been extensively studied and characterised. On the other hand, whether non-photosynthetic bacteria possess a clock remains a poorly investigated question. Only recently our group reported the existence of a circadian system in the free-living, non-photosynthetic bacterium Bacillus subtilis. We showed that a B. subtilis lab strain exhibits free-running rhythms in gene expression following entrainment with blue light/dark or temperature cycles, and we further explored entrainment properties to temperature as zeitgeber. Here, we extend these observations to undomesticated B. subtilis strains. Furthermore, using reporter gene assays, we characterise how the circadian clock responds to light as zeitgeber. Bacillus has photoreceptors for both blue and red light; we thus tested how the circadian system uses light of different spectral quality to entrain. We explored entrainment properties to light by applying classic circadian protocols in which we varied fluence intensity during entrainment and constant conditions. We observed systematic entrainment in photoperiod structures. We performed these experiments in wild-type reporter strains as well as in strains with genetic knock outs for the photoreceptor genes. In addition to clock-regulated gene expression, we studied colony morphology. Interestingly, we find that circadian organisation, response to light and physiology (biofilm formation) are entangled processes. Given that prokaryotes represent ca. 15% of the biomass on Earth, we predict that the characterisation of circadian systems in prokaryotes can have meaningful implications for health and industry.

14. The strong circadian influence in psychiatric disorders opens up for new opportunities to increase efficacy of personalized medicine.

Main author: John Axelsson
Affiliation: Karolinska Institute
Co-authors: Ben Holding, Predrag Petrovic, Leonie Balter

Keywords: chronotype, psychiatric traits, neuropsychiatric symptoms, individual differences, personalized medicine

For this to be successful, there is a need to better characterize when individuals are most likely to suffer from symptoms during the day. The objectives were to characterize how psychiatric traits relate

to chronotype, and how someone's chronotype and psychiatric profile predict when symptoms will be worse during the day.
In the pre-registered study, participants (N=503) completed scales measuring 13 psychiatric traits and chronotype (rMEQ) at baseline. Of those, 441 continued the next day with repeated ratings of psychiatric symptoms across the day (~08:00-01:00). Generalized additive mixed-effects models were used to assess the extent and shape of any non-linear interaction between transdiagnostic psychiatric constructs, time of day, and chronotype.
Ten psychiatric traits were associated with being an evening type (ET), mania was associated with being a morning type (MT), while autism and eating disorder were not related to chronotype. Symptoms varied across the day as a function of chronotype. Key findings are that MT were more active than ET, particularly in the morning, and that ET showed the expected shift in fatigue across the day compared to MT. ET also suffered from more emotional symptoms in the evening than MT, particularly in individuals high in depression-anxiety traits. Chronotype interacted with psychiatric traits in several other ways. For example, MT that were also high in traits of depression-anxiety or downregulatory problems had more ADHD-symptoms than ET throughout the day.
These findings show that virtually all psychiatric traits are strongly related to chronotype and that central psychiatric symptoms vary across the day depending on an individual's psychiatric profile and chronotype. In all, chronotype should be considered in personalized medicine and when tailoring treatments to optimize health and functioning across the day.

15. Chronogauge: a machine-learning framework for circadian phase inference using gene expression

Main author: Connor Reynolds
Affiliation: Earlham Institute, Norwich Research Park, Norwich, NR4 7UZ, UK
Co-authors: Joshua Colmer, Hannah Rees, Antony Dodd, Anthony Hall

Keywords: Circadian clock, transcriptomics, genomics, plants, machine learning, bioinformatics, computational biology

ChronoGauge is an open-source AI-based framework which can generate multiple sets of biomarker genes whose expression can be used to predict an organism's circadian time as a pseudo-phenotype for reverse- or forward-genetics. ChronoGauge has the potential to be applied to both plant and mammalian transcriptome datasets to assess circadian function.
The expression of genes within the circadian transcriptional network can potentially represent an organism's circadian time, thus allowing us to assay clock function based on the difference between the internal time and the actual time. Identifying a reliable subset of genes to act as circadian biomarkers however is challenging due to the multiplex nature of the overall network. In plants, thousands of genes are under circadian control, yet most do not act as reliable circadian biomarkers across diverse datasets.
Using Arabidopsis thaliana RNA-seq datasets for training and testing, ChronoGauge applies a sequential feature selection approach to build multiple unique circadian gene biomarker sets that can estimate the circadian time with an error as low as 23 minutes. ChronoGauge ensembles the predictions of different biomarkers to give a more reliable time prediction and can further evaluate different aspects of the circadian gene regulatory network.
ChronoGauge can be applied to previously published transcriptome datasets to test different types of hypotheses. Firstly, we can associate changes to the external environment, such as growth temperature with circadian clock function. As an example of reverse genetics, we used ChronoGauge to show errors in the predicted time in plants with different mutations of core circadian clock genes. We also used ChronoGauge in forward genetics, where the circadian time predictions across 158 Swedish ecotypes were used as a phenotype for a genome-wide-association-study.

16. Hypercaloric diet and time-restricted feeding reprogram microglial day-night immunity in the mediobasal hypothalamus and intermediolateral nucleus of spinal cord

Main author: Chun-Xia Yi
Affiliation: Amsterdam University Medical Centre, Location AMC
Co-authors: Milan M.H. Dorscheidt, Han Jiao, Jarne Jermei, Felipe Correa-da-Silva, Nikita L. Korpel, Ewout Foppen, Andries Kalsbeek and Chun-Xia Yi
Keywords: Microglia, sympathetic nervous system, metabolic disorders, time-restricted feeding, spinal cord.

Neurons in the mediobasal hypothalamus (MBH) are critical for the CNS to sense metabolic feedback from the periphery. These MBH neurons project to the paraventricular nucleus where pre-autonomic neurons are located and the pre-autonomic neurons further project to the intermediolateral nucleus (IML) in the spinal cord to control sympathetic outflow to metabolically active peripheral organs and regulate energy homeostasis. Microglia are the professional phagocytes in the CNS essential for maintaining a healthy microenvironment for neurons to function properly. Our previous studies found that under regular chow conditions microglial activity in the MBH fluctuates during L/D cycle, whereas in high fat diet (HFD)-induced metabolic disorders, it was constantly elevated, suggesting an important role for microglia in the CNS control of energy homeostasis in a time-of-day manner. In the current study, we explored whether HFD and/or time-restricted feeding would reprogram microglial day-night immunity in the MBH and IML. Male rats housed in a 12h-light/12h-dark cycle were fed chow or HFD for 8 weeks, either ad libitum or with food access restricted to the light (TRF-light) or dark (TRF-dark) period for the final 4 weeks before sacrifice. Rats were sacrificed every 4h over the 24h L/D cycle. In the MBH we found the highest microglial ramifications (indicating immune surveillant activity) in ad libitum chow-fed rats at the end of dark phase (ZT22), whereas this peak shifted to the beginning of dark phase (ZT14) in the TRF-light group, and unexpectedly further shifted to the end of the light phase (ZT10) in the TRF-dark group. In the spinal cord, we found that core-clock and immune gene expression was disturbed during the 24h L/D cycle in HFD rats, and that microglial ramification in the IML was decreased at the beginning of the dark phase (ZT14). Our on-going studies are exploring the mechanisms underlying these results.

17. Machine learning classification of temporal gating of temperature-responsive transcriptome dynamics in nature

Main author: Pirita Paajanen
Affiliation: John Innes Centre
Co-authors: Paige E. Panter, Tomoaki Muranaka, Genki Yumoto, Mie N. Honjo, Hiroshi Kudoh, Antony N. Dodd
Keywords: circadian gating, machine learning, cold response, natural conditions

The axial rotation and solar orbit of the Earth create predictable daily (24 h) and seasonal fluctuations in environmental conditions. These occur in combination with fluctuations in weather conditions that are becoming less predictable due to climate change. For sessile organisms it is incredibly important to be able to integrate the circadian and environmental stimuli to provide the appropriate response at each day and time of the year. The modulation by circadian timing of environmental signalling is thought to ensure that responses to stimuli are appropriate for the time of day, through a process known as gating. We have developed a new theoretical framework that defines two types of gating of this modulation, which we term discrete gating, when responses occur within well-defined windows

within each diel cycle and continuous gating, when the response changes throughout the day continuously. We have applied this framework to study transcriptome dynamics in response to transient temperature manipulations in a wild population of Arabidopsis halleri. Furthermore, we have developed a machine learning model to classify gating responses over time and across the seasons. From this, we found that diel cycles of gating are a dynamic process that depends on the season and specific environmental factors. We find that seasonal effects and clock effects are combined within the molecular processes that allow plants to respond to the environment, at any given time, within the diel and annual cycle.

18. The role of the gut microbiome in chronotype tuning

Main author: Eran Tauber
Affiliation: Department of Evolutionary and Environmental Biology, University of Haifa, ISRAEL
Co-authors: Bettina Fishman, Bayan Qadah

Keywords: -

Patterns of diurnal activity differ substantially between individuals, with early risers (“larks") and late sleepers (“owls”) being extreme examples of individuals who present diurnal preferences, also termed “chronotypes”. Given the bidirectional associations that have been discovered in recent years between the circadian clock and gut microbiota, we hypothesized that variation in chronotype is associated with variability in gut microbiota function and composition. We took advantage of Drosophila strains that show extreme chronotype that were generated by a process of artificial selection of wild-collected flies, selecting for diurnal and nocturnal flies. we have sampled feces from our artificial selection nocturnal and diurnal strains and carried out 16S rDNA microbiota profiling using the Illumina MySeq sequencing platform. Comparison of the diurnal and nocturnal microbiome indicated that 20% of variation among samples can be explained by the diurnal factor. We have cultured Acetobacter strain that was found to be enriched in diurnal flies and fed it to nocturnal flies. Following this treatment, the chronotype of the flies has become diurnal, demonstrating the causal role of the gut bacteria.
Inspired by our Drosophila studies, we have also profiled the gut microbiome composition of human, using metagenomic sequencing. The study, which consisted 133 established a distinct signature associated with chronotype based on two bacterial genera, Alistipes (elevated in “larks”) and Lachnospira (elevated in “owls”). We identified three metabolic pathways associated with the early chronotype, and linked distinct dietary patterns with different chronotypes. The lachnospira bacteria (which was also elevated in nocturnal Drosophila) is known to synthesize butyrate, suggesting an important role for short-chain fatty acid (SCFA) in inducing late chronotypes.

19. Searching for novel SCN enhancer marks that could drive daily timekeeping

Main author: Akanksha Bafna
Affiliation: Post-Doctoral Training Fellow, Neurobehavioral Genetics, MRC, Harwell , UK
Co-authors: Gareth Banks, Patrick Nolan
Keywords: SCN, Histone ChIP-Seq, Bulk RNA Seq, Enhancers , eRNA , genomics

Background/Objectives: Most organisms possess intrinsic circadian clocks that help to synchronize their molecular, behavioural and physiological processes to daily environmental conditions. In mammals, daily timekeeping is directed by the suprachiasmatic nucleus (SCN) also known as 'master pacemaker', located in the ventral hypothalamus. Here, we sought to identify novel regulatory elements that aid circadian gene transcription in the SCN.
Methods: To identify gene regulatory elements such as Enhancers and gene Promoters we carried out Histone-ChIP-Seq at six distinct time-points of the day. Histone mark H3K27ac is considered as a reliable marker for active enhancers whilst H3K4me3 can bait the gene promoters. We used SCN and Cortex mouse brain tissues for the Histone-ChIP-Seq to exclusively screen for SCN- enriched enhancer marks. We also carried out Bulk-RNA-Seq to understand the influence of SCN specific enhancer sites at rhythmic gene transcriptional machinery.
Results: Using comparative analysis between SCN and Cortex, we were able to identify SCN-enriched enhancer sites. Deep-analysis of the Histone-ChIP-Seq peak profiles revealed 1021 enhancer sites showing difference in enrichment at a distinct time-of-day. Of these, approximately a quarter showed rhythmic oscillation in H3K27ac (marker of active enhancer sites) occupancy. To establish an enhancer-gene relationship, we used the transcriptional read-out from the Bulk RNA-Seq. Intragenic differential enhancer site positively correlated with the gene transcription.
Conclusion: Here, for the first time we report a genome- wide pre-transcriptional regulation of the central circadian clock. We have successfully identified SCN specific enhancer marks, potentially addressing the long standing question of what makes the SCN distinct as a circadian central pacemaker. In conjunction we have also unveiled the occurrence of dynamic H3K27ac occupancy followed by enhancer RNA transcription and rhythmic gene transcription, imperative for daily timekeeping.

20. Disruption of the circadian clock component BMAL1 elicits an endocrine adaption impacting on insulin sensitivity and liver disease.

Main author: Céline Jouffe
Affiliation: Helmholtz Zentrum Muenchen
Co-authors: Céline Jouffe*, Benjamin D. Weger*, Eva Martin, Florian Atger, Meltem Weger, Cédric Gobet, Divya Ramnath, Aline Charpagne, Delphine Morin-Rivron, Elizabeth E. Powell, Matthew J. Sweet, Mojgan Masoodi, N. Henriette Uhlenhaut, and Frédéric Gachon
Keywords: circadian clock; nonalcoholic fatty liver disease; insulin resistance; growth hormone; estrogen

Obesity and liver diseases are associated with the disruption of the circadian clock that orchestrates mammalian physiology to optimize nutrient metabolism and storage. We show here that the activity of the circadian clock regulator BMAL1 is perturbed during liver fibrosis in humans. To understand the impact of BMAL1 perturbation in obesity and liver diseases, we assessed the impact of a high fat diet or leptin deficiency on Bmal1 knockout mice. While Bmal1 knockout mice were prone to obesity, they were protected against insulin resistance, hepatic steatosis, inflammation, and fibrosis. In addition to

direct transcriptional regulation of metabolic programs by BMAL1, we show that disruption of the growth hormone and sex hormone pathways plays a critical role in this protection. Similar endocrine perturbations correlate with the development of liver fibrosis in humans but were absent in hepatocyte specific Bmal1 knockout mice. This suggests that systemic endocrine perturbation associated with the disruption of BMAL1 activity is critical for the pathogenesis of metabolic and liver diseases.

21. The synchrony between chronotype and school timing explains adolescents' academic achievement

Main author: Guadalupe Rodriguez Ferrante
Affiliation: Universidad Nacional de Quilmes y Universidad Torcuato Di Tella
Co-authors: Andrea P. Goldin, Mariano Sigman and Maria Juliana Leone

Keywords: Adolescents, Chronotype, School start time, academic performance

Human chronotype is the expression of each individual's endogenous circadian timing under light-dark conditions. Interestingly, chronotype is modulated by several factors such as age and social timings (e.g. school schedules). In particular, chronotype becomes later during adolescence reaching a peak at the end of this developmental period. However, most school schedules start early in the morning causing a misalignment between adolescents' internal and social clocks, associated with unhealthy sleep habits and poorer cognitive and academic performance. Nonetheless, it is not clear whether this association is explained only by a negative effect of chronotype, a synchrony effect between chronotype and school timing or a combination of both effects. Complementary, little is known about whether, and how, other variables associated with academic achievement (i.e. grade retention) are modulated by the mentioned effects. Our group has previously studied this issue in secondary school students who attend school at different school timings (morning, afternoon, evening). Here, we present the results of the follow-up study, where the same students were evaluated both during their first year (13-14 y.o.) and, again, in their last year (17-18 y.o.) of school. We studied not only academic performances but also grade retention. We found that the synchrony effect, and not just the chronotype, predicts grade retention: morning-attending students with later chronotypes present higher odds of retaining a grade than earlier chronotypes and this difference is not observed in evening school timing. Regarding academic performance we found that both effects (i.e. a negative effect of chronotype and a synchrony effect) interacting with age explain students' grades. These findings show that students with late chronotypes are at a disadvantage, especially in the morning school timing. Consistently, these results should be considered to design and implement fairer educational public policies for adolescents.

22. Beneficial effects of timed exercise on the circadian system

Main author: Ayano Shiba
Affiliation: Netherlands institute of Neuroscience (NIN), Amsterdam UMC
Co-authors: Ayano Shiba[*1], P. de Goede[*1+], E. Foppen[1], N. L. Korpel[1,2], M.W. Jansen[1], C. Yi[2], J. Mul[3], D.J. Stenvers[2], A. Kalsbeek[1,2]
[1]Netherlands Institute of Neuroscience (NIN), Amsterdam, The Netherlands
[2]Amsterdam UMC, University of Amsterdam, Amsterdam, The Netherlands
[3]Swammerdam Institute for Life Sciences, University of Amsterdam, Amsterdam, The Netherlands
=+ Current affiliation: The Janssen Pharmaceutical Companies of Johnson & Johnson, Leiden, The Netherlands
* Equal contribution
Keywords: Exercise, Timing, Time restricted running, Circadian disruption, Glucose metabolism, Type 2 diabetes mellitus

Shift-work is associated with negative health effects such as obesity and type 2 diabetes mellitus, likely due to disrupted synchrony between different daily rhythms, such as the sleep-wake, nutritional and activity rhythm. We used voluntary time-restricted running (TRR) in rats to mimic chronic physical activity during the natural resting period, which is commonly experienced by people working in shift work situations. Rats were given first 2 weeks of unlimited access to get used to the wheel. For the next 4 weeks, they could either run ad libitum (ALR), only during their natural active period (dark runners: DR) or inactive period (light runners: LR). All animals liked to run in the wheel, however, the daily running distance was significantly less in LR than DR and ALR. Then, the rats were sacrificed at different time points. Our body scan data demonstrated that all animals with access to a running wheel showed a strong decrease in adiposity compared to sedentary controls, with light-restricted runners showing the smallest effect. The food intake per body weight was the highest in ALR and DR, lower in LR and the lowest in NR. Comparing DR and LR that ran similar cumulative distances revealed that the diminished effect of running on adiposity in LR was due to both the reduced amount of running as well as its timing. The day/night expression profiles of 8 clock genes and key metabolic genes involved in glucose and fat metabolism in three metabolically important tissues (soleus, gastrocnemius skeletal muscle, and liver) are currently being analysed. To investigate further the functional importance of timing, in new experiments we are investigating the effects of TRR on glucose metabolism. Overall, our results strongly support that the wrong timing of exercise is an important contributor to the negative health effects of shift-work conditions.

23. Rewiring of liver diurnal transcriptome rhythms by triiodothyronine (T3) supplementation

Main author: Leonardo V M de Assis
Affiliation: Researcher associate, Institute of Neurobiology, Center of Brain Behavior & Metabolism, University of Lübeck, Germany
Co-authors: Leonardo Vinícius Monteiro de Assis[1*], Lisbeth Harder[1,2*], José Thalles Lacerda[3], Rex Parsons[4], Meike Kaehler[5], Ingolf Cascorbi[5], Inga Nagel[5], Oliver Rawashdeh[6], Jens Mittag[7], Henrik Oster[1]
[1]Institute of Neurobiology, Center of Brain Behavior & Metabolism, University of Lübeck, Germany
[2]Current address: Division of Molecular Neurobiology, Department of Medical Biochemistry and Biophysics, Karolinska Institutet, Stockholm, Sweden

[3]Institute of Bioscience, Department of Physiology, University of São Paulo, Brazil
[4]Australian Centre for Health Services Innovation and Centre for Healthcare Transformation, School of Public Health and Social Work, Faculty of Health, Queensland University of Technology, Kelvin Grove, Australia
[5]Institute of Experimental and Clinical Pharmacology, University Hospital Schleswig-Holstein, Campus Kiel, Germany
[6]School of Biomedical Sciences, Faculty of Medicine, University of Queensland, Brisbane, Australia
[7]Center of Brain Behavior & Metabolism, Institute for Endocrinology and Diabetes – Molecular Endocrinology, University of Lübeck, Germany
Keywords: endocrine system, liver metabolism, thyroid hormones, hyperthyroidism, rhythmic analyzes, transcriptome

Diurnal (i.e., 24-hour) physiological rhythms depend on transcriptional programs controlled by a set of circadian clock genes/proteins. Systemic factors like humoral and neuronal signals, oscillations in body temperature, and food intake align physiological circadian rhythms with external time. Thyroid hormones (THs) are major regulators of circadian clock target processes such as energy metabolism, but little is known about how fluctuations in TH levels affect the circadian coordination of tissue physiology. In this study, a high triiodothyronine (T3) state was induced in mice by supplementing T3 in the drinking water, which affected body temperature, and oxygen consumption in a time-of-day dependent manner. 24-hour transcriptome profiling of liver tissue identified 37 robustly and time independently T3 associated transcripts as potential TH state markers in the liver. Such genes participated in xenobiotic transport, lipid and xenobiotic metabolism. We also identified 10 – 15 % of the liver transcriptome as rhythmic in control and T3 groups, but only 4 % of the liver transcriptome (1,033 genes) were rhythmic across both conditions – amongst these several core clock genes. In-depth rhythm analyses showed that most changes in transcript rhythms were related to mesor (50%), followed by amplitude (10%), and phase (10%). Gene set enrichment analysis revealed TH state dependent reorganization of metabolic processes such as lipid and glucose metabolism. At high T3 levels, we observed weakening or loss of rhythmicity for transcripts associated with glucose and fatty acid metabolism, suggesting increased hepatic energy turnover. In sum, we provide evidence that tonic changes in T3 levels restructure the diurnal liver metabolic transcriptome independent of local molecular circadian clocks.

24. Food entrainment modifies the quantity but not the circadian rhythm of neurotransmitter content in the rat spinal cord

Main author: Shantal Jimenez Zarate
Affiliation: Centro de Investigación y de Estudios Avanzados del Instituto Politécnico Nacional, Av Instituto Politécnico Nacional 2508, San Pedro Zacatenco, Gustavo A. Madero, 07360 – Ciudad de Mexico, Mexico
Co-authors: Celia Piña-Leyva, Marina Rodriguez-Sanchez, Benjamin Floran-Garduño, Ismael Jimenez-Estrada
Keywords: Neurotransmitter content, Circadian variability, Spinal Cord, temporal food restriction.

In a previous report, we showed that chronic food restriction (undernourishment) induces a decrement in the quantity and modifies the variability of the circadian content of various neurotransmitters in the spinal cord of male and female Wistar rats. In such study, we observed that food restricted rats ingest their pellets in almost 4 hours (between 8:00 and 12:00 hours, day time), so we believe that the timing

of the meal could influence the content of spinal neurotransmitters. In this study, we explore the effect of a 4-hour daily food access (FR) on the amount and circadian content variability of several spinal neurotransmitters (glutamate, GABA, dopamine, serotonin, norepinephrine, and epinephrine) and compared it with that of control (CONT) or chronic food restricted male and female rats, raised in our institutional animal facility. The content of spinal neurotransmitters was analyzed by HPLC, in groups of control and experimental male and female rats, randomly sacrificed at 4:00, 8:00, 12:00, 16:00, 20:00 and 24:00 hours, day time. Our results indicate that most of the spinal neurotransmitters analyzed in the FR group (glutamate, GABA, serotonin, adrenaline and dopamine) showed a significant increase in their spinal content but manifest a circadian variation similar to that observed in CONT rats. In contrast, chronic food-deprived rats showed a significant reduction in the amount and alterations in the circadian content variability of spinal neurotransmitters. It is suggested that the increased spinal content of neurotransmitters in the RF group of male and female rats might be associated to a hitherto unknown adaptive mechanism, while the changes in neurotransmitters content and circadian variability of chronically food-deprived rats were mainly associated to the imposed chronic food restriction.

25. Perinatal photoperiod influences adult period and locomotor activity

Main author:	Rick van Dorp
Affiliation:	Department of Cell & Chemical Biology, Leiden University Medical Center, Leiden, The Netherlands
Co-authors:	Meijer JH, Deboer T
Keywords:	photoperiod development mice C3H/HeN sex_difference locomotor_activity period

Seasonal variation in photoperiod is unavoidable in the non-equatorial parts of the world. However, the circadian system is not fully developed at birth and changes in light exposure due to photoperiod may affect this development. For the murine adult SCN it has been shown that exposure to varying perinatal photoperiods affects the SCN period. Additionally, small but significant effects of season at birth were shown in humans regarding chronotype and the occurrence of several disorders and pathologies.

To investigate this further we exposed male and female C3H/HeN mice (n≥8) to different perinatal photoperiods (LP=16h:08h; EqP=12h:12h; SP=08h:16h LD) and assessed, during adulthood, activity profiles in 12h:12h and in constant darkness (DD), locomotor activity period length (τ), rhythmic strength (RS), intraday variability (IV), and the response to a 15-minute light pulse at CT14 (PS).

LP-developed males showed a shorter τ in DD (23,38±0,36h) than males developed in SP (23,71±0,11h t-test, $p<0,028$) or EqP (23,70±0,14h $p<0,031$), but showed no differences in RS, IV or PS. Additionally, female mice showed more wheel running activity in LD when developed in SP, while male mice showed more wheel activity in LD when developed in LP (GLM $p<0,001$ interaction 'perinatal photoperiod' with 'time' in both sexes) and we observed a significant interaction between 'perinatal photoperiod' and 'sex' on wheel activity ($p<0,001$). In DD this effect persisted albeit smaller. We observed altered τ and wheel activity in adult mice after exposure to different perinatal photoperiods, indicating that photoperiod at birth affects the circadian system in adulthood. Interestingly, some of the effects depended on sex, showing inversed effects in males compared to females. We propose that perinatal photoperiod is an imprinting event that affects the development of the circadian system and motivated locomotor activity.

26. Time-memory in the bumble bee Bombus terrestris

Main author: Özlem Gönülkırmaz Çançalar
Affiliation: Hebrew University of Jerusalem, Israel
Co-authors: Oded Shertzer, Guy Bloch
Keywords: circadian rhythms, time-memory, bumble bee, foraging

Circadian clocks regulate many ecologically important behaviors in diverse animal species. Clock-controlled time-memory in honeybees allows foragers to precisely time flower visitation to periods of maximal pollen or nectar availability and reduces the high cost of arriving at a flower patch at the wrong time. Honey bees can forage up to ten kilometers away, which is very expensive as energy. It is unclear whether bumble bees that forage over shorter distances, and with a less sophisticated recruitment system than honeybees, are also capable of similar clock-regulated complex behaviors. To start addressing this question, we tested whether bumble bees (Bombus terrestris), who live in much smaller societies and forage over shorter distances, can associate a reward with the time of day. We marked bumble bee workers with individual number tags and trained them to forage for sugar syrup in a flight cage. The cage was equipped with yellow or blue feeders rewarding either during the morning or evening. After a two-weeks training session we filled blue and yellow feeders with water and recorded all feeder visitations from sunrise to sunset. We repeated this experiment twice, each time with a different colony. We found higher foraging activity during the morning and evening training sessions, compared to other times during the day. In addition, the bees were more likely to visit feeder with colors rewarding at the same time of day during the training sessions, and, with relatively few mistakes. Our results support the hypothesis that bumble bees can associate the time of day with both food rewards and colors. Thus, efficient time memory is not limited to species that evolved for sophisticated social foraging behaviors over large distances, such as honey bees.

27. Suprachiasmatic nucleus-mediated glucose entry into the arcuate nucleus determines the daily rhythm in blood glycemia

Main author: Betty Rodriguez-Cortes
Affiliation: Department of Physiology and Cellular Biology, Institute for Biomedical Research; Universidad Nacional Autónoma de México.
Co-authors: Gabriela Hurtado-Alvarado, Ricardo Gomez-Martinez, Luis Leon-Mercado, Masha Prager-Khoutorsky, Ruud M Buijs
Keywords: Tanycytes, Blood-hypothalamus barrier, glucose rhythm, GLUT1, Vasopressin

lycemia is maintained within very narrow boundaries with less than 5% variation at a given time of the day. However, over the circadian cycle, glycemia changes with almost 50% difference. How the Suprachiasmatic Nucleus, the biological clock, maintains these day-night variations with such tiny disparities remains obscure. We show that via vasopressin release at the beginning of the sleep phase, the suprachiasmatic nucleus increases the glucose transporter GLUT1 in tanycytes. Hereby GLUT1 promotes glucose entrance into the Arcuate Nucleus, leading to a lowering in peripheral glycemia. Conversely, blocking vasopressin activity or the GLUT1 transporter at the daily trough of glycemia increases circulating glucose levels to values usually seen at the peak of the rhythm. Thus, biological clock-controlled mechanisms promoting glucose entry into the arcuate nucleus explain why peripheral blood glucose is low before sleep onset.

28. The impact of time of day of ChAdOx1 nCoV-19 vaccine administration on SARS-CoV-2 anti-spike IgG antibody levels

Main author: Kyriaki Papantoniou
Affiliation: Medical University of Vienna
Co-authors: Astrid C. Erber*, Angelika Wagner*, Marianna Karachaliou, Maren Jeleff, Polyxeni Kalafatis, Manolis Kogevinas, Beata Pepłońska, Isabel Santonja, Eva Schernhammer, Hannes Stockinger, Kurt Straif, Ursula Wiedermann, Thomas Waldhör, Kyriaki Papantoniou
Keywords: time of day, circadian time, COVID-19 vaccine, SARS-COV-2 antibodies, chronomedicine

Data from human and animal studies are highly suggestive of an influence of time of day of vaccine administration on host immune responses, but to date evidence on COVID-19 vaccines is scarce. We aimed to investigate the effect of time of day of administration of a vector vaccine against SARS-CoV-2, ChAdOx1 nCoV-19 (AstraZeneca), on SARS-CoV-2 anti-spike S1 immunoglobulin (IgG) levels. Participants were 803 Medical University of Vienna employees who received their first vaccine dose in March 2021 and agreed to antibody measurement at baseline and after 3 weeks (21-22 days). Information on the exact time of vaccination was available based on predefined vaccination dates assigned between 9:00 and 16:00. Levels of the SARS-CoV-2 anti-S1 IgG were determined in binding antibody units (BAU/ml) using the anti-SARS-CoV-2 QuantiVac (IgG) ELISA (Euroimmun). Generalized additive models (GAM) and linear regression were used to evaluate the association of time of day of vaccination (continuous and hourly bins) with antibody levels adjusting for confounders. Explorative sex- and age-specific analyses were performed. Time of day of vaccination was associated non-linearly (U-shape or 'reverse J-shape') with antibody levels at 3 weeks. Morning vaccination was associated with the highest (9:00-10:00: mean 292.1 BAU/ml; SD 262.1), noon and early afternoon vaccination with the lowest (12:00-13:00: mean 217.3 BAU/ml; SD 153.6), and late afternoon vaccination with intermediate (14:00-15:00: mean 280.7 BAU/ml; SD 262.4) antibody levels. The differences between vaccination time points of 9:00-10:00 and 12:00-13:00 were the most pronounced, and statistically significant (beta coefficient -75.8, 95% CI -131.3, -20.4) after adjusting for potential confounders. Differences were larger among men and younger participants. Our findings show that optimizing the time of day of vaccination against SARS-CoV-2 has an impact on the magnitude of antibody levels. Whether this difference persists after the second vaccine dose or influences the protection state needs further evaluation.

29. Circadian clock genes are regulated by rhythmic corticosterone in the rat hippocampus

Main author: Martin Fredensborg Rath
Affiliation: Department of Neuroscience, Faculty of Health and Medical Sciences, University of Copenhagen, Panum Institute, Copenhagen, Denmark
Co-authors: Tenna Bering, Aurea Susana Blancas Velazquez, Tenna Bering, Aurea Susana Blancas Velazquez, and Martin Fredensborg Rath
Keywords: -

In the hippocampus, circadian clock gene expression seems to be important for memory and mood; however, the signaling mechanism controlling clock gene expression in the hippocampus is unknown. Recent findings suggest that circadian rhythms in circulating glucocorticoids driven by the SCN control rhythmic clock gene expression in extra-hypothalamic neurons; in addition, hippocampal neurons

express high levels of the glucocorticoid receptor. We therefore hypothesized that oscillations of clock genes in the hippocampus could be driven by SCN-controlled circadian rhythms in glucocorticoids.
To establish the temporal profile of hippocampal clock gene expression, qRT-PCR was performed on rat hippocampi. Daily rhythms were detected for Per1, Per2, Arntl, Nr1d1, and Dbp (p-values <0.05). Histological analyses confirmed clock gene products in both the hippocampal gyrus and the dentate gyrus. To determine the effect of rhythmic glucocorticoids on hippocampal clock gene expression, the SCN was lesioned in rats to abolish all circadian rhythms; subsequently, the adrenal glands were removed and a 24h corticosterone rhythm was reestablished by use of programmable micropumps (iPRECIO®) releasing corticosterone. Hippocampal gene expression was analyzed at ZT3 and ZT15 by qRT-PCR. For Per1 and Dbp, differential expression was detectable in sham controls (p-values <0.0001). Rhythmic gene expression in the hippocampus was abolished by SCN-lesion, suggesting that the hippocampus is a slave-oscillator governed by the SCN. However, most importantly, reestablishing the natural rhythm in corticosterone significantly restored differential expression between the analyzed time-points for both Per1 and Dbp (p-values <0.05).
In conclusion, our data show that rhythmic corticosterone can drive hippocampal clock gene rhythms suggesting that the SCN regulates the circadian oscillator of the hippocampus by controlling the circadian rhythm in circulating glucocorticoids.

30. The intestinal circadian clock drives microbial rhythmicity to maintain gastrointestinal homeostasis

Main author: Baraa Altaha
Affiliation: Technical university of Munich, School of life science, Chair of nutrition and immunology. Ziel institute for food and health
Co-authors: Marjolein Heddes, Yunhui Niu, Sandra Reitmeier, Karin Kleigrewe, Dirk Haller, Silke Kiessling

Keywords: Circadian rhythm, gut microbiota, microbial rhythmicity, microbial functionality, chronic jet lag, starvation, constant darkness, gut clock, gnotobiotic, germ free mice, Picrust, targeted metabolomic

Diurnal (i.e., 24-hour) oscillations of the gut microbiome have been described in various species including mice and humans. However, the driving force behind these rhythms remains less clear. In this study, we differentiate between endogenous and exogenous time cues driving microbial rhythms. Our results demonstrate that fecal microbial oscillations are maintained in mice kept in the absence of light, supporting a role of the host's circadian system rather than representing a diurnal response to environmental changes. Intestinal epithelial cell-specific ablation of the core clock gene Bmal1 disrupts rhythmicity of microbiota. Targeted metabolomics functionally link intestinal clock-controlled bacteria to microbial-derived products, in particular branched-chain fatty acids and secondary bile acids. Microbiota transfer from intestinal clock-deficient mice into germ-free mice altered intestinal gene expression, enhanced lymphoid organ weights and suppressed immune cell recruitment. These results highlight the importance of functional intestinal clocks for circadian microbiota composition and function, which is required to balance the host's gastrointestinal homeostasis.

31. Objective: Determine if there are trait-like individual differences in cardiovascular reactivity to cold pressor stress test after days of combined sleep restriction and circadian misalignment.

Main author: Sabrina Linton
Affiliation: Sleep and Chronobiology Laboratory
Co-authors: Sabrina K. Linton, Dana Withrow, Kate E. Sprecher, Christopher M. Depner, Tina M. Burke, Pieter C. Dorrestein, Monika Fleshner, Rob Knight, Christopher A. Lowry, Fred W. Turek, Martha H. Vitaterna, Kenneth P. Wright Jr

Keywords: cold pressor test, sleep restriction, circadian misalignment, cardiovascular reactivity, distal-proximal skin temperature gradient

Objective: Determine if there are trait-like individual differences in cardiovascular reactivity to cold pressor stress test after days of combined sleep restriction and circadian misalignment.
Methods: Healthy adults aged 25.6 ± 4.2 (mean ± SD) were enrolled in a 39-day protocol. Individuals kept self-selected 8h sleep schedules for 2 weeks prior to two 3.7 day lab visits. During their time in-lab, they received a baseline 8h sleep opportunity and then three 3h sleep opportunities, two during the biological day. The cold pressor test (CPT) was administered after baseline sleep and after the last daytime sleep opportunity; subjects submerged their hand in 0°C ice water for 3 min, and skin temperature on the contralateral hand and blood pressure, heart rate, pain, and salivary cortisol were measured.
Results: Linear mixed effects models revealed a main effect of time on systolic blood pressure (SBP), heart rate (HR), cortisol, pain, and distal-proximal skin temperature gradient (DPG); such that SBP, HR, cortisol, and pain were elevated and DPG was wider (i.e., more negative) in response to the CPT. Furthermore, SBP was higher 5 min before ($p<0.05$) and during the third min ($p<0.001$) of the CPT following combined sleep restriction and circadian misalignment compared to baseline. Additionally, a main effect of condition ($p<0.05$) showed a wider DPG after combined sleep restriction and circadian misalignment compared to baseline. Intraclass correlation coefficients (ICCs) revealed consistent individual responses with large variability between individuals.
Conclusions: Combined sleep restriction and circadian misalignment may worsen cardiovascular reactivity to a physiological stressor; SBP and DPG were elevated compared to baseline conditions indicating increased vasoconstriction. Furthermore, SBP was the most stable outcome for cardiovascular measures in response to CPT demonstrated by consistent ICC values across conditions and timepoints indicating it may be a robust measurement when assessing cardiovascular reactivity during sleep and circadian disturbances.

32. Run for your live(r): Exercise training at different times of day differentially modulates hepatic inflammation in early NAFLD

Main author: Artemiy Kovynev
Affiliation: Division of Endocrinology, Department of Medicine, Leiden University Medical Center, Leiden, The Netherlands
Co-authors: Artemiy Kovynev, Zhixiong Ying, Joost Lambooij, Amanda Pronk, Trea Streefland, Bruno Guigas, Patrick C.N. Rensen and Milena Schönke

Keywords: -

Introduction: Exercise effectively prevents obesity-related disorders, but it is unclear whether the beneficial health effects of exercise are restricted to unique circadian windows. We recently showed that late exercise training over four weeks reduces atherosclerosis and body fat mass whereas early training did not prevent fat mass gain, suggesting a greater improvement of hyperlipidemic and

inflammatory diseases with late training. Therefore, we now aimed to study whether the timing of exercise training differentially modulates obesity-related NAFLD development and progression.
Methods: We used male APOE*3-Leiden.CETP mice that were fed an obesogenic (high fat-high cholesterol) diet to induce NAFLD. These mice were endurance-trained on a treadmill for eight weeks (5x per week, 1 hour) either in the early (ZT13) or in the late (ZT22) active phase. Subsequently, NAFLD score (histology), hepatic inflammation (FACS), and inflammatory genes expression (qPCR) were compared to sedentary mice.
Results: Exercise training prevented an increase in body fat mass (+1.13 g and +1.06 g with early and late training) vs sedentary mice (+3.67 g) and fasting plasma glucose 7.0 mM and 6.9 mM in early and late training, compared to 7.7 mM in sedentary mice). Neither early nor late training affected liver triglyceride or cholesterol content compared to sedentary mice, likely due to a very early stage of hepatic steatosis. In line, hepatic expression of de novo lipogenesis genes (e.g., Fasn, Srebp1c) was similarly downregulated by early and late training. However, exercise had a distinct time-dependent effect on hepatic inflammation, as only early training promoted an influx of neutrophils and monocytes into the liver paired with increased expression of the pro-inflammatory cytokines (e.g. Tnfa, Il1b, F4/80).
Conclusion: Timing of exercise is a critical factor for the positive effect in cardiometabolic disease management. We currently investigate the effect of timed training on the development of advanced stages of NAFLD/NASH.

33. Thermoneutral zone, rhythm of activity, and circadian O2 consumption in Opn4 -/- mice

Main author: Giovanna Zanetti
Affiliation: Department of Physiology, Institute of Biosciences, University of São Paulo
Co-authors: Giovanna Zanetti, Daniela Dantas David, José Souto-Neto, Cristhian Cespedes, Thalles Lacerda, Maria Nathália Moraes, Ana Maria de Lauro Castrucci

Keywords: melanopsin; thermoneutrality; rhythm of activity; circadian O2 consumption; metabolism

Thermoneutral zone, rhythm of activity, and circadian O2 consumption in Opn4-/- mice: Background: Melanopsin (OPN4) is a retinal photopigment found in a subset of intrinsically photosensitive ganglion cells (ipRGCs), which play a role in central circadian pacemaker synchronization to the light-dark cycle. Peripheral tissues also express opsins which were shown to have light-independent sensory functions, such as thermoreception and metabolism. Aim: To evaluate the thermoneutrality range, the rhythm of activity, and the circadian O2 consumption in Opn4 knockout (KO) compared to wild type (WT) animals. Method: Activity was monitored at 1-minute intervals at 30°C and 22°C. The determination of thermoneutrality was performed at temperatures from 16 to 36°C for 3 consecutive days. For circadian O2 consumption, the animals were recorded for 36 h at 30°C, 22°C and 10°C. All protocols were performed under a light-dark cycle of 12:12 LD. Results: The thermoneutrality zone of WT animals lies between 26 and 36°C, for Opn4 KO between 23.5 and 36°C. The lack of OPN4 results in increased locomotor activity, compared to WT animals. The circadian O2 consumption at 30°C was identical for both genotypes. A remarkable increase in O2 consumption was observed at 22°C and 10°C in Opn4 KO animals compared to WT animals. Conclusion: We observed that Opn4 KO animals have a temperature difference of 2.5ºC in the initial thermoneutral temperature zone compared to WT. And the increase of O2 consumption in Opn4 KO animals exposed to low temperatures always occurred in the dark phase when the animals concentrate their activity and have an increase in metabolic rate. Therefore, we suggest that melanopsin may play an important role in the metabolism and temperature regulation of mammals. Financial support: CAPES, CNPq and FAPESP.

34. Chronic mistimed snacking promotes obesity in mice

Main author: Kimberly Begemann
Affiliation: [1]Institute of Neurobiology, University of Lübeck, Lübeck, Germany
[2]Center of Brain, Behavior, and Metabolism, University of Lübeck, Lübeck, Germany
Co-authors: Isabel Heyde, Pia Witt, Brinja Leinweber, Christiane E. Koch, Henrik Oster
Keywords: -

Frequent snacking influences body weight development and energy homeostasis. Furthermore, mistimed food intake disrupts the endogenous circadian clock network and promotes overconsumption of palatable, calorie-dense food, thus, also supporting obesity development. Mice and humans are especially susceptible for hedonic overconsumption in the early rest/late active phase. Therefore, we hypothesized that chronic mistimed snacking in the early rest phase is more likely to induce obesity. Mice received a daily chocolate snack over six weeks either in the early rest or early active phase in addition to normal chow provided ad libitum. The control group did not receive a daily snack. We show that snack timing is important for body weight development and metabolism in mice. Interestingly, rest time snacking increased body weight gain as well as fat mass and disturbed daily food intake rhythms without affecting cumulative caloric intake. In line with these data, we could show that rest time snacking affects daily temperature and activity rhythms as well as energy expenditure. Importantly, body weight development in mice receiving a daily snack in the early active phase was comparable to the control group. In conclusion, putting more focus on avoiding mistimed snacking should be considered in therapies of body weight regulation. Such approach could also improve compliance with weight loss regimens.

35. Circadian rhythms in melatonin and cortisol are disturbed in critically ill children

Main author: Arnout Cramer
Affiliation: Pediatric Intensive Care Unit, Erasmus MC Sophia Children's Hospital, Rotterdam, The Netherlands
Co-authors: Arnout B.G. Cramer, Ilse den Besten, Koen F.M. Joosten, Matthijs de Hoog, Rogier C.J. de Jonge, Sascha C.A.T. Verbruggen
Keywords: melatonin, cortisol, children, intensive care unit, paediatric intensive care unit

Background: A limited number of studies indicate that circadian rhythms may be disturbed in critically ill children, due to a combination of the underlying illness, lack of normal Zeitgebers, and iatrogenic influences. We aimed to verify these findings, as well as to explore the types of disturbances, and to determine how blood should be sampled to adequately evaluate circadian rhythm from melatonin and cortisol.
Methods: As part of a larger study regarding circadian rhythm and feeding in the pediatric intensive care unit, we took seven blood samples at four-hour intervals on several days of the admission. We included children from 37 weeks postmenstrual age up until 17 years of age and with various diagnoses. We excluded those with expected admissions shorter than 48 hours. This analysis concerns the admission day samples of the first twelve patients from whom five or more samples were successfully obtained.
Results: Different types of disturbances were found. Five patients showed low peak values of melatonin, one had high peak and trough values and in most patients the normal pattern was not seen. In eight out of twelve patients melatonin onset, the time at which melatonin levels start to rise, could be calculated, with only two patients showing a melatonin onset in the evening. In cortisol similar

findings of both high and low levels were obtained, again with no clear circadian rhythms. No consistent associations with underlying disease or administered medication were found.
Conclusion: Circadian rhythms of melatonin and cortisol are grossly disrupted in critically ill children. Further research is needed to identify the causes of different types of disturbances. The gross disturbances favor sampling throughout the day as opposed to frequent sampling in the evening which is used to determine subtle shifts in melatonin onset.

36. Circadian rhyme misalignment and risk of nonalcoholic fatty liver disease (NAFLD) in free-living adults

Main author: Yikyung Park
Affiliation: Washington University School of Medicine
Co-authors: Ningying Wu, Shu Jiang
Keywords: composite phase deviation, nonalcoholic fatty liver disease

Background: NAFLD is a fast-growing liver disease worldwide. Liver is one of the most circadian-regulated organs. Accumulating evidence from animal and controlled lab studies suggests that circadian rhythm disruption increases risk of metabolic dysfunction and liver diseases. Given a growing interest in identifying novel modifiable risk factors for NAFLD, we examined if circadian misalignment measured by a composite phase deviation (CPD) in free-living adults was associated with NAFLD.
Methods: CPD assesses the day-to-day variation in mid-sleep time and the difference between a given mid-sleep and the individual's preferred sleep timing (i.e., chronotype). We calculated a CPD using actigraphy with timestamps in adults in the US National Health and Nutrition Examination Survey 2005-2006 (n=751). Early, intermediate, and late chronotype was defined based on the distribution of mid-sleep time on weekends, corrected for oversleeping due to sleep loss on weekdays (<25th, 25-75th, and >75th percentile). NAFLD was defined by the Dallas Steatosis Index. Multivariable Logistic regression models adjusting for socio-demographics, smoking, physical activity, work schedule, and diet quality were used to estimate odds ratios (OR) and 95% confidence intervals (CIs) of NAFLD.
Results: Prevalence of NAFLD was 33%. Mean mid-sleep time and CPD were 2:45am and 1.83 hours, respectively. Compared with people in the lowest CPD category, people in the highest CPD category had more than a twofold increased risk of NAFLD (OR=2.33, 95% CI:1.44-3.77). The increased risk of NAFLD with higher CPD was more apparent in women, people 50+ years old, never smokers, and people with poor diet quality. Individuals with early (OR=2.18, 95% CI: 1.29-3.66) and late (OR=2.21, 95% CI:1.36-3.59) chronotype also had an increased risk of NAFLD compared with those with intermediate chronotype.
Conclusions: Chronic circadian rhythm disruption is a novel risk factor for NAFLD.

37. The role of clock genes in circadian function of the mammalian pineal gland

Main author: Aurea Susana Blancas-Velazquez
Affiliation: Department of Neuroscience, Faculty of Health and Medical Sciences, University of Copenhagen, Panum Institute, Copenhagen, Denmark
Co-authors: Tenna Bering and Martin Fredensborg Rath
Keywords: Pineal gland, primary cell culture, siRNA, clock genes

Background: The suprachiasmatic nucleus (SCN), the master clock of the body, signals information on circadian time to peripheral organs and other brain areas including the pineal gland, which synthesizes the nocturnal hormone melatonin. A defining feature of the SCN is the circadian expression of clock genes, altough clock genes are expressed in most tissues, including the pineal

gland. The melatonin profile is a major marker of circadian time; however, the local role of the clock-genes in mammalian pineal melatonin production is unknown. Therefore, the aim of this work is to determine whether clock-genes in the pinealocytes of the pineal gland regulate rhythmic melatonin production.
Methods: We here used a pinealoyte primary cell culture from male Sprague-Dawley rats. To determine the function of individual clock-genes, we transfected the cultures using small interference RNA (siRNA) to knockdown the clock genes Per1, Per2, Arntl or Clock. To mimic temporal signaling in the pineal gland, pinealocyte cultures were stimulated with norepinephrine (NE) for a period of 12 hours (to signal nighttime) during two cycles. We analyzed gene expression using qPCR.
Results: Clock genes in the pinealocyte primary cultures exhibited daily variations when they were synchronized by NE. Moreover, siRNAs effectively knocked down clock genes in the cultures. Per1, Per2 and Arntl knockdown had minor effects on the transcript of melatonin-producing enzyme AANAT, whereas knocking down Clock, decreased both Aanat transcript levels and amplitude.
Conclusion: Knocking down Clock reduces the expression of Aanat, thus suggesting a direct link between the circadian molecular clock work of the pinealocyte and rhythmic melatonin synthesis.

38. Therapeutic Nuclear magnetic resonance redirects the metabolism of NIH-3T3 cells

Main author: Viktoria Thöni
Affiliation: Viktoria Thöni, MSc, Institute of Zoology, University of Innsbruck, Technikerstr. 25, A-6020 Innsbruck, Tel: +43 676 561 4694
Co-authors: Viktoria Thöni[1°], David Mauracher[1°], Anil Ramalingam[1], Birgit Fiechtner[1], Adolf Michael Sandbichler[1] and Margit Egg[1*]
° both authors contributed equally
* corresponding author

Keywords: therapeutic Nuclear Magnetic Resonance, Cryptochrome, Hypoxia Inducible Factor-1α, radical pair mechanism, hypoxia, glycolysis, mitochondrial respiration, ischemia, therapeutic tool

Cryptochrome, known as the core protein of the circadian clock, enables magnetic sensing in migratory animals. Thereby, the protein is known to modulate intracellular reactive oxygen species (ROS) via the quantum based radical pair mechanism (RPM). Consequently, the protein has been suspected to be sensitive for weak electromagnetic fields (EMF) also in somatic cells. Recently, we reported the impact of therapeutic nuclear Magnetic Resonance (tNMR, 0.4mT; 17 kHz) on the circadian clock and associated Hypoxic Inducible Factor 1 alpha (HIF-1α) in mammalian NIH-3T3 cells. To further investigate the physiological consequences, we evaluated the metabolic situation in synchronized NIH-3T3 cells by combining the tNMR exposure either with normoxic or hypoxic oxygen conditions of 1% O2. We showed that tNMR treatment and normoxia / hypoxia altered cellular metabolism in NIH-3T3 cells. Normoxic tNMR exposure decreased glycolysis, lactate production, as well as the extracellular acidification rate and the ADP/ATP ratio, whereas mitochondrial and extracellular ROS, as well as cellular proliferation were elevated. Notably, compared to the sham treated cells these effects were amplified after hypoxic tNMR exposure. Hence, hypoxic tNMR treatment redirected cellular metabolism from glycolysis towards mitochondrial respiration, at the background of a highly controlled ROS signature. We therefore propose tNMR as a potential therapeutic tool that should be further investigated as treatment option for ischemia driven diseases like infarct, stroke and cancer.

39. Sleep as a major determinant for mental health outcomes in elite athletes

Main author: Elise Facer-Childs
Affiliation: Monash University, Australia, Turner Institute for Brain and Mental Health
Co-authors: Elise R. Facer-Childs (Presenter), Luis Mascaro, Daniel Hoffman, Darren Mansfield, Sean P.A. Drummond, Shantha M.W. Rajarathnam
Keywords: Sleep, Mental Health, Athletes, Elite, Actigraphy, Depression, Anxiety, Stress

Introduction: The link between mental health and sleep is well documented in the general population, with the majority of mental health disorders involving some type of sleep disturbance. There is, however, limited research investigating this relationship in elite athlete populations. The aim of this study was to identify whether sleep and mental health outcomes are associated in elite athletes, and if so, what measures of sleep were the most predictive of mental health outcomes.
Methods: A comprehensive assessment of sleep was conducted using both objective and subjective methods in 68 Australian Football League athletes (male, mean age = 23.3 ± 3.4y, range 18–32y). Rest-activity patterns were recorded using wrist actigraphy for an average of 13.8 ± 3.6 days (total 884 days data). Subjective sleep data were collected using daily sleep diaries and validated questionnaires. Validated mental health questionnaires were used to assess depression, anxiety and stress symptoms. Multiple linear regression modelling was used to investigate the relationship between sleep and mental health.
Results: Using a combination of sleep variables, poor sleep predicted 51% of the variation in clinical depression, 42% of the variation in stress, and 31% in clinical anxiety. Self-reported insomnia symptoms (using the Insomnia Severity Index), were the strongest predictors of poor mental health outcomes, followed by objective sleep monitoring via actigraphy. Sleep diary measures were the weakest predictors of mental health.
Conclusion: Our results present poor sleep as a major determinant of impaired mental health outcomes in a population that is constantly under pressure to perform at the highest level and may underreport mental health symptoms. These findings support the inclusion of sleep assessments as an initial screening tool as well as a core component of all routine health and rehabilitation programs.

40. Circadian rhythm of protein-protein interactions and post-translational modifications in the clock protein complex

Main author: Yuta Otobe
Affiliation: Graduate School of Science, The University of Tokyo, Japan.
Co-authors: Yuta Otobe, Shunsuke Ito, Michinori Koebis, Atsu Aiba, Yoshitaka Fukada and Hikari Yoshitane
Keywords: LC-MS/MS, Phospholylation, Protein interactions

The circadian clock drives daily biological rhythms, which are observed in a wide range of organisms. In cyanobacteria, KaiC protein is rhythmically phosphorylated not only under light-dark cycles but also under a constant dark condition and even in tubes, i.e., in vitro. However, KaiC protein is not conserved in the eukaryote. In mammals, clock genes and their encoded proteins form transcriptional/translational feedback loops, in which CLOCK and BMAL1 transactivate a series of target genes including their negative regulators, Per and Cry. Importantly, even in the absence of the transcriptional rhythms, several circadian events maintain their rhythmicity, indicating that protein dynamics such as posttranslational regulation, protein complex formation and conformation changes can drive the circadian oscillation also in the eukaryote. Here, we comprehensively identified posttranslational modification sites of clock proteins and their interacting proteins in cultured cells. We found in vivo circadian rhythms of these protein dynamics by generating Flag-BMAL1 knock-in (KI) mice and Flag-

PER2 KI mice and by subjecting a semi-purified clock protein complex to nano-LC MS/MS-based quantification.
Especially, CLOCK showed circadian phospholylation rhythms, whose phases were almost anti-phasic in a phosphosite-dependent manner. We revealed that the “phospho-switch of CLOCK protein” was governed by protein interaction with PER2 and by kinase activity of CK1. Our data suggest the presence of a circadian protein oscillator of mammalian clock components, which resembles the KaiC complex rhythm in vitro.

41. Synergistic effect of prenatal LPS and constant light during early postnatal development on circadian and immunity system.

Main author: Veronika Spišská
Affiliation: Department of Physiology, Faculty of Science, Charles University, Prague
Co-authors: Aneta Kubištová, Zdeňka Bendová, Veronika Spišská[1], Aneta Kubištová[1], Zdeňka Bendová[1,2]
[1]Department of Physiology, Faculty of Science, Charles University, Czech Rep.
[2]National Institute of Mental Health, Klecany, Czech Republic
Keywords: circadian system, immunity, LPS, constant light, development

It is already well established that the circadian clock and the immune system are mutually interconnected. In adult animals, long-term exposure to constant light leads to a desynchronisation of circadian rhythms and thus to an impairment of immunity functions and other physiological processes. In our previous experiments, we showed that prenatal injection of lipopolysaccharide (LPS) alters the expression of clock genes during early postnatal development of rat pups[1]. Our other results showed that constant light during postnatal development permanently alters circadian rhythms in expression of many genes in different brain regions in adulthood[2].
Our current research focuses on the synergistic effect of constant light in early ontogeny and prenatal exposure to LPS on circadian and immune system development. Our data suggest that postnatal housing in constant light enhances the effect of prenatal LPS injection and leads to a marked suppression of circadian rhythms in gene expression in suprachiasmatic nuclei, but also in hippocampus and spleen. Furthermore, constant light during ontogeny also alters circadian rhythms in peritoneal macrophages.
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[2]Kubištová et al. (2020) Constant Light in Critical Postnatal Days Affects Circadian Rhythms in Locomotion and Gene Expression in the Suprachiasmatic Nucleus, Retina, and Pineal Gland Later in Life. Biomedicines.

42. Acute sleep restriction predisposes the liver to inflammation by elevation of uric acid

Main author: Pawan Jha
Affiliation: 1 Department of Systems Pharmacology & Translational Therapeutics, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA 19104, USA.
2 Institute for Translational Medicine and Therapeutics, Perelman School of Medicine, University of Pennsylvania, PA 19104, USA.
Co-authors: Utham K. Valekunja, Shintaro Yamazaki, Jing Chen-Roetling & Akhilesh B. Reddy
Keywords: -

Sleep is essential for survival in mammals. Loss of sleep is associated with maladaptive physiological changes that lead to dysfunction of the metabolic, gastrointestinal, immune, and circulatory systems. Sleep restriction induces inflammation and cellular stress in peripheral organs. We found that 12 hours of sleep restriction in mice led to an inflammatory signature in the liver, which resolved after recovery sleep. We also found that xanthine oxidase (XO) expression was increased with the same profile. XO is an important enzyme for the production of uric acid. Uric acid is a damage-associated molecular pattern (DAMP), which can attract inflammatory cells (e.g., neutrophils) into tissues. We hypothesized that sleep restriction leads to sterile inflammation by the elevation of uric acid in the liver. To test this, we performed the sleep analyses by dividing the male C57BL/6J mice into three experimental groups: a control (normal sleep) group, a 12 h sleep-restricted group, and a recovery sleep group (24 h of recovery). We collected tissues for biochemical and histological analysis. We reported a rise in liver uric acid levels, and XO activity, following sleep restriction. These parameters returned to baseline during recovery sleep. Uric acid is synthesized in the liver as the final oxidation product of purine metabolism. Targeted metabolomics suggests that sleep loss enhances the conversion of nucleic acids, adenine, and guanine into uric acid in the liver. The concomitant rise of uric acid, and the neutrophil marker myeloperoxidase (MPO), during sleep-restriction suggests that a uric acid surge triggers neutrophil accumulation in the liver. Together, this indicates that sleep restriction modulates neutrophil trafficking into the liver, which may be either protective or maladaptive.

43. Combining lineage correlations and a small molecule inhibitor to detect circadian control of the cell cycle

Main author: Shaon Chakrabarti
Affiliation: Simons Centre for the Study of Living Machines,
National Centre for Biological Sciences
Co-authors: Anjoom Nikhat

Keywords: Lineage correlations, chronotherapy, circadian clock, cell cycle, interactions, KL001, mathematical modeling, cousin-mother inequality

Cancer chronotherapy has emerged as an exciting possibility for improving treatment regimens in cancer. However, one of the challenges in successful implementation of chronotherapy is improving our understanding of the nature of circadian clock – cell cycle coupling. While many molecular interactions have been discovered between the components of these two oscillators, recent work using time lapse microscopy surprisingly suggests that these interactions may not necessarily lead to an emergent control of the cell cycle by the clock. In this work, we explore a new strategy, based on measuring lineage correlations in cell cycle times, to probe the presence of circadian control of the cell cycle. We and others have previously suggested using phenomenological models, that the so called ‘cousin-mother inequality’ – an intriguing phenomenon where cousin cells show stronger cell cycle time correlations than mother-daughter pairs, could be leveraged to probe circadian effects on cellular proliferation. Here we computationally demonstrate that known molecular interactions that establish the cell cycle and circadian oscillations, along with some of their well-established connections, are sufficient to generate the cousin-mother inequality in cell cycle times. We demonstrate that cell cycle distributions and the larger cousin correlations as seen in HCT116 lineages can be quantitatively recapitulated by our models. Importantly, reverse coupling from the cell cycle to the circadian clock, cannot recapitulate these results. Finally, we explore the effects of the small molecule clock inhibitor KL001 to suggest an experimental test for probing circadian control: with increasing KL001 concentration, our models predict up to 50% decrease in the difference between cousin and mother-daughter correlations. In stark contrast, net cellular growth rates change only by about 5% with KL001 treatment. These results therefore suggest the exciting possibility of using lineage correlations as a fluorescent reporter-free probe of circadian clock – cell cycle interactions.

44. Rhythmic transcription of Bmal1 stabilizes the circadian timekeeping system in mammals.

Main author: Hikari Yoshitane
Affiliation: Tokyo Metropolitan Institute of Medical Science
Co-authors: Hikari Yoshitane, Yasuko O. Abe, Dae Wook Kim, Atsu Aiba, Jae Kyoung, Kim and Yoshitaka Fukada

Keywords: Genome editing, DNA cic-element, RRE, rhythmic transcription, mathematical modeling

In mammals, the circadian clock consists of transcriptional and translational feedback loops through DNA cis-elements such as E-box and RRE. The E-box-mediated core feedback loop is interlocked with the RRE-mediated feedback loop, but biological significance of the RRE-mediated loop has been elusive. In this study, we established mutant cells and mice deficient for rhythmic transcription of Bmal1 gene by deleting its upstream RRE elements and hence disrupted the RRE-mediated feedback loop. We observed apparently normal circadian rhythms in the mutant cells and mice, but a combination of mathematical modeling and experiments revealed that the circadian period and amplitude of the mutants were more susceptible to disturbance of CRY1 protein rhythm. Our findings demonstrate that the RRE-mediated feedback regulation of Bmal1 underpins the E-box-mediated rhythm in cooperation with CRY1-dependent posttranslational regulation of BMAL1 protein, thereby conferring the perturbation-resistant oscillation and chronologically-organized output of the circadian clock.

45. Self-organized macroscopic waves reveal intrinsic rhythms in a giant single-celled organism feeding on light

Main author: Eldad Afik
Affiliation: Division of Biology and Biological Engineering, California Institute of Technology
Howard Hughes Medical Institute
Co-authors: Tony J.B. Liu; Elliot M. Meyerowitz

Keywords: Light Entrainment; Spatio-temporal dynamics; Non-traditional models; Single-cell Dynamics; Time-Dependent Feeding

Living Systems often seem to follow, in addition to external constraints and interactions, an intrinsic predictive model of the world — a defining trait of Anticipatory Systems. Here we study rhythmic behaviour in Caulerpa, a marine green alga, which appears to predict the day/night light cycle.
Caulerpa consists of differentiated organs resembling leaves, stems and roots. While an individual can exceed a meter in size, it is a single multinucleated giant cell. Active transport has been hypothesized to play a key role in organismal development. It has been an open question in the literature whether rhythmic transport phenomena in this organism are of autonomous circadian nature. Using Raspberry-Pi cameras, we track over weeks the morphogenesis of tens of samples concurrently, while tracing at resolution of tens of seconds the variation of the green coverage. The latter reveals waves propagating over centimeters within few hours, and is attributed to chloroplast redistribution at whole-organism scale.
Our observations of algal segments regenerating under 12-hour light / 12-hour dark cycles indicate that the initiation of the waves precedes the external light change. Using time-frequency analysis, we find that the temporal spectrum of these green pulses contains a circadian period. The latter persists over days even under constant illumination, indicative of its autonomous nature. Our findings suggest the existence of distinct dynamical states, including a chaotic one, as well as an effect on

morphogenesis. We further explore the system under non-circadian periods, to reveal how the spectral content changes in response.
Time-keeping and synchronization are recurring themes in biological research at various levels of description — from subcellular components to ecological systems. We present a seemingly primitive living system that exhibits apparent anticipatory behaviour. This research offers quantitative constraints for theoretical frameworks of such systems and of biological self-stabilization far-from-thermal-equilibrium.

46. The abyss keeps time too

Main author: Audrey Mat
Affiliation: 1. Max Perutz Labs, University of Vienna, Vienna, Austria
2. Alfred Wegener Institute, Helmholtz Centre for Polar and Marine Research, Bremerhaven, Germany
Co-authors: -
Keywords: Deep-sea rhythms, wild clocks, hydrothermal vent mussel, marine rhythms

The deep sea (>200 m depth) represents ~93% of the biosphere in volume. However, it is also one of Earth's greatest ecological frontiers: the Moon and Mars surfaces have been mapped at high resolution, while less than 20% of the seabed surface has been surveyed at low resolution. But not only is our spatial understanding of the deep sea limited, we are also pretty ignorant about its temporal organisation. The main reason is that conducting experiments in the deep sea constitutes a technical challenge: it has a depth and pressure difficult to even imagine and no solar light. Yet, biological rhythms is present in the abyss. Indeed, my work recently showed that the valve behaviour and transcriptome of the hydrothermal vent mussel Bathymodiolus azoricus exhibit rhythmic patterns at 1700 m depth. Tidal cycles dominate the biology of mussels living on the Mid-Atlantic Ridge, where temperature and pressure also exhibit a tidal signal. Unexplored deep-sea ecosystems harbour fundamental biological mechanisms awaiting their disentanglement. From a chronobiological perspective, deep-sea ecosystems, blind to the day/night solar cycle but under tidal stimuli, can provide a unique window into the understanding of marine clocks and their evolution. Hydrothermal vents are generally considered to be either modern analogues of the primitive ocean or some of the first habitable environments on Earth. Besides their fascinating science, time is also ticking for deep sea ecosystem from a societal perspective. They are under severe anthropogenic threat, especially by mining and petroleum exploitation. Raising the scientific and public awareness for these places that harbour highly unique life forms in order to protect their values is therefore another major challenge for these ecosystems.

47. Metabolic effects of acute circadian desynchronization

Main author: Anhui Wang
Affiliation: Department of Endocrinology and Metabolism, Amsterdam University Medical Centers, University of Amsterdam, Amsterdam, Netherlands.
Co-authors:
Name: E. Foppen
Affiliations: 1, Department of Endocrinology and Metabolism, Amsterdam University Medical Centers, University of Amsterdam, Amsterdam, Netherlands.
2, Netherlands Institute of Neuroscience (NIN), Royal Dutch Academy of Arts and Sciences (KNAW), Amsterdam, Netherlands.
Name: S.F. Lam
Affiliation: Netherlands Institute of Neuroscience (NIN), Royal Dutch Academy of Arts and

Sciences (KNAW), Amsterdam, Netherlands.
Name: M.W. Jansen
Affiliation: Netherlands Institute of Neuroscience (NIN), Royal Dutch Academy of Arts and Sciences (KNAW), Amsterdam, Netherlands.
Name: A. Kalsbeek
Affiliations: 1, Department of Endocrinology and Metabolism, Amsterdam University Medical Centers, University of Amsterdam, Amsterdam, Netherlands.
2, Netherlands Institute of Neuroscience (NIN), Royal Dutch Academy of Arts and Sciences (KNAW), Amsterdam, Netherlands.
Name: D.J. Stenvers
Affiliation: Department of Endocrinology and Metabolism, Amsterdam University Medical Centers, University of Amsterdam, Amsterdam, Netherlands.

Keywords: Acute circadian desynchronization; Light inversion; Daily rhythms; glucose tolerance; Metabolism

Aims: Shift workers have an increased risk to develop type 2 diabetes. Recently, a human study showed that an acute 12h phase shift has acute negative effects on muscle insulin sensitivity at the onset of the active period. We aim to disentangle the underlying neuroendocrinological and metabolic mechanisms. Therefore, we subjected rats to acute circadian desynchronization and assessed the time-course of the behavioral and metabolic adaptation as well as the effect on the diurnal rhythm of glucose tolerance.
Methods: First, male Wistar rats (n=4) were placed in metabolic cages for 3 days in a 12h light: 12h dark (LD) cycle with standard chow available ad lib. Then a complete LD reversal (12h/12h DL cycle) was done at day1. Food and water intake, locomotor activity and RER were measured continuously for 7 days. In a second experiment, jugular vein cannulation surgery was performed in 8 rats, and 7-10 days later an intravenous glucose tolerance test (ivGTT) was performed at Zeitgeber Time 2 (ZT2) and ZT14. After 7-10 days rats underwent a complete 12h phase LD reversal, and after 3 days the ivGTTs were repeated.
Results: Wistar rats showed an expected gradual reversal of the daily rhythms of locomotor activity, food intake and energy expenditure, but food intake showed a slower adaptation. The glucose tolerance test showed that the daily rhythm of glucose tolerance had returned to normal in rats three days after the LD reversal.
Conclusion: In Wistar rats, the daily rhythm of glucose tolerance has adapted within three days of inversed light exposure, despite a slower adaptation of other daily rhythms. This may be due to an acute effect of light on glucose tolerance, or to a rapid adaptation of the tissue clocks. In future experiments we will address these questions as well as the impact of time-restricted feeding on adaptation speed.

48. Feasibility of time-restricted eating and impacts on cardiometabolic health in 24-hour shift workers: The healthy heroes randomized clinical trial

Main author: Emily N.C. Manoogian
Affiliation: Salk Institute for Biological Studies
Co-authors: Adena Zadourian, Hannah C. Lo, Nikko R Gutierrez, Azarin Shoghi, Ashley Rosander, Aryana Pazargadi, Cameron K. Ormiston, Xinran Wang, Jialu Sui, Zhaoyi Hou, Jason G. Fleischer, Shahrokh Golshan, Pam R. Taub, and Satchidananda Panda
Keywords: Shift-work, Time-Restricted Eating, Cardiometabolic Health

Shift work increases the risk of cardiometabolic disease, yet shift workers are often excluded from clinical studies to reduce this risk. As over 20% of the population is engaged in shift work, novel interventions are needed to reduce this risk. This randomized control trial assessed the feasibility of 10-hour time-restricted eating (TRE) in firefighters on 24-hour shifts and its impacts on cardiometabolic health. Participants completed a 2-week baseline/screening period and were randomized to a 12-week intervention of either (1) Standard of Care (SOC) of Mediterranean Diet counseling and allowed to eat ad libitum or (2) SOC and 10-hour Time-Restricted Eating (TRE). In the 137 firefighters who completed the study (13 Female, 23-59 years old), 10-h TRE was feasible, with TRE participants decreasing their eating window by 3 hours (baseline: mean 14.13h, 95% CI 13.78 to 14.47, intervention: 11.13h, 95% CI 10.73 to 11.54, p =3.29E-17). There were no adverse effects, yet the TRE group had a significantly better quality of life compared to the SOC group assessed via SF-36. The TRE arm also significantly decreased VLDL particle size compared to SOC (TRE: -1.34 nm, 95% CI -2.20 nm to -0.49 nm, SOC: -0.25 nm, 95% CI -0.86 nm to 0.36 nm, p=0.41, between groups p=0.044). In participants that had elevated cardiometabolic risks at baseline, the TRE arm had significant reductions compared to SOC in HbA1c (TRE: n=10, -0.21 %, 95% CI -0.55 % to 0.13%, SOC: n=11, 0.10 %, 95% CI -0.16 % to 0.036 %, between groups p=0.012), and diastolic blood pressure (TRE: n=9, -12.15 mmHg, 95% CI -17.56 mmHg to -6.73 mmHg, SOC: n=6, -3.77 mmHg, 95% CI -13.27 mmHg to 5.74mmHg, between groups p=0.033). For individuals working a 24-hour shift schedule, TRE is feasible and can improve cardiometabolic health, especially for individuals with increased risk.

49. Mechanical loading and hyperosmolarity as a daily resetting cue for skeletal circadian clocks

Main author: Michal Dudek
Affiliation: University of Manchester
Co-authors: Michal Dudek, Jayalath PD Ruckshanthi, Cátia F. Gonçalves, Beatriz Baño-Otálora, Craig Lawless, Dong Wang, Zhuojing Luo, Liu Yang, Farshid Guilak, Judith A Hoyland, Qing-Jun Meng

Keywords: entrainment, skeletal, mechanobiology, mTORC2

Light, temperature and feeding are among the most potent clock entrainment factors. Presence of niche-dependent physiological time cues has been proposed, allowing local tissues flexibility of phase adjustment. However, to date, such stimuli have remained elusive. Skeletal tissues such as the articular cartilage in joints and intervertebral discs of the spine are not innervated, do not possess direct blood or lymph supply and therefore are isolated from humoral clock synchronising factors. Existing evidence suggests that cartilage and intervertebral discs are highly rhythmic tissues experiencing a diurnal loading cycle, during which they are subjected to a prolonged period of compression (activity phase) followed by a period of low-load recovery (resting phase). Combining in vivo, ex vivo and in vitro methods in the form of mouse treadmill running, cartilage and disc explant culture and compression, primary chondrocyte assays and transcriptomics we show that cycles of mechanical loading and osmotic stimuli within physiological range drive rhythmic expression of clock genes and reset clock phase and amplitude in young and aged cartilage and intervertebral disc tissues. We identify the mTORC2-AKT-GSK3β pathway as a nodal point for mechano and osmo sensitive pathways regulating the circadian clock and leading to genome-wide induction of rhythmic genes. These results advocate mechanical loading and consequent daily surges in osmolarity as a bona fide tissue niche-specific time cue to maintain skeletal circadian rhythms in sync with animal's activity pattern and able to decouple the cartilage and disc clock from the central clock in the SCN.

50. In vivo characterization of candidate gene associated with Alzheimer's disease using Drosophila circadian rhythm and sleep assays

Main author: James Hodge
Affiliation: University of Bristol
Co-authors: Bangfu Zhu, Amy Preston, Edgar Buhl, James J. L. Hodge
Keywords: Circadian rhythms, Drosophila, Alzheimer's disease, sleep, clock neurons

Alzheimer's disease (AD) is the most common form of dementia and characterised by intracellular neurofibrillary tangles of hyperphosphorylated Tau and deposits of extracellular amyloid-beta (Aβ) plaques. This results in neurodegeneration, shortened life as well as disrupted circadian rhythms, sleep and memory. Recent genome-wide association studies (GWAS) and epigenome-wide association studies (EWAS) of AD have identified a number of potential genes associated with increased risk of AD. However, their causal role and contribution to disease pathology, remains largely unknown. We are using Drosophila as a system to in vivo screen through the top hits identified by EWAS or GWAS for AD, seeing which cause disease-relevant phenotypes. We employed a bioinformatic approach that prioritised novel genes nominated by GWAS and EWAS based on statistical significance, with human genes inputted to DIOPT, to identify the closest fly ortholog of the gene and targetable transgenic RNAi lines specific to the gene. We performed STRING analysis of protein-protein interactions and SCOPE analysis to probe progressive changes in gene expression in different neuronal and glia populations using the Drosophila single cell atlas of the ageing brain. Based on the expression pattern of the fly ortholog, promoter lines were selected to target expression RNAi. The effect of knockdown of the fly ortholog on neurodegeneration was quantified by expressing the RNAi in the eye and measuring photoreceptor degeneration comparing their effect to expression of human Tau 0N4R and Amyloid-Beta42. While the effect on longevity and progressive changes in locomotor behaviour was assessed by misexpression in all neurons or glia. Finally, candidate genes were also knocked down in clock and mushroom body neurons to determine their effect on circadian rhythms, sleep, memory, and neuronal excitability including their interaction with Tau and Amyloid pathways. Future work will screen for new drugs to the targets identified.

51. The peripheral muscle clock is not sufficient to prevent sarcopenia

Main author: Mireia Vaca-Dempere
Affiliation: Universitat Pompeu Fabra
Co-authors: -
Keywords: Skeletal muscle, sarcopenia, circadian rhythms

The peripheral muscle clock is not sufficient to prevent sarcopenia. Circadian rhythms are approximately 24h oscillations that can be found in all organisms at the molecular, physiological, and behavioral levels, which promote efficient organ function and systemic metabolic homeostasis. The function of most organs and tissues as well as their normal integrated behavior decline with aging, for largely unknown reasons. To ensure the correct functional coordination among different tissues, the suprachiasmatic nucleus (SCN) is considered the main synchronizer of all the peripheral behavior and clocks, although each tissue has its level of autonomy governed by the presence of local peripheral clocks. Skeletal muscle has an important role in the metabolic homeostasis of the body. During aging, muscles show a reduction in mass and force (sarcopenia), which is associated with disability, frailty, morbidity, and mortality. Mice deficient in the core clock machinery component Bmal1 (Bmal1-KO) show traits of premature sarcopenia, supporting a role of Bmal1 in skeletal muscle aging. However, Bmal1-KO mice do not provide information on which tissue clock is necessary to prevent the premature muscle aging phenotype. We studied the potential role of the skeletal muscle clock in preventing sarcopenia

in Bmal1-KO animals. By reconstituting Bmal1 expression in skeletal muscle, we found that the muscle autonomous clock is not sufficient to prevent the aging traits present in Bmal1-KO animals. These results indicate that distinct tissue clocks contribute to the homeostasis of skeletal muscle.

52. Determining the membrane circadian clock across evolution.

Main author: Edgar Buhl
Affiliation: School of Physiology, Pharmacology and Neuroscience, University of Bristol, UK
Co-authors: Mino Belle, Krasimira Tsaneva-Atanasova, Hugh Piggins, James Hodge

Keywords: daily electrical rhythm, electrophysiology, neuronal modelling, ion channels, ageing

Endogenous circadian rhythms are found in all lifeforms, from single cell organisms to plants, insects and mammals, including humans. They exist at the molecular, cellular and electrophysiological level allowing physiology and behaviour to be optimally aligned to 24h cycles of light and dark as well as seasons. Our health and wellbeing depend on appropriately timed and phased circadian rhythms. Ageing is known to cause daily rhythms in physiology and behaviour to dampen and fragment causing poor health and sleep in the elderly by mechanisms which appear conserved with rodents and flies. The molecular clock consists of clock genes, which are rhythmically expressed in clock neurons controlling the circadian expression of genes such as ion channels. Therefore, the molecular clock is coupled to neuronal electrical activity. Indeed, in clock neurons of mammals and flies, the molecular clock drives daily changes in electrical activity (referred to as the membrane or electrical clock) vital for communicating time-of-day information to the brain and body. Intriguingly, suppressing daily electrical activity of clock neurons with selective genetic and pharmacological modulators effectively stops the molecular clock and compromises behavioural rhythms. Thus, there are two interacting and mutually dependent timekeepers in clock neurons, one intracellular and molecular and the other in the membrane and electrical. Of these, the molecular clock is well-researched and understood, but there is a paucity of research and knowledge of the electrical timekeeper. Here we try to determine the components and mechanism of the membrane clock using flies, mouse and in silico models testing the hypothesis that there is a conserved set of ion channels that generate daily electrical variations in fly and mouse clock neurons. We believe this membrane clock may become weaker during ageing compromising circadian rhythms and the individual's health span, and we test this hypothesis using computational and Drosophila modelling.

53. Malaria parasite development is rhythmic and is synchronized with host feeding-fasting rhythms: How? Why? Huh?

Main author: Aidan O'Donnell
Affiliation: The University of Edinburgh
Co-authors: Sarah Reece

Keywords: Malaria, infection, rhythms, parasite, fitness

The roles of daily rhythms in infections are gaining recognition because explaining the regulatory mechanisms and fitness consequences of biological rhythms exhibited by parasites and hosts offers new avenues to treat infections. Malaria (Plasmodium) parasites exhibit ~24h developmental rhythms during replication in the mammalian host's blood and during transmission to insect vectors. The survival and transmission of malaria parasites is determined by whether these developmental rhythms are synchronised to the host's circadian rhythms, but how periodicity in these parasite traits is generated and maintained during infection is poorly understood. We address this using rodent malaria (Plasmodium chabaudi) infections of wild type (WT) and arrhythmic clock mutant (Per1/2 double knock

out) mice. We compare parasite and host rhythms in WT mice kept in LD and DD, with rhythms observed in mutant mice in DD. We use the mutant mice and a restricted feeding regime to decouple host rhythms in feeding from body temperature and locomotor activity and examine the consequences for parasite rhythms. Finally, we apply a 'phase-shift' to parasites and track the parasite schedule for over ten cycles to determine how they resynchronise with host rhythms. We show that (i) parasite rhythms match the phase of the host's feeding-fasting rhythm and not the phase of rhythms in activity or body temperature; (ii) the timing of the parasite replication cycle is independent of the canonical 'core' host clock; (iii) following perturbation, parasites reschedule to regain synchrony with the timing of the host's rhythm within 7 replication cycles by speeding up the replication rhythm by 2-3 hours per cycle. We discuss how it is beneficial for parasites to be in synchronization with their host's feeding-fasting rhythms and plasticity in their development duration facilitates this synchrony by enabling parasites to make small daily changes to their schedule when necessary.

54. Co-expression of diurnal and ultradian rhythms in the plasma metabolome of common voles (Microtus Arvalis)

Main author: Daan R Van der Veen
Affiliation: Chronobiology Section, Faculty of Health and Medical Sciences, University of Surrey, Guildford, UK
Co-authors: Andreas Psomas, Namrata R. Chowdhury, Benita Middleton, Raphaelle Winsky-Sommerer, Debra J Skene, Menno P Gerkema

Keywords: Ultradian, Diurnal, Metabolomics, Microtus, Entrainment

Metabolic rhythms include rapid, ultradian (hourly) dynamics, but it is unclear what their relationship to circadian metabolic rhythms is, and what role meal timing plays in coordinating ultradian metabolism. Here, for the first time, we characterised widespread ultradian rhythms in the plasma metabolome of the vole, an animal naturally expressing ~2-hour foraging rhythms throughout the day and night. These ultradian metabolite rhythms were co-expressed with diurnal 24 h rhythms in the same metabolites and did not align with food intake patterns. Under light-dark entrained conditions, we showed that an ultradian behavioural phase response curve to light-dark transitions synchronises vole ultradian behavioural patterns to diurnal light-dark cycles twice a day, and that these entrained ultradian behavioural patterns drive an ultradian eating pattern. We next used a unique approach to map this behavioural activity/feeding status to high temporal resolution (every 90 minute) measures of plasma metabolite profiles across the 24-hour light-dark cycle. A total of 148 known metabolites were detected in vole plasma. Supervised, discriminant analysis did not group metabolite concentration by feeding status, instead, unsupervised clustering of metabolite time courses revealed clusters of metabolites that exhibited significant ultradian rhythms with periods different from the feeding cycle. Two clusters with dissimilar ultradian dynamics, one lipid-enriched (period = 3.4 h) and one amino acid-enriched (period = 4.1 h), both showed co-expression with diurnal cycles. A third cluster was entirely comprised of glycerophospholipids, and expressed an 11.9 h ultradian rhythm without any diurnal rhythmicity. Our findings show coordinated co-expression of diurnal metabolic rhythms with rapid dynamics in feeding and metabolism, exposing that ultradian rhythms are integral to biological timing of metabolic regulation, and will be important in interpreting the impact of circadian desynchrony and meal timing on metabolic rhythms.

55. Clocks over two timescales: how is the clock rewired during ageing in Arabidopsis thaliana?

Main author: Ethan Redmond
Affiliation: Department of Biology, University of York, UK
Co-authors: Prof Seth Davis (1), Dr James Ronald (2), Dr Daphne Ezer (1)
(1): Department of Biology, University of York, UK
(2): Institute of Molecular, Cell and Systems Biology, College of Medical, Veterinary and Life Sciences, University of Glasgow, UK
Keywords: Plant clock, photoperiodic pathway, flowering, mathematical modelling, Arabidopsis thaliana, RNA-seq, omics

The plant circadian clock is linked to development on every scale, from control of the cell cycle to determination of flowering time. The photoperiodic pathway is a key example of this link since changes in day length must be interpreted accurately to maximise plant fitness. Circadian regulation of this pathway is generally studied in seedlings. This method allows researchers to apply standard circadian experimental designs to plants, such as release to free running conditions after entrainment. However, this approach fails to identify links between the circadian clock and other ageing-related processes, such as senescence.

In order to unravel the connections between the clock and ageing, we collected transcriptomic data from Arabidopsis thaliana at two different timescales: the 'developmental' timescale and the 'diurnal' timescale. For this, we synchronised the development of the plants and induced flowering, by shifting the photoperiod from short days to long days. We then sampled across the whole 24h at 2, 7, and 12 days after this photoperiod shift.

Using this approach, we can utilise information about the amplitude or phase of gene expression across each day. For example, senescence-associated genes have increased amplitude soon after the photoperiod shift. In terms of phase, many genes shift their time of peak expression, suggesting a widespread rewiring of the circadian clock's targets during the flowering transition. Further, we demonstrate how methods used in this project may be applied to similar datasets involving two timescales. These methods could be used to gain a deeper understanding of the age-varying effects of the circadian clock on diet and health in humans.

56. Sterols act on circadian expression of Wnt signalling but not on RORC signalling

Main author: Cene Skubic
Affiliation: [1]University of Ljubljana, Faculty of Medicine, Institute for Biochemistry and molecular biology
[2]Faculty of Computer and Information Science, University of LjubljanaCo-authors: Cene Skubic [1], Hana Trček [1], Tadeja Režen [1], Miha Mraz[2], Miha Moškon[2], Damjana Rozman[1]
Keywords: sterols, WNT-signnaling, RORC, circadian clock

The synthesis of cholesterol is a complex process composed of more than 20 reactions and is regulated also by the circadian clock. In the post-lanosterol part of cholesterol synthesis at least 13 different sterols are formed. Certain sterols between lanosterol and cholesterol were shown to be agonists of the RAR Related Orphan Receptor C (RORC) in immune cells. RORC is controlled by and itself controls the circadian genes (NPAS2, CLOCK, REV-ERB). Wnt signalling is another pathway shown to be affected by sterol molecules and interconnects the circadian clock with the cell cycle. The focus of our work was to determine the effect of different sterol molecules that accumulate in liver cells, on RORC signalling, Wnt-signalling, and the expression of core clock genes.

We used the liver cell line HepG2 with knocked-out (KO) genes from cholesterol synthesis (CYP51 KO, DHCR24 KO and SC5D KO). Due to depletion of enzyme activity, the substrates (upstream sterols) accumulate while the products (downstream sterols) are diminished. Single time point transcriptome of KO cell lines was obtained using microarrays to evaluate changes in gene expression and signalling pathways (KEGG and TransFac). To evaluate circadian changes, a 52h sampling of cells was done every 2h and targeted genes (core clock, Wnt and RORC signalling genes) were measured by qPCR. Circadian expression data was firstly pre-processed to remove MESOR accumulation, and then single component cosinor model was used to assess the rhythmicity of each gene and differential rhythmicity among selected pairs of genes. The period was fixed to 24 hours.
Even though we knocked out genes in the same metabolic pathway of cholesterol synthesis, transcriptome data showed large differences in differential expression of genes, which depended on accumulated sterols. There was no indication of expression changes in RORC targeted genes. On contrary, the Wnt signalling was changed highly in CYP51 KO, moderately in DHCR24 KO, but not in SC5D KO, which indicates a specific role of individual sterols on the Wnt pathway. PER2 gene was phase advanced in DHCR24 KO and phase delayed in SC5D KO compared to the native cells. RORC expression was not rhythmic at all. In contrast, DKK gene from Wnt signalling was rhythmic in all KOs with higher amplitudes compared to controls and was phase advanced. Interesting is also LEF1 (Wnt signalling gene) that showed circadian behaviour with high amplitude only in the CYP51 KO. In conclusion, our results do not confirm the sterol-RORC-circadian clock connection in hepatocellular carcinoma HepG2 cells but clearly indicate the role of different sterols in circadian regulation of Wnt signalling.

57. Gastrin-releasing peptide-producing neurons in the suprachiasmatic nucleus play an essential role in regulating behavioral and molecular circadian rhythms

Main author: Ruth Li
Affiliation: University of Tsukuba
Co-authors: Yoan Cherasse, Ran Inoue, Hisashi Mori, Arisa Hirano, Takeshi Sakurai

Keywords: GRP, SCN, behavioral rhythm, PER2, GABA, VIP

In mammals, the hypothalamic suprachiasmatic nucleus (SCN) is the master clock that regulates circadian rhythms. The SCN contains distinct subtypes of neurons expressing different neurotransmitters, such as gamma-aminobutyric acid (GABA), arginine vasopressin (AVP), vasoactive intestinal peptide (VIP), and gastrin-releasing peptide (GRP). In this study, we focused on unraveling the role of GRP-producing neurons in regulating circadian rhythm. We used the Grp-iCre knock-in (KI) mice and Cre-dependent adeno associate virus (AAV) to specifically manipulate SCN GRP-producing neurons. We traced the projections of these neurons and discovered that they mostly project to the thalamus and hypothalamus and receive inputs from within the SCN. When we inhibited the SCN GRP-producing neurons with tetanus toxin light chain (TeNTLC), mice displayed drastically attenuated behavioral rhythm. When we examined the core clock protein expression rhythm within the SCN of these mice, we discovered that the oscillation amplitude of PER2 expression was significantly smaller than in control mice. We next wanted to determine the key factor in the SCN GRP-producing neurons that is responsible for the observed phenomena, and the likely candidates were GRP, GABA, and VIP. We examined the behavioral rhythm in GRP deficient mice, but these mice exhibited relatively normal activity rhythm. We generated mice with vesicular GABA transporter (VGAT), a protein necessary for GABA release in the synapse, specifically knocked-out in the GRP-producing cells. These mice exhibited slightly but significantly lower Qp value of activity rhythm in DD compared to the control mice. We then specifically inhibited VIP production in the GRP-producing neurons by inducing the expression of VIP shRNA. These mice also displayed attenuated Qp value of behavioral rhythm in DD. We concluded that the SCN GRP-producing neurons are crucial for sustaining

behavioral and SCN molecular rhythm, and GABA and VIP in the GRP-producing neurons may both mediate rhythm expression to an extent.

58. Individual differences in subjective mood response to combined sleep restriction and circadian misalignment

Main author: Rebecca Cox
Affiliation: University of Colorado Boulder
Co-authors: Taylor E. Marshall, Dana Withrow, Hannah K. Ritchie, Kate E. Sprecher, Tina M. Burke, Alexandra N. Smits, Oliver A. Knauer, Molly K. Guerin, Ellen R. Stothard, Christopher M. Depner, Kenneth P. Wright, Jr.

Keywords: mood; sleep restriction; circadian misalignment

Previous research indicates sleep loss negatively impacts mood, and circadian misalignment may have similar adverse effects. For example, simulated shiftwork has been shown to decrease happiness and subjective well-being, and recent evidence suggests that combined sleep restriction and circadian misalignment (SR+CM) decreases positive affect. The present study sought to examine the impact of SR+CM on multiple subjective mood states.

Twenty healthy adults participated in a 39-day SR+CM protocol with 2 in-laboratory visits. The following protocol was repeated twice: habitual 8h sleep schedules at home for 2 weeks followed by a 4-day in-laboratory protocol with 4 sleep opportunities (8h on night 1, 3h on night 2, and 3h on days 3 and 4 [daytime sleep with nighttime wakefulness]). Ratings on 20 mood items were collected every 3h during scheduled wakefulness. Three factor scores representing fatigue, distress, and calm were extracted.

There were significant main effects of time ($P<0.05$) on fatigue, distress, and calm, such that all 3 mood states increased with sustained wakefulness and during biological night. Effects of SR+CM on mood were consistent between laboratory visits (ICCs .74-.91).

These findings support prior work showing detrimental effects of circadian misalignment on mood and extend these findings to include combined SR+CM and multiple mood states. Mood impairments may be trait-like features of cognitive deficits observed during combined SR+CM.

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59. The effect of estrogen on coupling in the SCN via gap junctions

Main author: Violetta Pilorz
Affiliation: University of Luebeck, Germany
Co-authors: Lina Schlaeger, Henrik Oster, Violetta Pilorz* corresponding author

Keywords: coupling, SCN, astrocytic and neuronal gap junctions, estrogen, female mice

The timing of many behaviours and physiological functions is controlled by the central clock in the suprachiasmatic nucleus (SCN) in hypothalamus. The basic mechanism that generates these patterns is the intrinsic rhythm of the individual cells. Circadian rhythms of rest and activity are extremely precise with day-to-day variations. This is achieved through coupling of individual circadian oscillations.

Gap junctions consisting of connexins (Cx) play an essential role in coupling within the SCN. The presence of estrogen-response-elements (EREs) in gap junctions suggests the modulatory effect of E2 on coupling in the SCN. To delineate the role of E2 in the SCN coupling, we assessed the activity of the neuronal and astrocytic gap junctions in the SCN in presence and absence of E2 in PER2::LUCIFERASE female mice. Using SCN explants we show that dampening of the PER2::LUC amplitude induced by antagonists for arginine vasopressin receptors can be prevented by E2. This prevention of amplitude reduction, however, cannot be accomplished by inhibiting neuronal Cx36 or astrocytic Cx43. Indeed, E2 can restore period length when combined with inhibition of Cx36, but not when Cx43 is inhibited.
This innovative finding, we confirmed by assessment of RNA expression in intact females with high estrogen levels and in

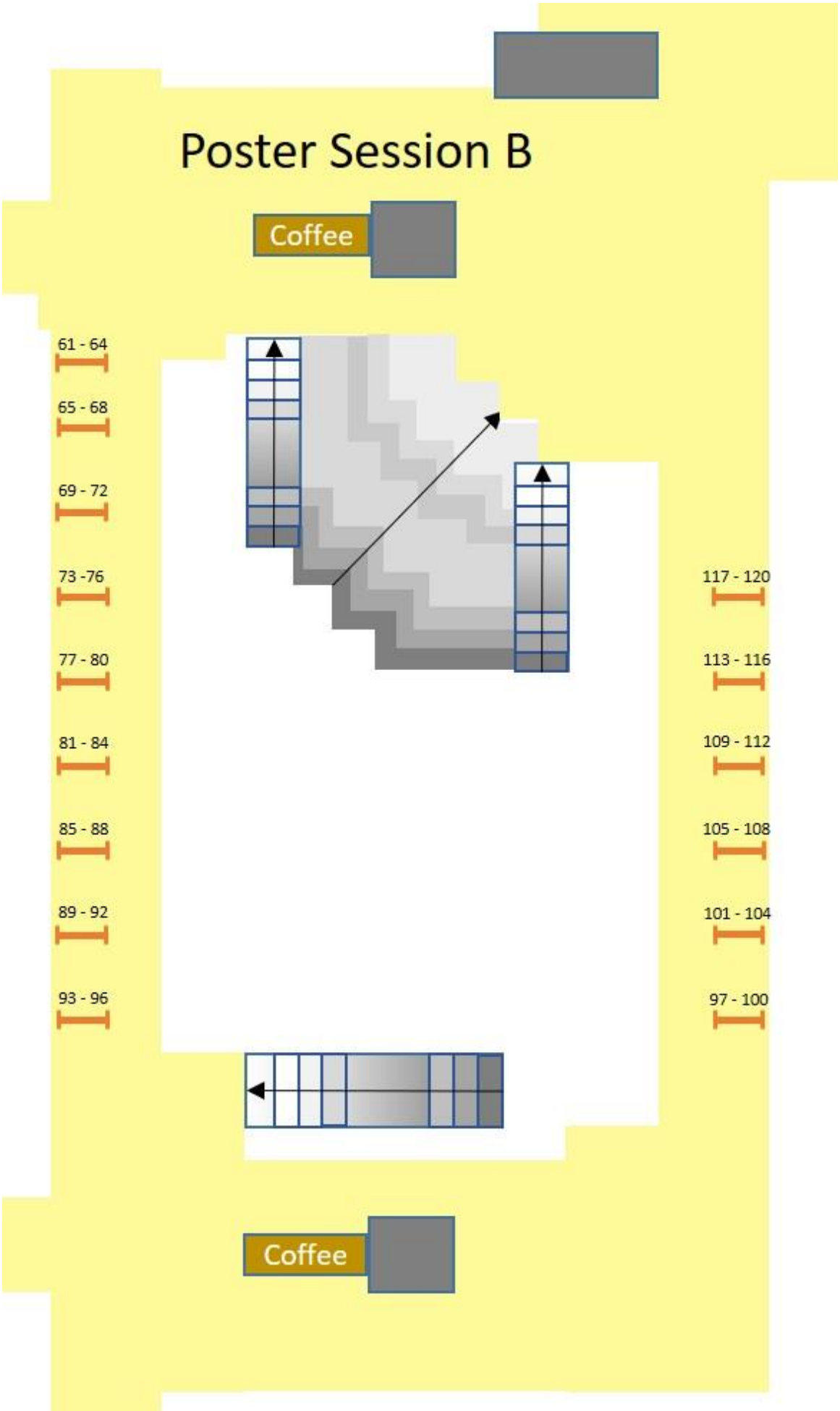
60. Post-transcriptional regulation of the proteome

Main author: Holly Kay
Affiliation: University of Edinburgh
Co-authors: Helen Feord, Ellen Grunewald, Babette Vlieger, Sergio Gil, Liangcui Chu, Sew Peak Chew, Alessandra Stangherlin, John O'Neill, Sander Granneman, Gerben van Ooijen

Keywords: RNA binding, metabolism, proteomics, organelle,

In order to predict and anticipate daily cycles in the environment, the cellular proteome must change dramatically over the 24h day. To assess the relative contributions of diurnal versus circadian regulation, we generated a deep-coverage proteome of Ostreococcus tauri under 12h:12h light:dark cycles compared to constant light conditions. The overlap between diurnally- and circadian-regulated proteins was small. Interestingly, transcript rhythmicity was poorly predictive of protein abundance rhythms. We therefore sought to obtain a circadian RNA-binding proteome to investigate temporal post-transcriptional regulation. Alongside the anticipated ribosomal and known RNA-binding proteins, we identified some key metabolic enzymes that rhythmically bind RNA in constant conditions. Post-transcriptional regulation by moonlighting metabolic enzymes must therefore be considered as a potential link reciprocally connecting metabolism to the circadian clock.

Poster Session B – Tuesday, 26.07.2022



Abstracts to poster session B on Tuesday 26.07.2022

61. Retinal organoids: The model to study the effects of light on the retinal circadian rhythms

Main author: Kamila Weissova
Affiliation: [1)]Institute of Animal Physiology and Genetics CAS, Veveří 97, 602 00 Brno, Czech Republic
[2)]Department of Histology and Embryology, Faculty of Medicine, Masaryk University, Kamenice 3, 62500 Brno, Czech Republic
Co-authors: Kamila Weissová[1,2], Jana Šebestíková[2], Canan Celiker[2], and Tomáš Bárta[1,2]

Keywords: retinal organoids, circadian rhythms, light

The circadian clocks have been deeply studied at the broad level of different peripheral tissue in various animal models. Nevertheless, extrapolation of results generated on animal models to humans has been always questionable. However, organoids derived from human pluripotent stem cells are unique, in that they are self-organizing 3D culture systems that are highly similar to human organs. But did they also develop circadian rhythms as the human's peripheral oscillators?
Human retina represents highly independent circadian oscillator in the circadian clock's hierarchy. As the light sensitive organ, it does not require synchronization inputs from suprachiasmatic nucleus, it has its own circadian machinery that can be entrained with light-dark cycles using its own neuro-humoral signals.
In this study, we use retinal organoids derived from human pluripotent stem cells as a new model to study retinal circadian rhythms. Retinal organoids can develop well-structured light sensitive neuronal retina that contains all of the retinal cell types. Using our unique in-house-developed light stimulation device we show that the exposure of retinal organoids to the light can synchronize the expression of crucial genes that are coupled to the circadian clock machinery as well as the presence of some of the retinal clock modulators. Our data shows that even in vitro developed retina display circadian oscillation that responds to light stimuli.

This study was supported by the Czech Science Foundation (GA21-08182S, GA21-05146S) the Grant Agency of Masaryk University (GAMU) - MUNI/G/1391/2018

62. RNA-sequencing unveils nuclei-specific patterns of transcription in seasonal siberian hamsters

Main author: Calum Stewart
Affiliation: University of Glasgow
Co-authors: Tyler Stevenson, Christopher Marshall

Keywords: -

Seasonal rheostatic changes in energy balance and reproductive status are common physiological programs used by animals to optimize fitness. Photoperiod-dependent changes in physiology are orchestrated by the hypothalamus, and communicated peripherally, in part, by pituitary signalling. In this study Siberian hamsters, a small seasonal rodent, were taken (n = 54) and held under either long photoperiod (LP; 16L:6D) (n = 6) or short photoperiod (SP; 8L:16D) (n = 48) for up to 32 weeks. At 4-week intervals, groups of SP animals (n = 6) were sacrificed, and brain and pituitary were extracted immediately. Body mass ($P < 0.05$), food intake ($P < 0.05$), testes mass ($P < 0.05$) and body temperature ($P < 0.05$) decreased after exposure to SP prior to recovering spontaneously after 20 weeks of SD exposure. Oxford nanopore sequence was used to generate transcriptomic data of pituitary, arcuate nucleus, and dorsomedial nucleus of the hypothalamus. BioDare2.0 bioinformatic

analyses identified several transcripts which were significantly rhythmic across the experiment (FDR < 0.05). Gene ontology analysis revealed different terms within the Arcuate, DMH, and pituitary gland with ‘heat shock proteins’, ‘membrane’ and ‘alternative splicing’ being the top enriched terms, respectively. This study has generated high resolution transcriptomic data from multiple neuroendocrine structures from a seasonal animal and identified high region-specific patterns for circannual interval timing. The findings reported here suggest that seasonality is orchestrated through dynamic changes occurring in individual nuclei which act together to generate the overall seasonal rhythm in physiology and behaviour.

63. Chronotype, sleep quality, and shift work preference among nurses

Main author: Linske de Bruijn
Affiliation: Netherlands Cancer Institute, Amsterdam
Co-authors: Vermeulen, R.C.H., Institute for Risk Assessment Sciences, Division of Environmental Epidemiology, Utrecht
Kromhout, H., Institute for Risk Assessment Sciences, Division of Environmental Epidemiology, Utrecht
Vlaanderen, J.J., Institute for Risk Assessment Sciences, Division of Environmental Epidemiology, Utrecht
Berentzen, N.E., Netherlands Cancer Institute, Department of Epidemiology, Amsterdam
Van Leeuwen, F.E., Netherlands Cancer Institute, Department of Epidemiology, Amsterdam
Schaapveld, M., Netherlands Cancer Institute, Department of Epidemiology, Amsterdam

Keywords: Shift work; night work; nurses; chronotype; sleep quality; circadian alignment; circadian rhythm

Background: Night shift work has been associated with an increased risk of type 2 diabetes mellitus and cardiovascular diseases due to circadian rhythm disruption. Chronotype (i.e. a marker for shift work tolerance) may modify these risks. This study assesses the associations of chronotype with shift type preference and sleep quality among nurses currently exposed to night work.
Methods: The current study used data from 37,731 Dutch female nurses (mean [SD] age: 53.8 [10.7] years) who participated in the Nightingale Study and completed a questionnaire including night work exposure, shift type preference, and sleep quality. Sleep quality in the last four weeks was assessed as a proxy of circadian disruption using the Medical Outcomes Study (MOS) sleep scale. Sleep quality and night shift preference (1-7, least to most preferred) were compared between chronotypes using one-way ANOVA in current night shift workers.
Results: Among nurses who never worked night shifts, 44.8% reported being morning types whereas 30.3% of the current night workers (n=7,626) considered themselves morning types. Within current night workers, those who considered themselves as evening types reported a higher preference for night shifts than intermediate types (4.3 vs. 3.9, $p<0.01$) but also reported higher MOS scores (32.9 vs. 29.4, $p<0.01$), indicative for poorer sleep quality. Morning types reported a lower night shift preference score and lower sleep quality than intermediate types (3.3 vs. 3.9, $p<0.01$; 30.8 vs. 29.4, $p<0.01$, respectively).
Discussion: This study shows that evening types more frequently worked night shifts and reported a higher night shift preference than intermediate and morning types, potentially indicating higher tolerance to night work. However, sleep quality was still lowest among evening types, suggestive of circadian disruption. This study highlights the importance of including chronotype as a potential effect modifier in studies of shift work as an exposure.

64. Evolution of the sensory inputs to the circalunar clock of Clunio marinus

Main author: Dušica Briševac
Affiliation: Max Planck Institute for Evolutionary Biology, August-Thienemann-Str. 2, 24306 Plön, Germany
Co-authors: Dušica Briševac, Tobias S. Kaiser
Keywords: biological clocks, moonlight, sensory nervous system, mechanotransduction, QTL mapping, whole-genome sequencing, development

The endogenous circalunar clock controls the development of the marine midge Clunio marinus. The clock is synchronized by moonlight and tidal cycles of mechanical agitation. We investigated the hierarchy of the two zeitgebers by applying them simultaneously while varying their phase relationship. The results showed that although there is some integration of the two, moonlight is the dominant zeitgeber.
Interestingly, two out of ten investigated lunar-rhythmic populations have lost the sensitivity to mechanical agitation, and one has lost the sensitivity to moonlight. Crossing experiments between insensitive and sensitive strains revealed that sensitivity to a zeitgeber is a genetically determined and a dominant trait. We aimed to study the genetic basis for this loss and discover the molecular components of the sensory inputs. Our approach was to combine quantitative trait loci (QTL) mapping and genome-wide screens of the ten differentially sensitive populations. QTL mapping suggested an oligogenic origin with one prevalent additive locus in two out of three insensitive strains. Genome-wide analysis brought to light a few candidate genes with known functions in the development of the sensory and central nervous system as well as light perception in the moonlight-insensitive strain. Our results support the notion that adaptive phenotypes have a complex genetic basis with mutations occurring at several loci. Nevertheless, by carefully screening for the most consistent signals, we were able to start unveiling the potential components of the sensory machinery.
As the timing of the tides varies in Clunio habitats, and with it the relationship of the zeitgebers that have an intricate hierarchy in the entrainment of the clock, we suggest that Clunio populations adapt locally to their timing niches by modulating their sensory inputs to detect the most informative zeitgeber.

65. Associations between night shift work and weight change among nurses within the prospective Nightingale Study

Main author: Henriëtte van Duijne
Affiliation: Netherlands Cancer Institute
Co-authors: Prof. dr. R.C.H. Vermeulen, Prof. dr. H. Kromhout, Dr. J. J. Vlaanderen, Dr. M.A. Rookus, Dr. N.E. Berentzen, Prof. dr. F.E. van Leeuwen, Dr. M. Schaapveld
Keywords: Night shift work, Weight change, BMI change, overweight, obesity

Introduction: While in many occupations night shift work is an integral part of the job, it has been associated with increased risk of diabetes and cardiovascular disease. These risks may be (partly) explained by weight gain following exposure to night work. This study aimed to prospectively examine associations of night shift work exposure with weight change.
Methods: The Nightingale Study, a prospective cohort study among 59,947 Dutch female nurses, was designed to study adverse health outcomes associated with night shift work. At baseline (2011), participants (median age 49 years) completed a questionnaire including job history and detailed assessment of night work. A follow-up questionnaire (2017), was completed by 63% of the cohort

(n=37,470). Associations of night work with weight change (kilogram) and development of overweight/obesity (≥25kg/m2) between 2011 and 2017 were assessed using linear and logistic regression analyses, adjusted for age.
Results: Women who never worked night shifts on average gained 3.5 kg (sd 5.5) during follow-up, while women who ever worked night shifts gained 3.6 kg (sd 5.8) (p=0.16). There was no trend in weight gain over categories of night work duration. Ever night workers had a statistically non-significant 10% higher risk of developing overweight/obesity than never night workers. The risk of developing overweight/obesity was slightly increased with longer duration of night work (OR 1.1 (95% CI 1.0-1.3) for 10-19 years and OR 1.3 (95% CI 1.1-1.5) for 20+ years).
Conclusion: Weight gain and risk of developing overweight/obesity in ever night workers and those with longer night work duration was not substantially increased after 6 years of follow-up compared with never night workers. Our findings may contribute to understanding mechanisms underlying health risks resulting from night shift work

66. Life beyond transcription: circadian rhythms in alternative splicing in Arabidopsis thaliana

Main author: Andres Romanowski
Affiliation: 1) Comparative Genomics of Plant Development, Fundación Instituto Leloir, Instituto de Investigaciones Bioquímicas Buenos Aires (IIBBA) – Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), C1405BWE, Buenos Aires, Argentina.
Co-authors: Rubén G Schlaen(1), Soledad Perez-Santangelo(1), Estefanía Mancini(1), Marcelo J Yanovsky(1).

Keywords: Arabidopsis thaliana, SPF30, alternative splicing, circadian clock, circadian rhythms, splicing factors

The circadian clock of Arabidopsis thaliana controls many physiological and molecular processes, allowing plants to anticipate daily changes in their environment. However, developing a detailed understanding of how oscillations in mRNA levels are connected to oscillations in co/post-transcriptional processes, such as splicing, has remained a challenge. Here we applied a combined approach using deep transcriptome sequencing and bioinformatics tools to identify novel circadian-regulated genes and splicing events. Using a stringent approach, we identified 300 intron retention, eight exon skipping, 79 alternative 3' splice site usage, 48 alternative 5' splice site usage, and 350 multiple (more than one event type) annotated events under circadian regulation. We also found seven and 721 novel alternative exonic and intronic events. Depletion of the circadian-regulated splicing factor AtSPF30 homologue resulted in the disruption of a subset of clock-controlled splicing events. Altogether, our global circadian RNA-seq coupled with an in silico, event-centred, splicing analysis tool offers a new approach for studying the interplay between the circadian clock and the splicing machinery at a global scale. The identification of many circadian-regulated splicing events broadens our current understanding of the level of control that the circadian clock has over this co/post-transcriptional regulatory layer.

67. Disruption of daily rhythms in metabolic pathways and energy metabolism by dim light at night in rats

Main author: Valentina Rumanova
Affiliation: Department of Animal Physiology and Ethology, Faculty of Natural Sciences, Comenius University, Bratislava, Slovakia
Co-authors: Monika Okuliarova, Ewout Foppen, Andries Kalsbeek, Michal Zeman
Keywords: metabolism, light at night, peripheral clocks, metabolic sensors, daily rhythms

Negative effects of dim artificial light at night (ALAN) on metabolism have been reported, but underlying mechanisms and disruption of daily rhythms are poorly documented. Therefore, in our study, we investigated effects of ALAN on energy metabolism and the expression of clock and metabolic genes. We exposed adult male rats to the ALAN (~ 2 lx) during the whole night (12L:12DL) for 2 weeks, and the control group was kept in the 12L:12D. Samples of blood, liver and epididymal adipose tissue were collected in 4-hour intervals over 24 hours. The locomotor activity displayed smaller peak during the daytime and dampened the peak at the beginning of night in the ALAN group as compared to controls. Elevated carbohydrate and decreased lipid oxidation mirrored changes in food intake during the daytime. Plasma metabolites (glucose, triacylglycerols, cholesterol) lost their rhythmic pattern and cholesterol levels were increased. The rhythms of hepatic metabolic sensors, interacting with the clockwork, were either phase-advanced (Ppara, Pgc1a, Nampt) or lost rhythmicity (Sirt1, Lxra) after ALAN. In addition, Ppara and Sirt1 expression became arrhythmic in the adipose tissue. Hepatic oscillations of Glut2 were phase-advanced and transcription factor Foxo1 lost its rhythmicity, probably contributing to the diminished amplitude of hepatic glycogen rhythm. Gene expression patterns in lipid metabolism showed either phase-advanced rhythms, arrhythmicity or even gained rhythmicity under ALAN regime. Clock genes in the liver and adipose tissue preserved their robust rhythmicity but showed shifts in the acrophase. In conclusion, ALAN profoundly disturbed daily rhythms of genes encoding key metabolic sensors and components of synthesis and degradation metabolic pathways. These changes were driven not only by shifted peripheral clocks, but the major role can be attributed to the eliminated rhythms in plasma metabolites, altered daily pattern of food intake and energy metabolism. Supported by APVV-17-0178 and VEGA 1/0492/19.

68. Infradian rhythms in cerebral oxygenation and blood volume in humans at rest: A 5 year-long study

Main author: Felix Scholkmann
Affiliation: University of Zurich, University of Bern
Co-authors: H. Zohdi, M. Wolf, U. Wolf
Keywords: Near-infrared spectroscopy, cerebral oxygenation, blood volume

Background: All parameters of human physiology show chronobiological variability. While circadian (cycle length ~24 h) rhythms of the neuronal, hemodynamic and metabolic aspects of human brain activity are increasingly being explored, infradian (cycle length > 24 h) rhythms are largely unexplored.
Aim: We investigated if cerebral tissue oxygen saturation (StO2) and blood volume ([tHb]) values measured over many years in many subjects during resting show infradian rhythmicity.
Subjects and methods: Absolute StO2 and [tHb] values (median over a 5 min resting-phase while sitting) were measured in 220 healthy subjects (age: 24.7 ± 3.6 years, 87 males, 133 females) 2 –4 times on different days over the right and left frontal lobe (FL) and occipital lobe (OL) by employing frequency-domain NIRS as part of different systemic physiology augmented functional near-infrared spectroscopy, SPA-fNIRS, studies. The data set consisted of 708 single measurements performed

over a time-span of 5 years (2017–2021). General additive models (GAM) and cosinor modelling were used to analyse the data.
Results: The GAM analysis revealed (i) a non-linear trend in the StO2 and [tHb] values over the 5-year-span, (ii) a circannual (cycle length ~12 months) rhythm in StO2 at the FL (amplitude (A): 3.4%, acrophase (φ): June) and OL (A: 1.5%, φ: May) as well as in [tHb] at the OL (A: 1.2 µM, bathyphase (θ): June), and (iii) a circasemiannual (cycle length ~6 months) rhythm in [tHb] at the FL (A: 2.7 µM, φ: March and September, respectively).
Discussion: We conclude that absolute values of StO2 and [tHb] show chronobiological variability on the group-level with a nonlinear long-term trend as well as circannual/circasemiannual rhythmicity. These rhythms need to be taken into account when defining reference values for StO2 and [tHb] and may correlate with the variability of cerebrovascular disease incidents over the year.

69. The developmental clock of C. elegans constitutes a rhythmic (phospho)proteome

Main author: Abhishek Upadhyay
Affiliation: Friedrich Miescher Institute for Biomedical Research (FMI), Basel, Switzerland
Co-authors: Abhishek Upadhyay[1,2], Maike Graf[1], Kathrin Baum[1], Dimosthenis Gaidatzis[1], Vytautas Iesmantavicius[1], Helge Grosshans[1,2]
[1]Friedrich Miescher Institute for Biomedical Research (FMI), Basel, Switzerland
[2]Corresponding authors

Keywords: Developmental clock, C. elegans, Molting cycle, Genetic oscillator, Phosphoproteomics

Many developmental processes repeat rhythmically. For example, rhythmicity in somite formation is ensured through a ‘clock’ mechanism where a Hes transcription factor inhibits its own transcription, yielding oscillatory activity. To what degree such genetically-encoded oscillators function in other developmental contexts is unknown. However, >20% of the transcriptome (~3700 genes) of the roundworm Caenorhabditis elegans accumulates rhythmically during larval development. Molecular genetic approaches have identified a set of transcription factors as putative core clock genes, including orthologues of circadian clock genes. Intriguingly, circadian protein phosphorylation plays an important role in setting the 24 h period. Moreover, nonlinear dynamical theory suggests that simple transcriptional feedback loops would not suffice to generate oscillations of the experimentally observed, 8-hour period. Hence, I proposed phosphorylation to be an additional key component of C. elegans oscillator. A synchronized population of worms were sampled hourly over the course of two cycles. To investigate whether and to which extent kinases and phosphatases contribute to the clock, the high throughput time course experiment was performed using 16-plex TMT-labelling combined with phosphopeptide enrichment and mass spectrometry (LC-MS/MS). Our quantitative data analysis (machine learning methods) discovered an extensively dynamic developmental (phospho)proteome. Notably, we identified 7924 proteins and 2553 phosphoproteins (10% and 5% of the proteome and phosphoproteome, respectively rhythmic). In addition, 10,602 phosphosites originating from 10,424 peptides were found to be phosphorylated. While temporal dynamics of marker proteins (GRH-1, LIN-42 etc) recapitulated the developmental clock, amplitude and phase analysis revealed potential hits. Furthermore, unbiased genetic screening-based approaches and mutational experiments will help characterize clock kinases and functionally relevant phosphosites, respectively. Our findings may provide insight into a developmental clock whose principle, recent findings suggest, may extend to rhythmic mammalian skin regeneration.

70. Clocks, COVID, CRISPR: tracing contemporary history of science through Wikipedia's data

Main author: Rona Aviram
Affiliation: LPI, Paris Cité University, France
Co-authors: Omer Benjakob[1,2]
[1]LPI, Paris Cité University, France
[2]The Cohn Institute for the History and Philosophy of Science and Ideas, Tel Aviv University, Tel Aviv, Israel
Keywords: Wikipedia, Clocks, History of science, Bibliometrics

Rapid changes in science coupled with methodological divides hinder the study of how knowledge accumulates, consolidates, and diffuses. Focusing on circadian clocks as our initial case study, we studied the field's contemporary history utilizing the open nature of English-language Wikipedia, the largest collectively written online encyclopedia. We employed a mixed method: qualitative reading of encyclopedic texts and their different versions over decades, interviews with key editors, and quantitative analyses of bibliometrics and edit patterns. We found circadian-related content on Wikipedia was up-to-date, cited high impact-factor references, and was maintained regularly by a small yet conscious group of scientists and laypeople. The edit history of the articles allowed us to trace the conceptual evolution of key ideas in chronobiology like the rise of non-transcriptional oscillators.

To further systemize our methods, we analyzed two additional case studies with unique links between science and society: CRISPR, and COVID-19. The latter study took an in-depth bibliometric analysis of Wikipedic pages, and showed how the pandemic and knowledge about it ballooned during the outbreak, but also made use of older science to safeguard accuracy just at a time when it was most needed. On CRISPR, we found Wikipedia documented the field's maturation from a "basic science" discovery to a scientific and technological revolution with wide social implications, mirroring key facets of the field's history, its effects on other bodies of knowledge, and the manner it has become a cultural talking point.

Overall, we have generalized a methodology and designed computational tools for using Wikipedia as a historiographical source in a way that can be applied to other fields and topics. This method, we propose, can turn Wikipedia into a digital, searchable, and free archive documenting the incremental growth of scientific research, tracking the stages of its transference from the lab and into the public sphere.

71. Deciphering the impact of the reversed restricted feeding on the circadian clock in choroid plexus

Main author: Tereza Mičkerová
Affiliation: Laboratory of Biological Rhythms, Institute of Physiology of the Czech Academy of Sciences, Prague, Czech Republic
Co-authors: Pavel Houdek, Alena Sumová
Keywords: circadian clock, choroid plexus, reverse restricted feeding, gene expression

The epithelial cells of the choroid plexus (CP) in the brain ventricles produce cerebrospinal fluid and act as a blood-cerebrospinal fluid barrier. Importantly, the CP cells harbor a robust circadian oscillator that may control the function of CP. Recently, the oscillator has attracted attention primarily because it represents a non-neural clock in the brain that may play an important role in the circuitry between brain clocks. Therefore, understanding the mechanism of how the CP clock is synchronized is of utmost importance. The CP clock has been found to respond to glucocorticoids, but its sensitivity to

other potential entraining signals has not been elucidated. In this study, we examined the effects of reverse restricted feeding (rRF) on the CP clock in mPer2Luc mice. Mice either had unlimited access to food (ad libitum group) or received food for 6 hours in the middle of their resting period for 10 days (rRF group). The CP was collected from the 3rd, 4th, and lateral ventricles at 4-hour intervals around the clock to determine daily gene expression profiles by RT qPCR. We found that rRF has significant and ventricle-specific effects on the amplitude and phase of CP clocks. In addition, rRF affects the expression of selected CP function-related genes and modulates the expression of inflammatory genes. The results provide the first evidence that rRF strengthens and entrains the clock in CP, indicating the importance of the feeding regime for the non-neuronal brain clock.

72. A 6-month time-restricted eating (TRE) intervention does not have an unfavourable impact on bone metabolism and health

Main author: Maria Papageorgiou
Affiliation: Division of Bone Diseases, Geneva University Hospitals (HUG) and Faculty of Medicine, University of Geneva, Geneva, Switzerland
Co-authors: Emmanuel Biver, Julie Mareschal, Nicholas Edward Phillips, Alexandra Hemmer, Emma Biolley, Nathalie Schwab, Emily N. C. Manoogian, Elena Gonzalez Rodriguez, Daniel Aeberli, Didier Hans, Caroline Pot, Satchidananda Panda, Felix Naef, Nicolas Rodondi, Serge Ferrari , Tinh-Hai Collet

Keywords: time-restricted eating, weight loss, bone health, bone turnover markers, bone mineral content, bone mineral density

Objective: It remains uncertain whether time-restricting eating (TRE) induces bone loss similar to that seen in response to conventional weight loss approaches (e.g., moderate caloric restrictions to very low-calorie diets). We aimed to investigate the effects of a 6-month randomized controlled trial of TRE vs. standard dietary advice (SDA) on bone metabolism and health. Methods: Adults with ≥1 component of metabolic syndrome were randomized to TRE (ad libitum eating within a self-selected 12h window) or SDA (food pyramid brochure) for 6 months. We analyzed data from 42 participants with available pre-/post-intervention bone turnover markers (BTMs) and total body bone mineral content (BMC) and density (BMD) by dual-energy x-ray absorptiometry (DXA). Statistical analyses were performed in the total population and according to weight loss responses to TRE/SDA. Results: In the total population [76% women, median age 47 years (IQR 31–52), median BMI 27.8 kg/m2 (IQR 24.9–30.6)], there were no between-group differences (TRE vs. SDA) in any bone outcome. Among weight loss responders (≥0.6 kg weight loss), the bone resorption marker CTX (carboxy-terminal type I collagen crosslinks) tended to decrease after TRE but increase after SDA (between-group differences p=0.041), whilst the bone formation marker P1NP (procollagen type I N-propeptide) did not differ between groups.Total body BMC decreased after SDA (p=0.028) but remained unchanged after TRE (p=0.31) (between-group differences p=0.028). Among non-responders to weight loss (<0.6 kg weight loss), responses of BTMs or total body BMC/BMD did not differ between TRE and SDA. Conclusions: Overall, our results suggest no adverse impact of TRE on bone outcomes, whilst, when resulting in weight loss, such an intervention may even be associated with some bone sparing effects relative to weight loss achieved by SDA.

73. Chronotype has been shown to depend not only on genetic and environmental factors, but also on age and sex.

Main author: Tali Sagiv
Affiliation: Noga Kronfeld-Schor lab, Zoology department, Tel Aviv University, Israel.
Co-authors: Noga Kronfeld-Schor
Keywords: Epidemiology of nocturnal and diurnal rodents' circadian clocks

Numerous studies found changes in the expression of the circadian rhythm with age, in both animals and humans. Chronotype in humans becomes later in adolescence and shift earlier thereafter, and is usually earlier in females compared to males at the same age, but females become earlier after the age of 40years old. Studies in rodents show additional changes with age with different characteristics of the biological clock, and sex differences are manifested in other features, such as quantity and distribution of daily activity, sleep time and entrainment ability. The source of these differences is not fully understood.
This experiment aims to study the changes in tau between age groups and sexes in nocturnal and diurnal spiny mice, and over the years in the same individuals.
Every year, starting 2018, we collected 10 male and 10 female common and golden spiny mice (Acomys cahirinus and A. russatus) from our breeding colony, and housed one species and sex each in a separate cage outdoors. Once a year, the animals were housed in individual cages under controlled laboratory conditions with 12:12 LD cycle for two weeks after which lights were turned off for two weeks. Activity was monitored continuously using IR detectors to measure tau and activity pattern.
The oldest spiny mice in the experiment are now 4 years old. In both species tau changes with age but the direction of the change differs between sexes; hance the difference between males and females is getting larger as the mice get older, with males having a longer tau compared to females at a later age – comparable to humans up to the age of 40 years. Further, activity is becoming more fragmented with age.

74. Artificial light at night disturbs time-of-day-dependent reactivity of neutrophils to lipopolysaccharide in rats

Main author: Viera Jerigova
Affiliation: Department of Animal Physiology and Ethology, Faculty of Natural Sciences, Comenius University, Bratislava, Slovak Republic
Co-authors: Michal Zeman, Monika Okuliarova
Keywords: artificial light at night, chronodisruption, neutrophils, inflammatory response

Chronodisruptive effects of artificial light at night (ALAN) are intensively studied in association with negative health outcomes. In this study, we investigated whether ALAN disturbs time-of-day-dependent inflammatory response in rats, focusing on the neutrophil numbers in the blood and renal cortex and their functional activity. Rats were kept either under the control light/dark cycle or exposed to low-intensity ALAN (~2 lux during the night). After 2 weeks of ALAN, animals were injected with lipopolysaccharide (LPS) at the beginning of their passive (ZT2) or active (ZT14) phase. Using flow cytometry, blood counts of total leukocytes and neutrophils, and respiratory burst of neutrophils were analysed. In the renal cortex, we evaluated neutrophil infiltration and the expression of proteins associated with renal inflammation using immunofluorescence and Western blot. ALAN eliminated daily variations of blood leukocytes and impaired their changes in the circulation following LPS challenge. ALAN-exposed rats also showed suppressed time-of-day-dependent response of circulating neutrophils, which represent the key effectors of the innate immunity. Increased production

of free radicals in blood neutrophils was observed upon LPS in both control and ALAN-exposed rats. Interestingly, LPS stimulation at ZT2 primed respiratory burst of neutrophils only in ALAN group, while no priming effect of LPS was observed in controls. In the passive phase, ALAN promoted neutrophil recruitment into the renal cortex independent of LPS administration, indicating disturbed rhythm in neutrophil trafficking into the kidney. Collectively, our data indicate that ALAN weakened circadian control of neutrophil numbers in the circulation and kidney, thereby affecting the time-dependent capacity of neutrophils to produce the oxidative burst under inflammatory conditions. Supported by APVV-17-0178 and VEGA 1/0565/22.

75. The role of light in commensalism vs. anthropophobia in wild mice

Main author: Mila Kazavchinsky
Affiliation: Kronfeld-Schor Lab, The School of Zoology
The George S. Wise Faculty of Life Sciences, Tel-Aviv University
Co-authors: Prof. Noga Kronfeld -Schor
Keywords: -

The house mouse is considered the most widespread mammalian species in urban areas. Its origin can be traced to the beginning of human civilization in the Levant. To this day, two sympatric species can be found in this area; the commensal house mouse, Mus musculus domesticus, and the mostly anthropophobic mouse, Mus macedonicus spretoides, which sometimes share the same habitat. Several studies demonstrate that M.musculus has adaptive traits that enable it to prosper also in the proximity of humans, though possible adaptations to artificial lights at night (ALAN) were not yet studied. During the last decades, the use of ALAN increases and changes the regular pattern of light exposure, more so in cities compared to rural environments; the novelty, intensity, and prevalence of the phenomenon cause unprecedented selective pressure. We hypothesized that adaptations to ALAN exist in M. musculus, enabling it to thrive in human settlements, but not in M. macedonicus.The research is conducted on wild-caught mice, belonging to the two species described above, captured in the wild in rural habitats, where they coexist. The captured mice were housed in individual cages under controlled lab settings, with 12:12 LD cycle, with ad-lib. food and water. After a few days acclimation, the mice were were tested in three behavioral tests: 1) nest building test – to check their general well-being; 2) light/dark box – testing stress and photophobic response; 3) open filed test – evaluating stress and mobility. The behavioral tests were followed by activity measurement using infrared motion detectors under 12:12 LD cycle and constant darkness (DD). The two species behaved differently in the light/dark box test but not in the open field test, suggesting that non-commensal M. macedonicus are more photophobic than urban-adapted M. musculus, which also exhibited higher activity levels during the light phase (LD) and subjective day (DD).

76. Regulation of PER1 phosphorylation and its interactome

Main author: Antoine Torchiat
Affiliation: University of Fribourg, Albrecht lab
Co-authors: Albrecht Urs, Ripperger Juergen
Keywords: SCN, circadian rhythm, PER1, Kinase, Phosphorylation

The circadian clock is an evolutionary adaptation to the daily lightdark cycle generated by the sun. It allows organisms to organize behavior and physiology over the 24 hr day to adapt and optimize, body function to predictably recurring daily events. Malfunction or disruption of the circadian clock in humans results in various pathologies including obesity, cancer, and neurological disorders.
Circadian oscillations emerge from transcriptional and post-translational feedback loops. An important step in generating rhythmicity is the translocation of clock components into the nucleus regulated in many cases by kinases.
PER1 gene is important to maintain circadian rhythms in cells. We have identified that Cyclin-dependent kinase 5 (CDK5) interacts with PER2 and phosphorylates it at Ser394 to maintain its stability and transport it into the nucleus. PER1 & PER2 genes are involved in regulating distinct and opposite biological functions. Most of PER proteins phosphorylation sites are not characterized, especially for PER1.
We aim first to find the potential CDK5 dependent phosphorylation site in PER1 & investigate the interactome of PER1 depending on CDK5 phosphorylation site. Our preliminary data indicate that PER1 and CDK5 can interact in vitro in cells. We plan to do invitro kinase essay followed by mass spectrometry to identify PER1 amino acid(s) phosphorylated by CDK5.
The phosphorylation site(s) will be mutagenized followed by a kinase essay. We will produce an appropriate antibody to test CDK5-dependant PER1 phosphorylation, address the molecular meaning of the identified CDK5-dependant phosphorylation site of PER1, the protein stability, its half-life and its period length. We will also see its impact on protein oscillations of clock genes such as CLOCK, BMAL1, PER2, CRY and potential changes in the transcriptional activity.
Finally, we will characterize CDK5 dependent phosphorylation site of PER1 in term of temporality and spatial nature in the suprachiasmatic nuclei (SCN) in vivo in mice.

77. Changing daylight length on sleep-wake regularities at high latitude

Main author: Katharina Wulff
Affiliation: Departments of Radiation Sciences and Molecular Biology,
Umeå University, Sweden
Co-authors: Zoe Darrasse, Aleksi Ala-Prinkkilä, Leo Pasturel, Frida Hector, Magnus Domellöf, Richard Lundberg, Wayne Davies
Keywords: Light, latitude, photoperiod, actigraphy, sleep, wakefulness, circadian, behaviour, children

The aim of this ongoing project is to explore the influence of changes in daylight length, direction and amplitude on human behaviour by employing a high latitude in the design (latitude: about 64° N). Three-year old children from the Northpop birth cohort at Västerbotten region in Sweden are being monitored 24hrs a day for eight consecutive days in the field using quantifiable actigraphic techniques to simultaneously capture time patterns of ambient light and sleep-wake behaviour. Patterns are enriched with discrete diary entries by parents. A tool for quantifying the amount and course of light exposure broken down in long-format has been developed specifically for wrist-worn actigraphs. About 500 good quality datasets have been collected so far, which are at various stages of analysis. Here we focus on motor activity during sleep recorded every 30 seconds with the MotionWatch 8 in 145

children. Data grouped by photoperiod into four seasons reveal lowest sleep percentage (87.63%, 95% CI 87.1 – 88.16) and highest number of minutes moving (88 min, 95% CI 80.2-95.6) during the sleep period (sleep-onset to sleep-offset) in Spring. The shortest sleep period across 24 hours occurs in Summer (604 min, 95% CI 566-642) and the longest in Spring (661 min, 95% CI 654-669), both significantly different from each other ($p<0.003$). From the viewpoint of the circadian system, 'advancing' daylight by daylength during Spring of over 6 min daily from February to April (1st Feb to 30th April: from 7h 11min to 13h 30min) runs against the endogenous clock's mechanism that naturally lengthen its period in diurnal species, which is resembled by the Autumn course of 'delaying' daylight length. Early morning light itself may trigger more movements, alternatively 'advancing' daylight might be a photoperiodic trigger for motor restlessness during sleep in Spring.

78. An animal model of chemotherapy related fatigue shows misalignment of behavioural and SCN electrical activity

Main author: Yumeng Wang
Affiliation: Department of Cell and Chemical Biology, Leiden University Medical Center
Co-authors: Yumeng Wang, Mayke M.H. Tersteeg, Anouk W. van Beurden, Stephan Michel, Jos H.T. Rohling, Jacques Neefjes, Johanna H. Meijer, and Tom Deboer

Keywords: Cancer related fatigue, SCN, MUA, doxorubicin, behavior

Background: Cancer-related fatigue (CRF) is a devastating long-term side effect of many cancer survivors that confounds the quality of life for months to years after treatment. The cause of CRF is poorly understood. We recently showed that the central circadian pacemaker in the suprachiasmatic nucleus (SCN) is likely affected by chemotherapy. We applied SCN neuronal activity recordings to further investigate the link between CRF and the circadian clock.
Methods: 7 male C57BL/6J mice were treated with either doxorubicin or vehicle (control). 4 weeks after treatment, brain slices containing the SCN were used for 24-48 h ex-vivo multiunit activity recordings. Another 12 male C57BL/6J mice were implanted with electrodes in the SCN, and neuronal activity was recorded before and after the mice were treated either with doxorubicin or vehicle in light dark (LD) conditions and in constant darkness (DD).
Results: The ex-vivo recordings of electrical activity rhythm in the SCN slices from doxorubicin-treated mice show unaltered amplitude but a broader range of peak times (CT 22.28 – 8.3h) compared with recordings in control mice (CT 5.2 - 6h) indicating a disruption of the timing of clock function. Furthermore, the in-vivo results from doxorubicin-treated mice show that the SCN is still rhythmic, but the phase angle of entrainment of the behavioral activity shows increased variability compared to control treated animals.
Conclusion: We have shown previously that doxorubicin-treated mice display behavior analogous to the symptoms of cancer-related fatigue in human patients. The present results suggest that chemotherapy-induced fatigue may be due to lower precision of circadian timing, weaker downstream signaling from the SCN and misalignment of rest-activity behavior with the central pacemaker.

79. Diurnal regulation of hepatic metabolism by the glucocorticoid receptor

Main author: Konstantinos Makris
Affiliation: [1] Institute for Diabetes and Endocrinology (IDE), Helmholtz Center Munich (HMGU) and German Center for Diabetes Research (DZD), Ingolstaedter Landstr. 1, 85764 Neuherberg (Munich), Germany
[2] Institute for Diabetes and Cancer (IDC), Helmholtz Center Munich
Co-authors: Konstantinos Makris[1], Kinga Balazs[1], Céline Jouffe[1,2], Kenneth Allen Dyar[2], Fania Feby Ramadhani[1], Vlera Fonta[1], Fabiana Quagliarini$_1$, and Nina Henriette Uhlenhaut[1,3]
Keywords: -

Glucocorticoids (GCs) are adrenal steroid hormones, which are secreted in a diurnal and clock-controlled manner. They bind to the glucocorticoid receptor (GR), a transcription factor that controls the expression of enzymes, related to hepatic processes, such as bile acid production, lipid homeostasis and carbohydrate catabolism. Aberrant molecular clock function or glucocorticoid secretion causes profound metabolic dysregulation, leading to obesity, type 2 diabetes, and non-alcoholic fatty liver disease.
In our recent work, we found that GCs diurnal secretion leads to rhythmic binding of the glucocorticoid receptor to the chromatin at the onset of the feeding phase, at ZT12 (Zeitgeber 12; 6 p.m.). Moreover, there is a significant overlap between hepatic glucocorticoid receptor binding profile and other circadian clock factors, such as BMAL1, CRY1, CRY2 and PER1, jointly controlling transcriptional rhythms. Hepatic deletion of the GR in mice dampens over 50% of rhythmic genes and lowers their amplitude. Interestingly, high-fat diet increases the glucocorticoid receptor binding amplitude, which is driven by STAT5 occupancy. Absence of murine hepatic glucocorticoid receptor affects triglyceride and glucose homeostasis, causing hepatic steatosis.
In hypocaloric conditions, caloric restriction elevates the secretion of glucocorticoids at the peak of hormones (ZT12), without causing metabolic abnormalities and protecting from obesity, diabetes, and aging. Therefore, caloric restriction not only sharpens the rhythmicity of the circadian clock, but also boosts the diurnal secretion of glucocorticoids. For this reason, we have characterized GR binding with associating gene expression around the clock between caloric restricted wildtype and GR liver specific knockout mice. We have found a deep reprogramming of oscillatory programs in the GR liver specific knockout mice upon caloric restriction. Here, we will present mouse liver ChIP-seq, RNA-seq, bioinformatics and metabolic phenotyping data elucidating the impact of increased glucocorticoid action during caloric restriction.

80. Sub-regions of the SCN receive a heterogeneous synaptic input from the retina

Main author: Hugo Calligaro
Affiliation: Salk Institute for Biological Studies, La Jolla, CA, USA
Co-authors: Shoghi A, Chen X, Kim KY, Khov B, Finander B, Le H, Ellisman MH, Panda S
Keywords: melanopsin, Suprachiasmatic Nucleus, retinal ganglion cells; connectome

The suprachiasmatic nucleus (SCN) in the hypothalamus of the vertebrate brain is the central pacemaker regulating the circadian rhythmicity of tissues throughout the body. The SCN receives photic information through melanopsin-expressing retinal ganglion cells (mRGC), signals known to synchronize the body with environmental light cycles. The SCN is a complex nucleus composed of several subtypes of neurons receiving synaptic input from mRGC as well as from synapses supplied by neurons residing in other brain regions. Determining how these different inputs map into the network of synaptic connections on and between SCN neurons is key to help propel our understanding

of the regulation of the circadian clock by light as well as to unravel the relevant local circuits within the SCN. This level of detail required to map these connections requires the application of 3D electron microscopy and compatible genetic probe labeling methods. To propel this work we developed a Cre-dependant electron microscopy reporter, APEX2, allowing us to specifically label mitochondria of mRGC axons using serial blockface scanning electron microscopy (SBEM) to resolve and quantify the fine structure of mRGC in 3D volumes of the SCN. The maps thus created provide a first draft of the patterns of connectomic organization of SCN in two specific regions, the core and the shell, known to be composed of different neuronal subtypes, and here shown to differ with regard to the patterning of their mRGC input. Our results indicate that a network of neurons forming dendro-dendritic synapses is present in both sub-regions of the SCN and receives denser mRGCs synaptic input in the shell compared to the core. This challenges the presently held view that photic information coming directly from the retina is mainly integrated by the core region of the SCN.

81. Chronic inflammatory arthritis drives systemic changes in circadian energy metabolism

Main author: Polly Downton
Affiliation: University of Manchester, UK
Co-authors: Fabio Sanna, Robert Maidstone, Toryn M. Poolman, Edward A. Hayter, Suzanna H. Dickson, Nick A. Ciccone, James O. Early, Antony Adamson, David G. Spiller, Devin A. Simpkins, Matthew Baxter, Roman Fischer, Magnus Rattray, Andrew S.I. Loudon, Julie E. Gibbs, David A. Bechtold, David W. Ray

Keywords: Rheumatoid arthritis, Circadian clock, Inflammation, Mitochondria, Ceramides

Rheumatoid arthritis (RA) is a debilitating chronic inflammatory disease in which symptoms exhibit a strong time-of-day rhythmicity. RA is commonly associated with metabolic disturbance and increased incidence of diabetes and cardiovascular disease, yet the mechanisms underlying this metabolic dysregulation remain unclear. Inflammatory and metabolic processes vary through circadian time, suggesting an important temporal crosstalk between these systems.
Using an established mouse model, we show that chronic inflammatory arthritis results in rhythmic inflammation and extensive changes in rhythmic gene expression in the inflamed joint. Inflammation also drives major changes in muscle and liver energy metabolism and rhythmic gene expression. Transcriptional analysis at tissue and single cell level, combined with phosphoproteomic analysis, reveal chronic inflammation results in dysregulation of fatty acid metabolism and mitochondrial dysfunction. These changes are associated with increased EGFR-JAK-STAT3 signalling. Metabolomic analysis confirms rhythmic metabolic rewiring and impairment to lipid β-oxidation, and reveals a pronounced shunt towards sphingolipid and ceramide accumulation. The arthritis-related production of ceramides was most pronounced during the day, coinciding with the time of peak inflammation and increased reliance on fatty acid oxidation. We conclude that localised joint inflammation drives a time-of-day dependent build-up of bioactive lipid species, driven by rhythmic inflammation and altered EGFR-STAT signalling.

82. Genetic regulation of chromatin accessibility regulation during sleep deprivation

Main author: Carlos Neves
Affiliation: Université de Lausanne, Switzerland
Co-authors: Jimenez Sonia, Hor Charlotte, Franken Paul
Keywords: Sleep Deprivation, Epigenetics, Gene Regulation

Sleep is known to be driven by the interplay of a circadian rhythm and a homeostatic pressure. Though the molecular basis of circadian rhythms is well studied, understanding of sleep homeostasis is still lacking in comparison. In this study, we explore the regulatory regions of the genome associated with response to 6h sleep deprivation using the BXD genetic reference population of mice (34 BXD/RwwJ recombinant inbred lines), including the C57BL/6J (B6) and DBA/2J (D2) parent strains. Chromatin accessibility changes were estimated from cortical samples by ATAC-sequencing and correlated with our previously published multi-omics dataset.
We find widespread changes in chromatin accessibility with 36'447 genome regions affected by sleep deprivation (3.5% of the regulatory genome), 73.5% of which with increased accessibility. Moreover, 60% of these regions are correlated with expression of genes within the same topological domains, with 21% located within 5Kb of a transcription starting site. Given the well-described differences between BXD lines' response to sleep deprivation both behaviorally and molecularly, we analyzed influence of genetic variants on chromatin accessibility, estimating that genetic background (B6 vs. D2 genotype) strongly affects accessibility of 10'308 regions (1.3% of the regulatory genome). Furthermore, 88% of these regions showed significant correlations with the expression of at least one gene within the same topological domain, with 10% being located within 5Kb of a transcription starting site. Interestingly, 25% of the regions showing allelic-specific accessibility overlap with those with changes induced by SD, suggesting a potential mechanism for genetic regulation of response to SD. Our results point to a pervasive and complex interplay between epigenetic and transcriptional factors during sleep deprivation. We additionally show that genetic background has widespread effects on the epigenetic landscape and transcriptional state, which is fundamental to understanding differences in sleep behavior across individuals.

83. Eastward jet lag is associated with impaired performance and game outcome in the National Basketball Association

Main author: Josh Leota
Affiliation: Turner Institute for Brain and Mental Health, School of Psychological Sciences, Monash University, Australia
Co-authors: Daniel Hoffman, Mark É. Czeisler, Luis Mascaro, Sean P.A. Drummond, Clare Anderson, Shantha M.W. Rajaratnam, & Elise R. Facer-Childs.
Keywords: circadian disruption, phase advance, elite athletes, sport, sleep, travel, NBA, time zone travel.

Introduction: Elite athletes are often required to travel across time zones for national and international competitions, causing frequent jet lag. Chronobiological models of rapid travel have generated conflicting results and associated hypotheses about which direction of travel is more detrimental to athletic performance. The aim of this study was to examine whether the direction of travel-related jet lag is associated with performance in the National Basketball Association (NBA), and if so, to explore potential mechanisms.
Methods: Ten seasons comprising of 11,481 games of NBA data from the 2011/2012 to the 2020/2021 regular season were analyzed using multi-level mixed models with one fixed factor (three levels; jet lag direction: eastward vs. westward vs. no jet lag) and three random factors (team, opponent, game

time). Predicted circadian resynchronization rate was accounted for, and home and away games were analysed separately. Mediation analyses were performed to examine potential mechanisms.
Results: Among home teams, eastward (but not westward) jet lag was associated with reduced winning (Δ (i.e., change) = -6.03%, p = .051), points differential (Δ = -1.29 points, p = .015), rebound differential (Δ = -1.29 rebounds, p < .0001), and effective field goal percentage differential (Δ = -1.2%, p < .01). As the magnitude of eastward jet lag increased, home team points differential decreased (2 hr Δ = -4.53 points, p < .05; 1 hr Δ = -0.72 points, p = .07). No significant associations were found between jet lag and away team performance.
Conclusion: Eastward jet lag was associated with impaired performance for home (but not away) teams. Sleep and circadian disruption associated with advancing phase following eastward travel may have significant adverse consequences on performance in the NBA, particularly when recovery time is limited. Sports organisations could consider chronobiology-informed scheduling and interventions to maximise recovery and performance of their athletes.

84. Resetting the clock is a molecular tug-of-war

Main author: Nina Rzechorzek
Affiliation: MRC Laboratory of Molecular Biology, Cambridge, UK
Co-authors: Nina M Rzechorzek, Marrit Putker, Aiwei Zeng, Andrew Beale, Celine Goh, Priya Crosby, John S O'Neill

Keywords: Circadian phase, glucocorticoids, temperature, entrainment, shift work, jet lag, mouse, human

To be understood, oscillatory phenomena need a temporal reference point. When cellular circadian rhythms are synchronized by external timing cues, it is rarely clear to what phase they are set, due to a paucity of unambiguous molecular markers against which subsequent events might be precisely measured. We sought to define a reference standard for cellular circadian time and determine the number of discrete circadian phases in mammalian cells.
Across 7 independent mouse and human fibroblast lines, we noted marked variation in circadian phase after serum synchronization. To isolate a true reference stimulus, we performed a systematic analysis of external cues on circadian timing in culture. Glucocorticoid consistently minimized temporal variation within and between cell lines, defining our reference as GCT0—the circadian phase cells are set to by glucocorticoid. Validating GCT0 in 15 independent lines and organoids, we found that (1) circadian phase resetting in response to other cues is partly species- and cell-line dependent, (2) a molecular timetable constructed from GCT charts at least 3 distinct circadian phases, and (3) this timetable reliably predicts cellular phase and rhythmic robustness in the context of temporal coherence, temporal conflict, and mistiming of cues. Translating our results to mice, we found that rapid circadian re-entrainment after simulated shift work relies on the optimal timing and order of potent cellular resetting cues.
We propose GCT0 as a reference standard for cellular circadian time and present a molecular map of the cellular circadian cycle. This resource can be used to generate hypotheses, test predictions, and enhance reproducibility across labs and model systems. Highlighting its utility, we exploited our timetable to simulate shift work in vitro and accelerate re-entrainment in mice. The RESET trial incorporates these findings into a novel intervention aimed at reducing the impact of jet lag and shift work on circadian health.

85. Chronophenotyping atrial fibrillation using machine learning

Main author: Jonathan Sobel
Affiliation: Technion/Rambam, UNIL
Co-authors: Jonathan Sobel, Shany Biton, Erez Marcusohn, Mahmoud Suleiman, Kenta Tsutsui and Joachim A. Behar

Keywords: Cardiac arrhythmia, Atrial fibrillation, Machine learning, Chronophenotype

Background: Atrial fibrillation (AF) is the most prominent type of cardiac arrhythmia, which affects an estimated 4% of the adult population over 60 and this percentage increases with age. Circadian variation in the disease presentation has previously been reported at the population level. However, it is unknown whether there exist chronophenotypes and what their clinical outcomes are.
Methods: A collection of 24h Holter recordings and patients clinical information were collected at Rambam hospital in Haifa (Israel) and Saitama Medical University (Japan). A deep learning method called ArNet2 was developed to classify 60 beats windows into normal sinus rhythms or AF. We used ArNet2 on both databases to detect episodes of AF. Unsupervised learning was used to identify AF patient subgroups according to AF episodes temporal distribution. Finally, we describe the difference in demographics (age, BMI, and mortality), comorbidities, and heart rate at the population level in each identified AF chronophenotype.
Results: Our work confirmed on two independent databases (geographically and ethnically distinct) diurnal variation of AF at the population level and suggested the existence of several chronophenotypes. These were: persistent AF (high burden), low AF, morning AF, mid-day AF, evening AF and night exclusive AF. The characterization of these clusters was associated with different proportions of comorbidities, age, sex, BMI, and distinct prognosis.
Conclusions: We developed a novel unsupervised learning method for chronophentyping through the analysis of the temporal distribution of AF episodes during 24 hours long ECG recordings. A total of 6 phenotypes was discovered. These sub-groups of AF patients depict distinct prognoses and characteristics which may suggest alternative treatment plans . Our work paves the way to chronophentyping based on the automated analysis of Human long term continuous physiological recordings.

86. Office lighting and cognitive functions: can it be too bright?

Main author: Mirjam Münch
Affiliation: Centre for Chronobiology, University Psychiatric Clinics Basel & Transfaculty Research Platform Molecular and Cognitive Neurosciences, University of Basel, Switzerland
Co-authors: Marta Benedetti, Lenka Maierova, Christian Cajochen, Jean-Louis Scartezzini, Mirjam Münch* (presenting author)

Keywords: light conditions, work performance, cognition, working memory, melanopic EDI, daylight

Innovative daytime lighting conditions can impact wellbeing and productivity and enhance cognitive functions in office workers. This study aimed to test whether optimized office lighting over several days increases vigilant attention, reaction time and working memory.
Thirty-four young participants spent 5 consecutive days (for 8 hours) in an office room equipped with an automated controller for blinds and electric lighting and a larger window surface (=Test room). Separated by one week, they also spent 5 consecutive days in a control office room without a controller (=Reference room) in a balanced-cross over design. Every 2.5 hours, participants had to complete an auditory cognitive test battery containing the Psychomotor-Vigilance Test (PVT) and the

0-2-3-back task with spoken letters, followed by subjective assessments of mood, alertness, temperature, and glare. Light exposures were assessed with stationary devices for photopic illuminance and irradiance.
Photic illuminance in a vertical plane at eye level was, on average, 320lux higher in the Test than the Reference room reported recently, which corresponded to a difference in melanopic EDI of ≈ 200 lux (Benedetti et al. 2022). Unexpectedly, reaction times assessed during the PVT (median, 10% fastest, and 10% slowest) were significantly slower in the Test than in the Reference room (mean difference: 7.5ms for the 10% slowest; $p<0.05$). Similarly, accuracy in the 2-back test was worse in the Test room compared to the Reference room ($p<0.05$). The office lighting with an automated control system did not lead to better cognitive performance when compared to the Reference room. Most likely because already in the Reference room, [with approximately 730 melanopic EDI (lux)], the lighting was far above the minimum recommended daytime lighting levels (Brown et.al. 2022). Hence, there is a need to explore the upper lighting thresholds for these functions to define the optimal dose of light in the office.

87. Astrocytes regulate spatiotemporal circadian patterns of neuronal activity in the suprachiasmatic nucleus

Main author: Natalie Ness
Affiliation: UK Dementia Research Institute at Imperial College London, London, UK; Dept of Brain Sciences, Imperial College London, London, UK
Co-authors: Marieke Hoekstra, Renaud Bussiere, Marco Ferrari and Marco Brancaccio

Keywords: astrocytes, glutamate, GABA, SCN network function, synchronization

Clock gene expression and neuronal activity follow a precise spatiotemporal progression in the suprachiasmatic nucleus (SCN), travelling from the dorsomedial to the ventrolateral aspect within 2-4h, a pattern previously associated with photoperiodic responses and time-appropriate engagement of downstream targets. Astrocytes can cell-autonomously initiate neuronal rhythms in the SCN and circadian behaviour, however whether and how astrocyte activity is coordinated across SCN regions and circadian time is uncertain. We utilised unsupervised K-Means clustering to parametrise patterns of clock gene expression, neuronal and astrocytic activity in SCN slices transduced with AAVs expressing astrocyte- and neuron-specific genetically encoded fluorescent and bioluminescent indicators. Our pipeline faithfully reproduced spatiotemporal circadian patterns of PER2::LUC expression and neuronal calcium. In contrast, we did not detect any spatiotemporal pattern in astrocytic calcium (detected by gfaABC1D-lck-GCaMP6f), thus suggesting a distinct, independent regulation of astrocytic temporal activation. We have previously proposed that glutamate released by astrocytes would play a major role in determining timing of GABA release by SCN neurons. To investigate this relationship further, we recorded extracellular glutamate (by gfaABC1D-iGluSnFr) and GABA (by Synapsin-GABASnFr) in SCN slices. As expected, glutamate was in phase with astrocytic calcium, and anti-phasic to neuronal calcium. Intriguingly, GABA was also in anti-phase to neuronal calcium and followed spatiotemporal patterns akin to astrocytic calcium and glutamate, suggesting that GABA levels strongly correlate with astrocyte activity, rather than with the spatiotemporal organisation of neuronal activity in the SCN. To further assess the role of astrocytes in mediating the spatiotemporal organisation of the SCN, we ablated Bmal1 in astrocytes and showed that while overall circadian oscillations of neuronal calcium were preserved, their spatiotemporal profiles were strongly disrupted. Independent experiments of astrocytic connexin-43 inhibition confirmed such findings, and further highlight a key role for astrocytes in encoding spatiotemporal organisation of circadian time in the SCN circuit.

88. Clock-to-clock communication in the adrenal gland

Main author: Iwona Olejniczak
Affiliation: Universität zu Lübeck
Co-authors: Iwona Olejniczak, Mariana Astiz, Henrik Oster
Keywords: Adrenal gland, glucocorticoids, circadian clock

The circadian clock influences the physiology of living organisms and allows them to anticipate and adapt to daily environmental changes. The main pacemaker, located in the suprachiasmatic nucleus (SCN) of the hypothalamus, is entrained by light. The SCN resets peripheral clocks via hormonal and neural signals. However, more recent research suggest that peripheral clocks can entrain directly to relevant environmental cues - independent of the SCN - and may communicate within and between tissues.
Glucocorticoids (GCs) are an important hormonal signal that regulates many aspects of physiology including the entrainment of peripheral clocks. GCs are produced in the cortex of the adrenal gland. This organ consists of two parts: the outer cortex and inner medulla. Both compartments differ functionally and morphologically. In this project we demonstrate that both cortex and medulla harbor functional clocks that are sustained ex vivo. Under such conditions, in absence of systemic cues, the period of the medulla clock is longer than that of the cortex, causing the clocks to drift apart. This effect is less pronounced when medulla is cultured in the absence of the cortex suggesting that a cortex-derived signal contributes to the regulation of the medulla period. We further show that glucocorticoids are likely involved by observing a phase shift and a period shortening of medulla clocks under glucocorticoid receptor blockage with mifepristone.
Overall, this project strengthens the case of a federated circadian organisation by highlighting a specific example of peripheral clock-to-clock communication.

89. Daily profile in expression of components of SARS-CoV-2 entrance pathway in the lungs and colon of male Wistar rat during 24h LD cycle and how it is influenced by 17β-estradiol administration

Main author: Iveta Herichova
Affiliation: Department of Animal Physiology and Ethology, Faculty of Natural Sciences, Comenius University, Bratislava, Slovak Republic
Co-authors: Soňa Jendrisková, Paulína Pidíková, Lucia Kršková, Lucia Olexová, Martina Morová, Katarína Stebelová, Peter Štefánik
Keywords: ACE2, ADAM17, TMPRSS2, BMAL1, PER2, ESR1, ESR2, GPER1, Covid

The study was aimed to elucidate interferences between 17β-estradiol (E2) signalling and the circadian system in the regulation of the expression of molecules facilitating SARS-Cov-2 entrance into the cell. E2 was administered at a dosage of 40 μg/kg/day for 7 days to adult male Wistar rats and sampling of the lungs and colon was performed during a 24-h cycle. Responsiveness of gene expression to E2 was also tested in DLD1 cell line. The daily pattern of expression of ACE2, ADAM17 and TMPRSS2 along with E2 receptors ESR1, ESR2 and GPER1 and clock genes was analysed. E2 plasma levels exerted a distinct daily rhythm in both groups with increased mesor and amplitude in E2 treated rats. We did not observe a significant difference in daily pattern of locomotor activity between groups. As a consequence of E2 administration, a rhythm in ACE2 and TMPRSS2 mRNA expression emerged in the lungs. On the other hand, a rhythmic pattern in ACE2 and TMPRSS2 mRNA expression was detected in the colon only in control group. ADAM17 mRNA expression showed a pronounced rhythmic pattern in both tissues that was not influenced by E2 treatment. ESR1 mRNA expression showed a rhythmic pattern, which was diminished by E2 treatment in both tissues.

The influence of E2 administration on ESR2 and GPER1 mRNA expression was greater in the lungs than in the colon as a significant rhythm in ESR2 and GPER1 mRNA expression appeared only in the lungs after E2 treatment. E2 administration also increased the amplitude of bmal1 expression in the lungs, which implicates altered functioning of peripheral oscillators in response to E2 treatment. The daily pattern of components of the SARS-CoV-2 entrance pathway and their responsiveness to E2 should be considered in the timing of pharmacological therapy for Covid-19. Supported by grants APVV-16-0209, APVV-20-0241 and VEGA 1/0679/19.

90. The molecular oscillators of the protochordate Botryllus schlosseri

Main author: Rachel Ben-Shlomo
Affiliation: Department of Biology and Environment, Faculty of Natural Sciences, University of Haifa-Oranim, Tivon, Israel
Co-authors: Yotam Voskoboynik, Aidan Glina, Mark Kowarsky, Norma F Neff, Katherine J Ishizuka, Karla J Palmeri, Stephen R Quake, Debashis Sahoo, Ayelet Voskoboynik
Keywords: evolution, circadian cycle, circatidal cycle, clock gene paralogous, clock-Bmal1 transcription activators, Casein Kinase I

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 sessile diurnal colonial marine organism that inhabit the intertidal zone and thus may displays a tidal cycle (12.4hrs) as well as circadian cycles. We combined genome and transcriptome sequencing analyses to identify circadian clock genes, and their expression. Profound search for the Period (Per) and Cryptochrome (Cry) canonical clock genes orthologues was unsuccessful. Currently, the genome of 15 species from seven families and three orders of tunicates has been sequenced and none of them is showing either the Per or the Cry canonical circadian clock genes. Therefore, it seems that the subphylum Tunicata is missing the negative regulatory elements (genes) of the circadian oscillator.
We detected at least five different paralogous of the transcription activators clock-Bmal1 genes, six Casein Kinase I (CKI) paralogous and about 20 Rev-erba/Ror-a,b paralogous. RNA-seq analysis collected along the day, as well as along short daily cycles of 16hr, indicated that two of the clock-Bmal1 transcripts showed lower level during the light phase. We also detected two CKI transcripts that oscillated along the day, in the same phase as the transcription factors. Several other transcripts expressed a ~12hr cycling of transcription and may be associated with a possible circatidal pacemaker. These include a homolog of the transcription factor clock-bmal1 and a homolog of CKI, that oscillated at the same phase, however were not affected by the light-dark daily cycle. We suggest that a tidal oscillator in B. schlosseri is operated in a comparable way to the circadian one, but using other homologs.

91. Why are circadian clocks also ultradian clocks?

Main author: Monika Stengl
Affiliation: University of Kassel
Co-authors: Jenny Plath, Pablo Rojas, Huleg Zolmon, Susanne Neupert, Martin Garcia
Keywords: accessory medulla, insect circadian clock, multiscale timing, neuropeptides, coupling

The accessory medulla (AME) in the brain´s optic lobes is the circadian clock of the nocturnal Madeira cockroach. It consists of clock neurons with an unusual abundance of colocalized neuropeptides. The clock controls sleep wake cycles via timed release of neuropeptides, but specific cellular, molecular

mechanisms are unknown. Extracellular recordings from the isolated AME in vitro measured circadian as well as ultradian rhythms of action potential activity. At dusk and dawn, the middle of the day and the middle of the night peaks of activity changes occur with unknown origin. In vivo recordings of the cockroach circadian clock confirmed that the clock ticks not only with a circadian, but also with ultradian rhythms at different frequency bands occurring at defined phases of the circadian cycle. Ensembles of synchronously spiking neurons underlie these daytime-dependent ultradian rhythms of different frequencies. Since it is known from mammalian cortex that synchronized neuronal activity is necessary for gating and binding of multisensory information, we suspected that the same mechanisms play a role in the insect clock circuits. Ultradian oscillations of the membrane potential allow for fast and energy conserving synchronization of neuronal populations as basis of fast information transfer at synapses. Indeed, application of the clock´s neuropeptide pigment-dispersing factor (PDF) transiently formed a synchronized ensemble of neurons that persisted over seconds to minutes after washout. We are currently examining clock mechanisms in long-term multiple loose patch recordings, and with Ca2+ imaging of isolated clock neurons at multiple time scales. In summary, we provide evidence for a new hypothesis suggesting that the membrane of circadian clock neurons is a multiscale master clock as basis of fast information transfer via resonance and transient synchronization at circadian and ultradian time scales. [Supported by DFG grants STE531/18-1,2,3; STE531/26-1; STE531/27-1; NE911/5-1; GRK 2749/1 “multiscale clocks”; P/979 Univ. Kassel]

92. The role of biological sex in the relationship between circadian alignment and well-being in elite athletes

Main author: Luis Mascaro
Affiliation: Turner Institute for Brain and Mental Health, Monash University
Co-authors: Shantha M.W. Rajaratnam, Josh Leota, Daniel Hoffman, Sean P.A. Drummond, Elise R. Facer-Childs
Keywords: circadian rhythms, phase angle, dlmo, elite athletes, sex differences, sleep, psychological strain

Introduction: Circadian rhythms govern physiological timing and influence sleep, well-being, and performance. Desynchrony between circadian timing and behaviours (circadian misalignment) can compromise daily functioning. To date, the role of biological sex in objective circadian timing has not yet been investigated in elite sports. Here, we investigated sex differences in sleep and circadian timing variables, and their relationship with sleep and mental health outcomes.
Methods: Participants were elite Australian Rules Football (AFL) athletes (N = 141, 43% female; M-age = 23.8 ± 4.0 years). Data were collected across two time-points prior to the start of the 2021 and 2022 AFL seasons. Circadian phase was assessed via salivary melatonin (collected hourly from 5hrs pre- and 1h post-habitual bedtime to analyse dim light melatonin onset; DLMO). A questionnaire battery of sleep and mental health measures was completed, in addition to actigraphy and sleep diaries collected across two weeks.
Results: Female athletes had a significantly later circadian phase (DLMO; 20:42 vs 20:13) and midsleep time (03:24 vs 02:58), and worse self-reported athlete psychological strain (APSQ), daytime sleepiness, and insomnia, relative to male athletes. There was no sex difference in phase angle (interval between sleep onset and DLMO times; M = 2.5hrs ± 49 mins). DLMO time did not predict sleep efficiency, sleep latency, insomnia, sleepiness, or APSQ. Phase angle predicted APSQ for female athletes only via a quadratic trend: psychological strain was worse among female athletes with shorter and longer phase angles.
Discussion: Our results suggest female athletes have a later circadian phase, which is not associated with adverse outcomes, except for higher psychological strain for those with greater circadian misalignment. Future research should consider the role of sex within factors such as scheduling,

athlete management, and sport professionalism. Sports practitioners should see value in striving for circadian alignment and protecting at-risk athletes.

93. Uncoupling of behavioural and metabolic rhythms in an arctic ruminant

Main author: Sara Meier
Affiliation: University of Zurich
Co-authors: Melanie Furrer, Nora Nowak, Monica A. Sundset, Reto Huber, Renato Zenobi, Steven A. Brown*, Gabriela Wagner* (*co-corresponding)
Keywords: Metabolomics, Seasonality, Arctic physiology, Rumination

Background/Objectives: Studies in mice and humans report 10-30 % of blood metabolites display circadian rhythms of abundance, but these rhythms can be disrupted by altered daily schedules. Adapting to the extreme changes in environmental light in the Arctic, Reindeer (Rangifer tarandus) display a 24-hour activity rhythm following the light-dark signal in spring and fall, but become behaviourally arrhythmic in constant light and darkness during arctic summer and winter. Little is known about how these changing seasonal light conditions affect metabolic rhythms.
Methods/Results: Four Eurasian tundra reindeer (R. t. tarandus) kept under semi-natural conditions were brought indoors for 2-hourly blood plasma collection across 24 hrs in four seasons at The Arctic University of Norway (69°N). Using liquid chromatography-mass spectrometry (LC-MS) we measured and putatively identified 893 metabolic features, and determined their 24-hour rhythms of abundance for each season. Half the detected features were rhythmic during at least one season (q-value ≤ 0.1). A core set of 66 features remained rhythmic in at least 3 seasons. More rhythmic metabolites (315 features) were observed in winter and spring and fewer rhythmic metabolites (65 features) were observed in summer and fall. Different pathways were rhythmic among those groups. Examining the loss of metabolic rhythmicity in summer and fall, some metabolites instead show ultradian sleep-wake/feeding dependence.
Conclusions: Remarkably, seasonal changes in daily metabolic rhythms appear to be uncoupled from behavioural rhythms. We propose more metabolic pathways are rhythmically employed during seasons when endogenous energy stores are being utilised, and likely ultradian metabolism is employed during seasons when these stores are being replenished, analogous to circadian disruption and weight gain in other rhythmic species. This flexibility allows maximal utilisation of available resources while simultaneously allowing reindeer to adjust to the extremely diverse metabolic needs across the arctic seasons.

94. Are the benefits of bright light therapy dependent on the activity of the SCN?

Main author: Dan-Adrian Epuran
Affiliation: University of Fribourg
Co-authors: Urs Albrecht, Jürgen A. Ripperger
Keywords: Seasonal Affective Disorder, Bright Light Therapy, Period1, Lateral Habenula, SCN, Phase Advance

Mood disorders represent a major toll on the health of people, severely deteriorating the quality of life, with seasonal affective disorder (SAD) being such an example. As a first line of treatment for SAD, bright light is administered. Light therapy has been shown to affect mood in humans. Meanwhile, the involvement of the circadian clock on the molecular mechanisms that translate positive effects of light in mood remain poorly understood. Using mice as an animal model, our lab has identified clock gene Period1 (Per1) as a component required to mediate the benefits of light. Per1 deletion in the lateral habenula, a region involved in controlling mood-related behaviors, led to suppression of the valuable

effects of light applied at zeitgeber time (ZT) 22. A light pulse at ZT22 (analogous to bright light therapy) is associated with a phase advance which has been proven to ameliorate mood in depressive patients. Hence, we are interested in seeing if mice who do not present this advance still profit from the light pulse. To that extend, we will be using mice that have a Per1 knock-out in the suprachiasmatic nuclei (SCN). While their locomotor activities will be recorded, a light pulse will be applied at ZT14 (phase delay) and ZT22 (phase advance) and compared to a control group. We hypothesize that Per1 knock-out group will not show any phase advance. Both groups will then undergo the behavioral experiments of Forced Swim Test (FST), sucrose preference test (SPT) and the O-maze test. These results will be consolidated at the biochemical level by looking at changes in the rate limiting enzymes involved in the dopamine metabolism, tyrosine hydroxylase and monoamine oxidase. Taken together, this experimental design can help us conclude if the light induction of Per1 in the lateral habenula is sufficient for the beneficial effects of light or whether phase advances mediated by the SCN are necessary.

95. Spatiotemporal organisation of PER2::LUC expression in the mouse dorsal vagal complex – a multicomponent circadian timing centre

Main author: Lukasz Chrobok
Affiliation: University of Bristol; School of Physiology, Pharmacology, and Neuroscience
Co-authors: Jake Ahern, Charlotte Muir, Alan Champneys, and Hugh D. Piggins

Keywords: dorsal vagal complex; extra-SCN oscillator; PER2::LUC; bioluminescence; phase oscillator model

The dorsal vagal complex (DVC) is a multicomponent hindbrain centre processing metabolic and cardio-vascular cues, many of which exhibit daily and/or circadian variation. The DVC is composed of three neuronal structures: the area postrema (AP; a circumventricular organ lacking functional blood-brain barrier), the nucleus of the solitary tract (NTS; a hindbrain satiety centre and a major recipient of vagal afferent signals), and the dorsal motor nucleus of the vagus nerve (DMV; a source of vagal efferents). The DVC is in close proximity to the fourth ventricle/central canal which is lined by an ependymal cell layer formed by tanycyte-like cells (referred to here as 4Vep). Recently, we found robust circadian timekeeping properties in the mouse DVC as manifested in rhythmic PERIOD2::LUCIFERASE (PER2::LUC) expression ex vivo in the AP, NTS, and 4Vep (Chrobok et al. Comms Biol 2020). Here, with the use of further bioluminescence recordings and mathematical modelling, we explored the spatiotemporal patterning of PER2::LUC expression in the DVC. We determined that AP is the most robust oscillator, with its cellular components maintaining synchrony and dampening much more slowly than those of adjacent NTS and DMV. Interestingly, compared to the AP/NTS, the non-neuronal oscillators of the 4Vep were phase delayed (about 8h) and exhibited a significantly shorter period. These experimental observations served as a basis for a phase oscillator model of the DVC the outcome of which supported the view that the AP acts as the coordinator of other DVC oscillators. Collectively, our experimental and modelling studies revealed interactions of neuronal and non-neuronal circadian oscillators in the hindbrain which will need to be taken into account for furthering understanding of the functional significance of circadian timekeeping in the DVC.

96. Light-dependent rhythms in Per2 knock-out mice

Main author: Andrey Lazopulo
Affiliation: Université de Lausanne
Co-authors: Francesco LaSpada, Yann Emmenegger, Cecilia Diniz Behn, Paul Franken
Keywords: Circadian clock, Period2, Light, Mathematical model, Mouse

Background. Period2 (Per2) encodes one of the key circadian clock proteins. Knowledge about the role of PER2 in generating rhythms in overt behaviors is, however, based largely on mice carrying mutant Per2 alleles, which still express (altered) PER2 protein. Upon release in constant darkness (DD) Per2 mutants initially show circadian rhythms of short period that deteriorate with time. Here we describe a null allele Per2[tm1Ccl] (Fu et al., 2002) that has not been behaviorally characterized in detail.
Results. Mice homozygous for the Per2[tm1Ccl] allele (KO) showed a more severe phenotype than the widely used mutants and we found that free running rhythmicity strongly depended on the history of light exposure prior to DD; i.e., when kept under standard LD12:12 KO mice immediately became arrhythmic, while after LD11:11 or after LD12:12 followed by 1 day under LD 10:14 mice free ran with short period (22.2 h). A single light pulse could restart rhythmicity in arrhythmic KO mice. Under constant light conditions (LL), KO mice maintained rhythmicity with a longer period than under DD (25.3 h). Curiously, after 10 days under LL period abruptly shortened to 23.5 h. We constructed a mathematical model that relied on two differential equations that undergo Hopf bifurcation. The bifurcation parameters decreased during light exposure and were reset at light onset. The model could recapitulate many aspects of the effect of light on circadian behavior in KO mice.
Conclusions. Behavioral analysis of Per2 null allele revealed a unique set of circadian phenotypes. Rhythmicity under DD highly depended on prior light/dark schedules suggesting that PER2 is involved in processing light duration in the circadian clock. The model explains the KO phenotypes and can be further utilized to uncover the role of PER2 in the overt rhythms.

97. Plasma metabolite rhythms in entrained vs constant routine protocols using targeted LC-MS/MS metabolomics

Main author: Namrata Chowdhury
Affiliation: PhD student
Co-authors: Namrata R. Chowdhury[1], Victoria L. Revell[1,2], Daan van der Veen[1], Benita Middleton[1] and Debra J. Skene[1]
[1]Chronobiology, Faculty of Health and Medical Sciences, University of Surrey, Guildford, UK.
[2]Present address, Surrey Sleep Research Centre, Faculty of Health and Medical Sciences, University of Surrey, Guildford, UK.
Keywords: -

Our previous studies[1,2] have shown that a large proportion of metabolites (55-75%) are rhythmic under entrained laboratory conditions. We have now conducted a constant routine study; comparison of plasma metabolite rhythms between protocols will not only allow us to differentiate circadian and diurnal rhythms but also the acute effect of timed meals, sleep and fasting on metabolite profiles.
The constant routine (constant dim-light (<5 lux), semi-recumbent posture, continual wakefulness, hourly isocaloric snacks) and the entrained protocol (light/dark cycle, standardised meals, postural changes, sleep) involved an equal number of healthy, young males and females (n=30 in CR and n=24 in entrained; mean±SEM 23.7±0.5 years, females taking oral contraceptives). Targeted LC-MS/MS metabolomics1,2 measured approximately 130 metabolites from five different metabolite

classes (amino acids, biogenic amines, acylcarnitines, glycerophospholipids and sphingolipids) in 2-h plasma samples collected across 28 h and 34 h in the 40-h CR and entrained protocol, respectively. In the entrained protocol, in both sexes, most amino acids (~80%) showed a direct response to meals but subsequently declined in concentration during the sleep/fasting period. By contrast, in the CR protocol, amino acids peaked during the night of wakefulness. Metabolites from the other chemical classes did not show an obvious response to the large meals.
In the CR protocol, a small proportion of metabolites, 21% (n=27/127) in males and 18% (n=23/127) in females, were found to be rhythmic (mainly amino acids and lyso PCs) in contrast to the entrained protocol (69% (n=97/141) in males; 73% (n=95/130) in females). The majority of acylcarnitines, phospholipids and sphingolipids lost their rhythmicity in the CR protocol. These findings suggest that exogenous factors (feeding/fasting, sleep/wake, rest/activity and light/dark cycles) predominately drive circulating metabolite rhythms. Funded by BBSRC (BB/I019405/1)

[1]DAVIES, S.K. et al., 2014 PMID: 25002497
[2]HONMA, A. et al., 2020 PMID: 30929284

98. Alterations in Adar2 rhythm expression and RNA editing of Kcna1, 5-HT2CR and Gria2 in the hippocampus in adulthood, as the result of constant light during early postnatal development

Main author: Aneta Kubištová
Affiliation: Department of Physiology, Faculty of Science, Charles University, Czech Republic
Co-authors: Veronika Spišská, Zdeňka Bendová, Aneta Kubištová[1], Veronika Spišská[1], Zdeňka Bendová[1,2]
[1]Department of Physiology, Faculty of Science, Charles University, Czech Republic
[2]National Institute of Mental Health, Klecany, Czech Republic
Keywords: circadian clock; light at night; hippocampus; Adar2; RNA editing

Exposure of rats to constant light conditions during early postnatal development leads to a series of systemic changes that persist into adulthood. Previous studies describe long-term changes in mammalian physiology and circadian system, such as changes in the rhythms of clock gene expression, an increased incidence of anxiety-like behaviour, or a reduced ability of the circadian system to phase shift in response to light pulses. Our previous study demonstrated the circadian rhythmicity of adenosine deaminase acting on RNA 2 (ADAR2)-dependent adenosine-to-inosine editing (A-to-I) in Gria2 in several brain structures including the hippocampus [1]. Furthermore, we have shown that housing in constant light in early postnatal development induces long-lasting changes in gene expression profiles in the brain [2]. In this study, we examined the Adar2 expression rhythm and the changes in A-to-I editing in its substrate genes Kcna1, 5-HT2CR and Gria2 in the hippocampus of animals maintained in constant light from day P0 to P20. Animals were then released to LD12:12 conditions and samples of the hippocampus were collected from 30-day-old rats. Our data suggest several changes in the A-to-I editing ratio of Kcna1, 5-HT2CR and Gria2 mRNA, as well as changes in the expression rhythm of Adar2 in the hippocampus. These results suggest long-term changes in the post-transcriptional editing process by constant light, which could have potential implications for hippocampal physiology.

1. Míková, H. et al. (2021). Circadian Regulation of GluA2 mRNA Processing in the Rat Suprachiasmatic Nucleus and Other Brain Structures. Molecular neurobiology

2. Kubištová, A. et al. (2020). Constant Light in Critical Postnatal Days Affects Circadian Rhythms in Locomotion and Gene Expression in the Suprachiasmatic Nucleus, Retina, and Pineal Gland Later in Life. Biomedicines

99. The multiverse of human light exposure analyses

Main author: Anna M Biller
Affiliation: Chronobiology & Health, Technical University Munich, Germany
Co-authors: Dr. Grégory Hammad, Prof. Dr. Manuel Spitschan
Keywords: human light exposure, dosimetry analyses, analytic flexibility

Background: Light exposure can both improve and disrupt health and well-being. Most evidence concerning the impact of light exposure comes from well-controlled laboratory studies employing parametric light stimuli. However, personalised light exposure in the real world is time-dependent and highly variable. Consequently, we lack a nuanced understanding of the statistical properties of personalised light exposure. This knowledge gap is partially due to the following challenges: (1) How to best measure human light exposure, including the choice of measurement devices, their properties, and their placement, (2) how to best quantify and summarise time-dependent light exposure, (3) how to achieve reproducible and robust results given a high degree of analytic flexibility.
Methods: Here, we focus on challenges (2) and (3). We subject the wrist-referenced light dosimetry data from the Multi-Ethnic Study of Atherosclerosis data set (MESA; Zhang et al., 2018; Chen et al., 2015; n=2155, mean recording duration 7 days) to a range of light-related metrics implemented in pyActigraphy (Hammad et al., 2021), including summary statistics over intensity (e.g., linear and log mean, median) and timing (e.g., time above threshold). We then analyse how these metrics are statistically related to each other to understand which metrics capture overlapping information. To address the question of analytic flexibility, we develop a “multiverse” analysis in which we vary various parameters, including bin size. Finally, we simulate missing data of different durations to understand the sensitivity of metrics to incomplete data.
Results: We hypothesise to find considerable statistical overlap in various metrics. We also characterise the analytic flexibility inherent in light dosimetry analyses using a specification curve analysis, which visualises the outcome variable as a function of various choices of analytic parameters.
Conclusion: We provide a systematic and data-driven method for exploring and characterising light exposure patterns in humans.

100. Chronic shift impairs the daily reproductive rhythms of female mice

Main author: Marine Simonneaux
Affiliation: Institute of Cellular and Integrative Neuroscience (CNRS UPR 3212) and University of Strasbourg
Co-authors: Mathilda Kretz, Thibault Bahougne, Paul Klosen, Nathalie Jeandidier, Valérie Simonneaux
Keywords: circadian disruption, female reproduction, kisspeptin, luteinizing hormone, shift work

In female mammals, the timing of the preovulatory LH surge depends on the combination of the positive estrogen feedback and a circadian signal which synchronizes the LH surge with the transition between the resting and active period at the end of the follicular phase, when arousal is maximal. Since the correct timing of the LH surge is critical for optimal fertility, we are exploring how a chronodisruptive environment alters the female mammals’ gonadotropic axis. Adult female mice were

either kept in regular light/dark schedules or exposed to a chronic shift (successive rotations of 10-hour phase advance for 3 days followed by 10-hour phase delay for 4 days, a model of shift work conditions). Daily LH secretion and daily activity of the anteroventral periventricular nucleus kisspeptin neurons, as well as fertility parameters, were then compared between both groups of mice. The chronodisruptive protocol abolished the preovulatory LH surge and the activation of kisspeptin neurons typically observed at the light/dark transition of the day of proestrus. Furthermore, when shifted female mice were mated with a control male, their fertility was significantly decreased. The results show that chronic exposure to shifted light/dark schedules severely alters reproductive activity, with an impaired kisspeptin-regulation of the preovulatory LH surge leading to a reduction in gestational success. We are currently investigating the effect of this experimental model of shift work results in changes on the activity of the vasopressin-containing neurons located in the suprachiasmatic nucleus known to transmit the daily information to the kisspeptin neurons. In future experiments, we will investigate whether the peripheral clocks within the gonadotropic axis are also altered by chronic shift. Altogether, these experiments will provide a better understanding of the potential impact of circadian disruption on the daily reproductive rhythms of female mammals.

101. Circadian rhythms of RNA-Binding Motif 3 (Rbm3) in the suprachiasmatic nucleus (SCN)

Main author: Marieke Hoekstra
Affiliation: UK Dementia Research Institute at Imperial College London, London, UK; Department of Brain Sciences, Imperial College London, London, UK.
Co-authors: Natalie Ness, Marco Brancaccio

Keywords: temperature, SCN, RBM3

Cold-inducible RNA-Binding Motif 3 (RBM3) mRNA levels shows daily variations and is reduced following sleep loss (Maret et al., PNAS 2007). RBM3 overexpression can revert synaptic and behavioural symptoms in mouse models of neurodegeneration (Peretti et al., Nature 2015). RBM3 manipulation could therefore be a therapeutic target to counteract the negative consequences of sleep loss. However, little is known about the circadian regulation of RBM3 function in the brain.
We therefore designed a Rbm3 transcriptional reporter driving expression of luciferase (Rbm3-luc) and used adeno-associated vectors (AAV) to deliver this to ex vivo SCN slices. AAV-Rbm3-luc showed high-amplitude circadian oscillations in the SCN peaking 4hrs after neuronal calcium.
With this tool, we compared to what extent neuronal and astrocytic cellular clocks in SCN slices contributed to Rbm3-luc circadian oscillations. Neuronal but not astrocytic Bmal1 deletion reduced Rbm3-luc rhythm robustness, as reflected by a doubling of the relative amplitude error, and halved its amplitude.
Because RBM3 is a cold-induced protein, we next investigated how changing temperature from 37°C to 32°C and vice versa affected Rbm3-luc activity and rhythms. Decreasing temperature to 32°C led to a strong increase in overall Rbm3-luc activity, and when increasing temperature back to 37°C, Rbm3-luc's activity is initially suppressed for the first two cycles. Surprisingly, the decrease in temperature specifically elongated Rbm3-luc but not PER2::Luc oscillations, suggesting a misalignment of the two rhythms with temperature changes.
Taken together, Rbm3 transcriptional activity has neuronal-driven circadian oscillations in the SCN, with overall expression levels and periodicity sensitive to changes in temperature. These results show that circadian modulation of RBM3 may be an additional aspect to consider in the development of therapeutics when aiming to manipulate the endogenous expression of this neuroprotective protein.

102. The circadian clock and tight junctions interact in epithelial cells

Main author: Nemanja Milićević
Affiliation: Faculty of Medicine and Health Technology, Tampere University, Finland
Co-authors: Elina Mäntylä, Bobin George Abraham, Teemu O. Ihalainen, Soile Nymark
Keywords: clock genes, tight junctions, transepithelial electrical resistance, epithelia, RPE

Tight junctions (TJs) are multiprotein complexes that bind epithelial cells creating barriers for various solutes. A number of TJs are regulated by the clock resulting in daily variations of epithelial barrier permeability. Recently, TJs have been implicated in regulating gene expression. We tested the hypothesis that the circadian clock and TJs mutually interact. We found that serum-shock synchronization induces a peak in transepithelial electrical resistance (TEER) in embryonic stem cell-derived retinal pigment (hESC-RPE) and Madin-Darby Canine Kidney II (MDCK) epithelial cells. By RT-PCR, we found rhythmic expression of clock genes BMAL1, PER1, PER2 in hESC-RPE and MDCK cells. Bioinformatics analysis of published RPE and kidney (for MDCK) transcriptomics datasets revealed lists of clock, clock-controlled and time-affected tight junction genes. STRING analysis revealed networks of interacting genes that link the clock and TJs involving ACTB, CLDN2, CLDN4 in the RPE and SEC13, CGNL1, CLDN16 in the kidney. We validated these pathways by RT-PCR. In real-time TEER measurements (by CellZscope), TEER was suppressed by the ROR agonist Nobiletin in MDCK cells, but enhanced in hESC-RPE cells. Conversely, the REV-ERBα agonist SR9009 enhanced TEER in both MDCK and hESC-RPE cells. RT-PCR analysis revealed that Nobiletin suppressed the mRNA expression of CLDN2, and SR9009 suppressed TJP1 mRNA expression in hESC-RPE at the peak TEER time-point. Thus, the clock-mediated regulation of TJs in epithelia is most likely tissue-specific and is also regulated by yet unknown post-transcriptional pathways. The potential reciprocal link between TJs and the clock was tested in MDCK cells lacking TJs ZO1 and ZO2. MDCK ZO1-/-ZO2-/- cells showed shorter and attenuated oscillations of BMAL1 gene expression compared to WT; and single knock-outs of ZO1 and ZO2 in MDCK cells. These preliminary observations suggest that TJs ZO1 and ZO2 extend periods and amplify amplitudes of clock gene oscillations.

103. Mechanical control of the fibroblast circadian clock via YAP/TAZ

Main author: Juan F. Abenza
Affiliation: Institute for Bioengineering of Catalonia (IBEC)
Co-authors: Juan F. Abenza (presenter), Leone Rossetti, Malèke Mouelhi, Javier Burgués, Ion Andreu, Keith Kennedy, Pere Roca-Cusachs, Santiago Marco, Jordi García-Ojalvo, Xavier Trepat
Keywords: Mechanics, circadian, Rev-erb, YAP, Hippo, mechanotransduction, cell density, peripheral clocks

Cells sense and respond to the mechanical properties of their external environment. This sensing is accomplished thanks to a diverse set of biochemical pathways which impact on gene expression, subsequently affecting key cellular processes like proliferation and differentiation. Very recently, mechanics has been observed to also affect circadian rhythms, further broadening its importance in tissue homeostasis.
Our project aims to clarify the influence of mechanobiological hallmarks on the regulation of the fibroblast circadian clock. We have used NIH3T3 cells expressing Venus fluorescent protein under the promoter of the circadian gene Rev-erbα (RevVNP), confocal microscopy and customised computational analysis. Our results indicate that RevVNP basal and circadian expression depends on

cell density. By performing gap closure experiments, we observed that RevVNP expression, typically low and rhythmic, is perturbed upon cell migration.
To disentangle the pathway that influences Rev-erbα transcription upon cell density changes, we used fibronectin micropatterning. Confined cells on single cell-sized areas displayed RevVNP circadian oscillations like those of confluent cells. Next, we stopped the migration of cells at low density by altering their actin dynamics with jasplakinolide and latrunculinA and observed the striking emergence of robust circadian oscillations, unlike the case of untreated single cells.
We then checked the localization of two prototypical mechanosensitive transcriptional regulators, YAP/TAZ and MRTFA, in the aforementioned compendium of conditions. We observed a strong anticorrelation of RevVNP circadian robustness and YAP/TAZ nuclear levels but not with those of MRTFA. To test if YAP/TAZ regulate the clock directly, we overexpressed dominant positive mutants of YAP/TAZ. This caused a huge impairment of the RevVNP oscillations, which demonstrates a novel role of YAP/YAZ as a circadian modulator.
Considering the role of YAP/TAZ as core mechanosensors and the metabolic importance of REV-ERB, our findings provide a fundamental link between the largely disconnected fields of chronobiology, metabolism and mechanobiology.

104. Spontaneous and GRP-evoked activity in the tuberoinfundibular dopaminergic network of the rat mediobasal hypothalamus

Main author: Jake Ahern
Affiliation: School of Physiology, Pharmacology and Neuroscience, University of Bristol
Co-authors: David Lyons, Alan Champneys and Hugh D. Piggins

Keywords: tuberoinfundibular dopaminergic neurons, TIDA, prolactin, GRP, multi-unit arrays, circadian

The brain contains an array of oscillators, with periods ranging from milliseconds and hours (ultradian) to 24h (circadian) and longer (infradian). These oscillators are pivotal for generating and shaping rhythms in physiology and behaviour. The neuroendocrine axis is no exception and here we focused on a distributed network in the mediobasal hypothalamus containing the arcuate nucleus (ARC) and surrounding ventromedial hypothalamus (VMH). Particular attention was paid to the tuberoinfundibular dopaminergic (TIDA) neurons which display a distinct ultradian pattern of phasic neuronal activity. The TIDA population controls pituitary prolactin secretion via the release of inhibitory dopamine into the hypophysial vasculature. Under baseline conditions, there is a daily rhythm in both neuroendocrine dopamine and prolactin concentration, however, the basis of these nychthemeral rhythms is incompletely understood. The master circadian clock in the suprachiasmatic nucleus (SCN) could be a potential driver of prolactin rhythmicity via its interaction with the TIDA circuit and its presynaptic partners. Gastrin-releasing peptide (GRP) is a peptide output of the SCN and we investigated if GRP could alter TIDA network activity to contribute to the daily oscillation in neuroendocrine dopamine and corresponding prolactin release.

Multi-electrode grids were used to record the extracellular action potential activity from the ARC and VMH of male rats both spontaneously and following application of GRP and the related peptides neuromedin-B and -C. Spontaneous activity was used to quantitatively characterise the network organisation of TIDA-like oscillations, which we found to be more prominent in the medial-rostral ARC, adjacent to the wall of the third ventricle. The peptides GRP and neuromedin-B and -C induced a dramatic change in TIDA-like network activity across the ARC, characterised by a switch from phasic to tonic discharge. Further, we show that TIDA-like units reside in a wider GRP-responsive network extending across the ARC and VMH. This switch in activity may constitute a multi-

component mechanism by which GRP is able to tune TIDA inhibitory output, and hence prolactin release, to circadian time.

105. Selection for synchronised development using timeseries properties of the circadian clock

Main author: Sarah Lock
Affiliation: University of York
Co-authors: Marina Knight, Seth J. Davis and Daphne Ezer
Keywords: -

It is important to ensure that crops are uniform at harvest time to maximise yields. However, even isogenic plants grown in the same environment may develop asynchronously. We hypothesised that circadian clock and photoperiod entrainment genes would help control developmental synchrony, by helping plants synchronise their daily gene expression pattern and adapt to the change in seasons. To identify genes that jointly control photoperiod response and developmental synchrony, we investigated the effect of photoperiod changes in a recombinant inbred line (RIL) population between two Arabidopsis ecotypes adapted to living at extreme latitudes. This work uses branch of statistical modelling called functional data analysis (FDA) in conjunction with QTL identification to investigate the way in which properties of the circadian clock influence how well plants sense and respond to changes in photoperiod, and how this ultimately impacts the stochasticity of developmental traits.
The data consisted of circadian rhythm measurements that were expressed through the CCR2 gene measured by luciferase imaging of living plants. Plant circadian rhythms such as CCR2 adjust their phase and amplitude over time directly in response to changes in photoperiods, ultimately this means these rhythms are a result of a non-stationary oscillator. For this reason, it is suitable to view these data using FDA approaches, where the circadian rhythms are transformed from discrete observations to clearly defined functions. These mathematical functions of time are then able to be explored to reveal numerous otherwise unidentifiable traits. Viewing these data as functions allowed for a thorough and detailed exploration including identification of time dependent QTLs. This analysis suggests additional genetic loci that influence photoperiod detection and developmental synchrony, suggesting targets for future breeding efforts.

106. Ultradian excitation-inhibition temporal dynamics in dorsal hippocampus across the circadian day

Main author: Nicolette Ognjanovski
Affiliation: University of Michigan, Leiden University Medical Center
Co-authors: David S. Kim, Emma Charlett- Greene, Sofie van Koppen, Jos H.T. Rohling, Sara J. Aton, Brendan O. Watson, Stephan Michel
Keywords: Hippocampus, Ultradian, network connectivity, temporal dynamics, sleep, electrophysiology,

Twenty-four-hour rhythmicity in physiology and behavior is driven by changes in neuronal activity that varies across the light–dark cycle. This circadian rhythmicity is most prominent in neurons of the brain's central pacemaker the suprachiasmatic nucleus but is also found in other brain regions. This includes the hippocampus, which shows circadian rhythmicity in both clock-gene expression and synaptic plasticity. However, it is currently unknown whether electrical properties of the hippocampus are modulated by the light-dark cycle. Since new experimental data is converging towards a critical role of oscillations in connecting activity to sensory processes- such as light perception, we implanted tetrodes into CA1 of C57BL/6J mice to identify circadian patterns in electrical activity in the

hippocampus across the day. We recorded individual cells' firing rates, local field potentials, and assessed connectivity and E/I balance for 24hr across a 12:12 cycle. We then quantified dynamics on an hour-by-hour basis to report the following findings: I. Fast-spiking inhibitory interneurons show ultradian rhythmic activity across the day, with peaks in firing at light transitions, while excitatory principal cells' firings remain relatively static. II. Sleep-associated NREM delta (0.5-3Hz) and sharp-wave ripples (SPWRs- 150-250Hz) as well as REM and waking theta (4-10 Hz) are temporally dynamic. Delta and SPWRs were comparatively higher during NREM occurring in the light phase. III. Measures of connectivity, stability, and E/I balance show that hippocampal network structure is not static across the day but fluctuates with inhibitory cell firing (peak at ZT6). These results show for the first time the dynamic baseline structure of hippocampal electric activity. Network structure seems to reorganize over time, possibly according to the behavioral demands of the rest-activity cycle. Specifically, stability and E/I balance are shown to be indicative of a critical network state, which may indicate an optimal timing of learning in the hippocampus.

107. Effects of the postnatal methamphetamine administration on the clock and immune status of suprachiasmatic nuclei and extra-SCN brain oscillators

Main author: Kateryna Semenovykh
Affiliation: Laboratory of Biological Rhythms, Institute of Physiology, Czech Academy of Sciences, Prague, Czech Republic
Co-authors: Houdek P., Honzlová P., Šlamberová R., Sumová A.

Keywords: methamphetamine, development, choroid plexus, glial cells, neuroinflammation, suprachiasmatic nuclei

Methamphetamine (MA) is one of the most widely used psychostimulants and is becoming increasingly popular among pregnant women and nursing mothers. However, the effects of maternal MA abuse on offspring brain function later in adulthood have not been well recognized. The aim of this study was to investigate the impact of MA exposure during the early postnatal period on the clock and/or immune status of various brain regions, including the suprachiasmatic nuclei (SCN), dentate gyrus, and barrier complexes of various ventricles (choroid plexus and cerebrospinal fluid-brain barrier). Rat pups received SHAM or MA injections daily for 12 days from birth and were sacrificed at two different time points (midday and midnight) two weeks and three months later, respectively. Analyses of expression patterns of clock gene and anti-/proinflammatory markers by RT-qPCR revealed that 2 weeks after the treatment, MA significantly suppressed expression of the clock genes and activated the proinflammatory response in the SCN. The clock in the barrier complex of the 3rd ventricle was slightly downregulated, and interestingly, the barrier complex of the 4th ventricle was affected less. Furthermore, immunohistochemical analysis confirmed that treatment with MA activated microglia and astrocytes in the SCN and dentate gyrus. Consistent with the RT-qPCR results, MA increased IBA1 immunopositivity in the barrier complex of the 3rd but not the 4th ventricle. Although MA treatment in pups had no effect on their locomotor activity rhythm in adulthood, some of the changes detected in the SCN and third ventricle barrier complex persisted even 3 months after the MA withdrawal. The results demonstrate that early postnatal exposure to MA has significant acute and long-lasting effects on the clock and immune status of neuronal and non-neuronal cells in the brain.

108. Synaptic plasticity induces sleep-wake transitions in large-scale computational models

Main author: Guanhua Sun
Affiliation: University of Michigan
Co-authors: Daniel Forger
Keywords: sleep-wake transition, synaptic plasticity, calcium channel, computer simulation

Understanding sleep has been a central topic in sleep research. Researchers have raised different hypotheses and computational models to explain sleep and sleep-wake transitions. However, most of these models achieve sleep-wake transitions by directly changing the electrophysiology of neurons and ignore many electrophysiological or synaptic processes that are known to affect sleep dynamics. Using a newly built simulation platform that can parallelly simulate hundreds of thousands of neurons with full electrophysiology as fast as in real time, we simulate a network of spiking cortical neurons that include calcium signaling. In this network, we also simulate a calcium synaptic plasticity model that regulates the synaptic weights between the neurons. Surprisingly, we observe that the spontaneous fluctuating calcium dynamics can affect the synaptic weight distribution, which further causes the whole network to transition between sleep and wake. Alternating extracellular calcium concentrations can also induce sleep-wake transitions of the network, a phenomenon that has been known from past experiments.

109. Endurance capacity is shaped by clock proteins and exercise training in a day-time dependent manner

Main author: Yaarit Adamovich
Affiliation: Department of Biomolecular Sciences, Weizmann Institute of Science, 7610001, Rehovot, Israel
Co-authors: Vaishnavi Dandavate, Saar Ezagouri, Gal Manella, Ziv Zwighaft, Jonathan Sobel, Yael Kuperman, Marina Golik, Asher Auerbach, Maxim Itkin, Sergey Malitsky, and Gad Asher
Keywords: Exercise, Metabolism, Training, Glycogen

Exercise and circadian biology are closely intertwined with physiology and metabolism, yet the functional interaction between circadian clocks and exercise capacity is only partially characterized. Here we tested different clock mutant mouse models to examine the effect of the circadian clock and clock proteins, namely PERIODs and BMAL1, on exercise capacity. We found that day-time variance in endurance exercise capacity is circadian clock controlled. Unlike wild type mice, which outperform in the late compared to the early part of their active phase, PERIODs and BMAL1 null mice do not show day-time variance in exercise capacity. As it appears that BMAL1 impairs and PERIODs enhance exercise capacity in a day-time dependent manner. Analysis of liver and muscle glycogen stores as well as muscle lipid utilization suggested that these day-time effects mostly relate to liver glycogen levels and correspond to the animals' feeding behavior. Furthermore, given that exercise capacity responds to training, we tested the effect of training at different times of the day and found that training in the late compared to the early part of the active phase better improves exercise performance. Overall, our findings suggest that clock proteins shape exercise capacity in a day-time dependent manner through changes in liver glycogen levels, likely due to their effect on animals' feeding behavior.

110. Lack of Per2 increases aquaporin-4 localisation to astrocytic endfeet contacting the peri-vascular space of the glymphatic system

Main author: Katrin Wendrich
Affiliation: University of Fribourg
Co-authors: David A. Parker, Jürgen A. Ripperger, Felix Meyenhofer and Urs Albrecht
Keywords: Aquaporin4, glymphatic system, Period2 KO, astrocytes

The glymphatic system plays a critical role in brain metabolic homeostasis and waste clearance. These prcesses are time of day dependent and involve the water channel aquaporin-4 (AQP4), a component of the dystrophin-associated complex (DAC). AQP4 channels are highly enriched at astrocytic endfeet ensheating the perivascular space of the gylmphatic system to promote movement of the cerebrospinal fluid (CSF). Interestingly, perivascular localization of AQP4 is highest during the rest phase. How the daily change of AQP4 localization in astrocytes contacting the perivascular space is regulated is not well understood. In order to investigate a role of the circadian system in this process we analyzed AQP4 distribution in mice lacking the clock gene Period 2 (Per2) during light and dark with immunohistochemistry and examined gene expression levels of AQP4 and other DAC proteins in specific brain regions around the clock. We found an overall increase of AQP4 localization to the astrocytic endfeet in Per2 KO mice compared to controls in the light phase (rest), as well as in the dark phase (activity). Correspondingly, gene expression of AQP4 and proteins of the DAC around the clock were significantly increased in the cortex of Per2 KO mice. This indicates that Per2 in the brain is involved in the regulation of AQP4 localization in astrocytes potentially affecting CSF flow. This opens new avenues in studying the regulation of the glymphatic system.

111. Circadian regulation of protein turnover by muscle peripheral clock is required for muscle mass homeostasis

Main author: Jeffrey Kelu
Affiliation: King's College London
Co-authors: Jeffrey J. Kelu[1], Siegfried Labeit[2,3], and Simon M. Hughes[1]
[1]Randall Centre for Cell and Molecular Biophysics, King’s College London, SE1 1UL, London, UK
[2]Department of Anesthesiology, Medical Faculty Mannheim, University of Heidelberg, 68169 Mannheim, DE
[3]Myomedix GmbH, 69151 Neckargemünd, DE
Keywords: circadian, muscle, protein turnover, ubiquitylayion, proteasome, autophagy

Muscle tissue displays circadian variations in function, physiology, and metabolism. We previously showed that muscle grows faster during the day than at night, and such diurnal difference 1) persists under constant conditions, 2) requires the function of the molecular clock, and 3) is independent of physical activity and nutrition. Mechanistically, TORC1 signalling contributes to day-time growth; whereas proteasomal activities constrain night-time growth. To test the cell-autonomous role of the muscle peripheral clock, we expressed dominant-negative CLOCK protein specifically in muscle tissues (mΔCLK) using zebrafish. mΔCLK perturbed muscle clock output, reduced muscle size, and altered circadian muscle growth by enhancing muscle growth at night (but no effect during the day). This suggests nocturnal muscle protein turnover and growth are regulated by the muscle clock. Treatment with either 1) muscle-specific E3 ubiquitin ligase MuRF inhibitor myomed-205, or 2) autophagy inhibitors MRT68921/ bafilomycin A1 phenocopied the effect of mΔCLK by specifically augmenting night-time muscle growth. This implicates the involvement of ubiquitin-proteasome system (UPS) and autophagy in nocturnal protein turnover. We showed that genes code for MuRFs

and autophagy processes exhibit nocturnal oscillations, but such oscillations were disrupted in mΔCLK fish, leading to defective proteasomal and autophagic flux. Muscle function was reduced, though sarcomeric structures appeared unaffected, after muscle clock inhibition. Together, we conclude that circadian regulation of protein turnover via UPS and autophagy by the muscle clock is crucial for the optimal growth and function of muscle.

112. Disruption of dorsomedial hypothalamic rhythms under high-fat diet can be prevented by restricted nighttime feeding

Main author: Anna Sanetra
Affiliation: Jagiellonian University in Krakow, Poland
Co-authors: Palus-Chramiec K, Chrobok L, Jeczmien-Lazur JS, Gawron E, Klich JD, Pradel K, Lewandowski MH

Keywords: restricted feeding, hypothalamus, food entrainment, high-fat diet

Circadian regulation of feeding behaviour is controlled by the food-entrainable oscillator, the main part of which is considered to reside in the Dorsomedial Hypothalamus (DMH). This brain structure not only participates in the metabolic state-dependent enhancement of or decline in food intake, but also incorporates the circadian timing phase into the regulatory processes, thanks to day/night changes in its cellular activity. In this study we investigated whether and how the physiological circadian rhythms of DMH neurons change under high-fat diet (HFD), the most commonly used model for the induction of obesity. As we were looking to study the possible causes, rather than effects of obesity, we fed the animals either control (CD) or HFD for 3-4 weeks only, which precedes excessive weight gain of HFD-fed rats. Following, we performed both immunohistochemical and electrophysiological experiments, which revealed an impairment of the day/night rhythm in cFOS immunoreactivity and neuronal activity, as well as sensitivity to various metabolically relevant peptides under HFD. Parallelly we observed that HFD disrupts the feeding activity rhythm, stimulating food intake during the non-active daytime. Since the DMH is known to entrain to feeding schedule, we checked if its neuronal activity is simply a reflection of the animals' feeding behaviour, and whether it is still observed if the animals eat exclusively during nighttime. Our results clearly indicate, that the DMH activity disruption can be prevented by feeding restricted to the active phase, highlighting the importance of temporally regulated feeding behaviour on the physiological functioning of the hypothalamic metabolic centre. The work was supported by the programme "Excellence Initiative–Research University" at the Jagiellonian University in Kraków, Poland as well as by the Polish National Science Centre (2017/25/B/NZ4/01433).

113. Characterization of transcriptionally active clock complexes in time and space using quantitative proteomics

Main author: Fatih Aygenli
Affiliation: Systems Chronobiology, Institute of Medical Psychology, LMU - Munich
Co-authors: Maria Robles

Keywords: Mammalian Circadian Clock, BMAL1/CLOCK Complex, Proteomics, ChIP-MS

The circadian clock is a self-sustainable internal timing system that drives daily oscillations of molecular, metabolic, and physiological events. In mammals, the molecular mechanism of the clock is based on interconnected transcriptional and translational feedback loops with the transcription factors BMAL1 and CLOCK at their core. By rhythmically binding to DNA, BMAL1/CLOCK heterodimer drives the cycling expression of more than 20% of genes in each tissue, with minimal overlap across different tissues. Consequently, the precise mechanism of interaction and action of BMAL1/CLOCK

at the chromatin, and how this is differentially shaped in diverse tissue, is not completely understood. In this study, we combine mass spectrometry-based quantitative proteomics with a chromatin immunoprecipitation protocol to establish a ChIP – MS method to isolate and characterize BMAL1/CLOCK complexes bound to chromatin. We have analyzed and compared the protein composition of BMAL1/CLOCK complexes at the chromatin in three mouse tissues (liver, lung, kidney) and two timepoints (ZT4, ZT7) during the transcriptional active phase. Our data revealed that BMAL1/CLOCK complexes have extensive tissue specific constituents, such as transcription factors, chromatin remodelers and post-transcriptional regulators. Whereas common interactors across times and tissues seem to be part of the core complex as those proteins have been reported to play a role in circadian clock function in a variety of cell lines and organs. Overall, our analysis yielded the first comprehensive map of spatiotemporal interactors of CLOCK and BMAL1 at the chromatin in mouse tissues and our data would aid to comprehend how rhythmically gene expression in different tissues is regulated.

114. Space-time organization of liver zonation

Main author: Tomaz Martini
Affiliation: EPFL
Co-authors: Nagammal Neelagandan, Cedric Gobet, Felix Naef

Keywords: liver, single-cell sequencing, pathophysiology, sexual dimorphism

The liver is a highly perfused organ, commonly recognised for its role in metabolism and xenobiotic detoxification. Due to its role, gene expression patterns vary dramatically throughout the day. Recent advances in single-cell sequencing and spatial reconstruction have revealed that hepatocytes are much more heterogenous than previously thought, with zones within the liver's repetitive unit, the lobule, specialized for specific tasks. At the same time, the liver is also highly sexually dimorphic. However, there is very limited data on how these variables integrate and how hepatic physiology at the single-cell level changes in function of circadian time, lobular zone and sex.
Our results show that there are considerable differences not just in overall gene expression between males and females, but that there are also considerable differences in lobular zonation and spatially confined gene expression between the two sexes. For some genes, these observations are time-of-day dependent. Many of the sexually dimorphic genes are those involved in pathologies, therefore our observations can explain why there are differences in disease prevalence between males and females. These differences, previously lost with bulk approaches, can allow us to work towards the identification of potential novel pharmacological targets.

115. Using multiple wearable devices to extract personalised dynamic and circadian parameters that describe glucose levels, heart rate and heart rate variability

Main author: Nicholas Phillips
Affiliation: Hôpitaux universitaires de Genève - HUG
Co-authors: Nicholas Phillips, Tinh-Hai Collet, Felix Naef

Keywords: Wearable devices, glucose levels, circadian rhythms, mathematical modelling, Bayesian inference

Wearable biosensors and mobile applications can now measure physiological variables over multiple days in free-living conditions, revealing circadian rhythms and responses to external stressors such as meals and physical activity. Here we present a statistical framework to extract interpretable, personal parameters from these complex time series. Over a two-week period we measured food and drink ingestion events, glucose dynamics, physical activity, heart rate and heart rate variability for 25

participants from a healthy population. By modelling the effect of meal consumption of glucose levels, we infer how quickly glucose returns to baseline after meal consumption for each individual. This global meal response half-life parameter and the average post-meal glucose increase both show significant associations with the glycaemic coefficient of variation (R-squared = 0.63) but provide a more interpretable metric of individual glucose dynamics in response to meals. The underlying circadian rhythms in glucose levels show high amplitudes of up to 1 mmol/L in some individuals, while this additional circadian component is virtually undetectable in others. We found that physical activity and circadian rhythms are consistently useful for predicting heart rate (HR), explaining between 40 and 65% of variance across participants. In contrast, the ability to predict heart rate variability (HRV, a proxy for parasympathetic nervous activity) was more heterogenous between individuals, where physical activity, circadian rhythms and HR explain between 20 and 80% of HRV variability, according to the participant. Finally, adding activity, HR and HRV to the model of glucose levels and meals could explain up to 10% of glucose variability in some individuals, showing that the inclusion of additional physiological signals are helpful for a better understanding of glucose dynamics. By capturing the interactions between different subsystems, the multi-wearable approach can offer a more dynamic, personalised description of cardiometabolic health states.

116. Basic helix-loop-helix transcription factors CLOCK-BMAL1 and MYC-MAX leverage histone contacts for DNA motif recognition

Main author: Alicia Michael
Affiliation: Friedrich Miescher Institute for Biomedical Research
Co-authors: Alicia K. Michael, Lisa Stoos, Kelly L. Healy, Kristina Makasheva, Martina Minnich, Priya Crosby, Joscha Weiss, Georg Kempf, Simone Cavadini, Lukas Kater, Jan Seebacher, Luke Isbel, Ralph S. Grand, Florian Andersch, Jennifer L. Fribourgh, Dirk Schübeler, Carrie L. Partch, Andrew C. Liu, Beat Fierz, Johannes Zuber, Nicolas H. Thomä

Keywords: chromatin, CLOCK-BMAL1, E-boxes, nucleosome, MYC-MAX

The family of basic helix-loop-helix (bHLH) transcription factors (TF) consists of over 100 members that recognize E-boxes (CANNTG). We investigated how chromatinized E-boxes can be engaged throughout the nucleosome using two structurally diverse members of the bHLH family; the circadian TF, CLOCK-BMAL1 and the proto-oncogene MYC-MAX. Both preferentially bind E-boxes near the nucleosomal entry/exit sites and structural studies reveal that they trigger DNA-release from histones to gain access to nucleosome-embedded E-boxes. The CLOCK-BMAL1 PAS dimerization domains interact with the histone-octamer disc atop the H2A/H2B acidic patch, an interaction critical for robust circadian cycling. At a more internal E-box, the MYC-MAX leucine zipper dimerization domain binds histones H2B/H3. This binding is enhanced by an additional TF, OCT4, revealing structural and mechanistic insights into how TFs cooperate on chromatin. Our study provides mechanisms underlying E-box recognition throughout the nucleosome shared by bHLH family members and identifies factor-specific contacts between histones and the bHLH dimerization domains critical for their function.

117. Adaptation to critical metabolic conditions is dependent on the circadian clock: a study in the circadian model organism Neurospora crassa

Main author: Krisztina Káldi
Affiliation: Department of Physiology, Semmelweis University, Budapest, Hungary
Co-authors: Orsolya Sárkány, Anita Szőke, Géza Schermann, Axel C. R. Diernfellner, Michael Brunner, Norbert Gyöngyösi and Krisztina Káldi
Keywords: metabolism, starvation, Neurospora crassa, White Collar Complex, Frequency, transcriptome

Circadian clocks are closely linked to metabolism. On the one hand, the clock rhythmically modulates different metabolic pathways, and on the other hand, nutrients and metabolic cues are important Zeitgebers driving the circadian machinery. It is therefore not surprising that in human, conditions involving circadian rhythm dysfunction, such as shift work or jetlag, are associated with an increased risk of metabolic disorders including obesity, metabolic syndrome and type 2 diabetes. Using the circadian model organism Neurospora crassa, we examined how the molecular clock is affected by drastic changes in the nutrient level and whether adaptation to starvation is influenced by the circadian clock. We show that molecular timekeeping is robust even under severe limitation of carbon sources, however, stoichiometry, phosphorylation and subcellular distribution of the key clock components display significant alterations. Protein kinase A, protein phosphatase 2A and glycogen synthase kinase are involved in the molecular reorganization of the clock. RNA-seq analysis reveals that the WCC has a striking impact on nutrient-dependent expression of a large set of genes, including enzymes and regulators of carbohydrate, amino acid and fatty acid metabolism. Moreover, our molecular and phenotypic data indicate that a functional clock facilitates recovery from starvation. We suggest that the molecular clock is a flexible network that allows the organism to maintain rhythmic physiology and preserve fitness even under long-term nutritional stress.

118. How does daylight saving time affect patients with delayed sleep-wake phase disorder?

Main author: Cátia Reis
Affiliation: IMM - Faculdade de Medicina, Universidade de Lisboa
Co-authors: Luísa K. Pilz, Achim Kramer, Luisa Vaqueiro Lopes, Teresa Paiva, Till Roenneberg
Keywords: DLMO, local time, sun time, chronotype, DSWPD, phase angle

During Standard Time (ST), differences between Local and Sun Time (∆LAST) may occur depending on the location within the time zone. Except for locations east of the time-zone meridian, these differences are increased by one hour during Daylight Saving Time (DST). We hypothesized that patients with Delayed Sleep-Wake Phase Disorder (DSWPD) suffer from sleep deficits during DST.
We analysed clinical records of 162 DSWPD patients (52.5% male; median [Q1, Q3] age: 35.5 [26.0, 50.3]; age range:16-92) from a Centre for Sleep Medicine in Lisbon, Portugal (GMT zone). Dim Light Melatonin Onset (DLMO) was measured as a marker for circadian phase in 82 patients (54 from DST and 28 in ST) and we calculated the phase angle difference between DLMO and Sleep Onset (SO), Mid-sleep (MS) and Sleep End (SE) on work- (w) and work-free days (f). ST and DST data were compared using Mann-Whitney or Student's t tests. The association between ∆LAST and SOw was assessed using Spearman's correlation. ∆LAST was computed using the R package "solartime". Analyses were performed with SPSS v.27 and R; the significance level was set at 5%.

On a weekly average, patients slept an hour shorter during DST than under ST (62 min. p<0.01), mainly due to sleep on workdays (SDw, p<0.01). SDw correlated with ΔLAST (rsp=0.35, p<.01). The phase angles SOw-DLMO and SOf-DLMO were not different in ST and DST. However, the phase angles for MSw-DLMO (p=.005), MSf-DLMO (p=.025), SEw-DLMO (p<.001) and SEf-DLMO (p=.027), were larger in ST. The average SE-DLMO phase angles in ST were close to the biological night length described in the literature. Our results favour perennial ST and suggest assigning time-zones close to sun time as was decided at the International Meridian Conference in Washington in 1884. These conditions would prevent circadian misalignment and sleep deprivation.

119. Effect of time restricted feeding on the rhythmic behavior of peripheral tissues

Main author: Zsófia Búr
Affiliation: Department of Physiology, Semmelweis University, Hungary
Co-authors: Krisztina Ella, Ágnes Sűdy, Bence Koós, Ármin Szabolcs Kisiczki, Zalán Lumniczky, Krisztina Káldi

Keywords: metabolic rhythm, peripheral tissues, adipokines, cytokines

Introduction: It is well-known, that food intake entrains the circadian clock in certain peripheral tissues and parallel alters the metabolic rhythm. In line with this, irregular timing of food intake leads to disruption of the metabolic rhythm. Metabolic disturbances (like diabetes mellitus or obesity) are usually associated with changes in inflammatory responses.
Aims: Our previous experiments showed, that timed feeding increases the amplitude of the oscillation of blood leukocyte count and dampens autoimmune responses. Our aim was to investigate whether time-restricted feeding (TRF) affects the inflammatory markers of the bone marrow, the spleen and the adipose tissue.
Methods: Two feeding schedules were set in a mouse model. In the ad libitum (AL) group food was constantly available, whereas in the TRF group food availability was limited to the first 10 hours of the active phase of the animals. Bone marrow, spleen and adipose tissue samples were isolated after 4 weeks conditioning. Absolute leukocyte counts and percentage of leukocyte subsets were determined by flow cytometry. The relative expressions of clock (per2, reverba), inflammatory (il-6, tnfa, il-1b, nlrp3) and adipokine (leptin, adipsin) genes were determined.
Results: In the TRF group neutrophil levels in the bone marrow oscillated according to the rhythm of the blood cell count. In addition, in the AL group the neutrophil count was markedly elevated at the beginning of the active phase. TRF increased the amplitude of time-dependent clock gene expression in all investigated tissues. In the spleen and in the adipose tissue both the oscillation and the average levels of cytokines and adipokines were enhanced.
Conclusion: TRF modifies the rhythmic functions of all the investigated tissues, and this might contribute to the milder reactivity of the immune system. Our observations suggest that timed food intake could be effective as a supplementary therapy in autoinflammatory diseases.

120. Sex and circadian timing modulate oxaliplatin hematological and hematopoietic toxicities

Main author: Sandrine Dulong

Affiliation: Chronotherapy, Cancer and Transplantation, University of Paris Saclay, Faculty of Médecine, Bat A 3rd Floor, Campus CNRS, 7 rue Guy Moquet, 94800, Villejuif, France
INSERM UMR-S 900, Institut Curie, MINES ParisTech CBIO, PSL Research University, 92210 Sai

Co-authors: Sandrine Dulong, Lucas Eduardo Botelho de Souza, Jean Machowiak, Benoit Peuteman, Gaelle Duvallet, Déborah Boyenval, Elise Roth, Afag Asgarova, Yunhua Chang, Xiao-Mei Li, Adlen Foudi, and Annabelle Ballesta

Keywords: oxaliplatin, sex, circadian rhythms, hematological toxicity, hematopoietic toxicity, personalized medicine

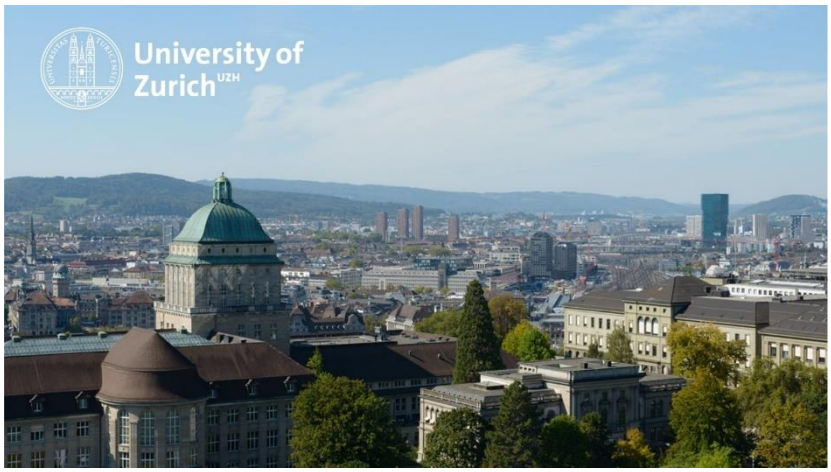
Oxaliplatin was nearly twice as hematotoxic with optimal timing differing by 6h in women as compared to men with colorectal cancer, in randomized trials. In male mice, hematological toxicities displayed circadian rhythms. We here investigated the sex- and timing-related mechanistic determinants of oxaliplatin hematopoietic toxicities.

B6D2F1 male and female mice, synchronized in LD12:12, were treated once with oxaliplatin at 6 different ZTs with 0, 5 or 10mg/kg for males and 0 or 5mg/kg for females. Body weight loss (BWL), circulating blood cell counts, bone marrow cellularity (BMC) and 7 flow cytometry-monitored populations of immature progenitors were evaluated 3 days after injection.

In untreated animals, circadian rhythms of circulating white blood cells (WBC) were similar in both sexes with a peak at ZT5. BMC also displayed circadian variations, with a peak at ZT20in males, a phase shift of 17h between males and females, and higher amplitudes in females. All BM progenitor counts presented robust rhythms with aligned phases around ZT3 in females whereas only 3 out of 7 populations were rhythmic in males with lower amplitudes and a male-to-female phase shift of ≈6h.

In animals treated with 5mg/kg, rhythms were observed in BWL and WBC for females, but not for males. Regarding BMC, toxicity rhythms of toxicity were shown in BMC between both sexes with higher amplitude in females. An important depletion of bone marrow progenitors was observed in treated males for all circadian timing whereas, in females, large amplitude toxicity rhythms were obtained for all progenitors with worst timing around ZT3. Increasing the dose to 10mg/kg in males induced circadian toxicity rhythms in BWL and WBC but not in BMC or progenitor counts.

Sex-related differences were found in circadian rhythms of the hematopoietic system and of the response to oxaliplatin, suggesting complex and sex specific clock-controlled mechanisms.



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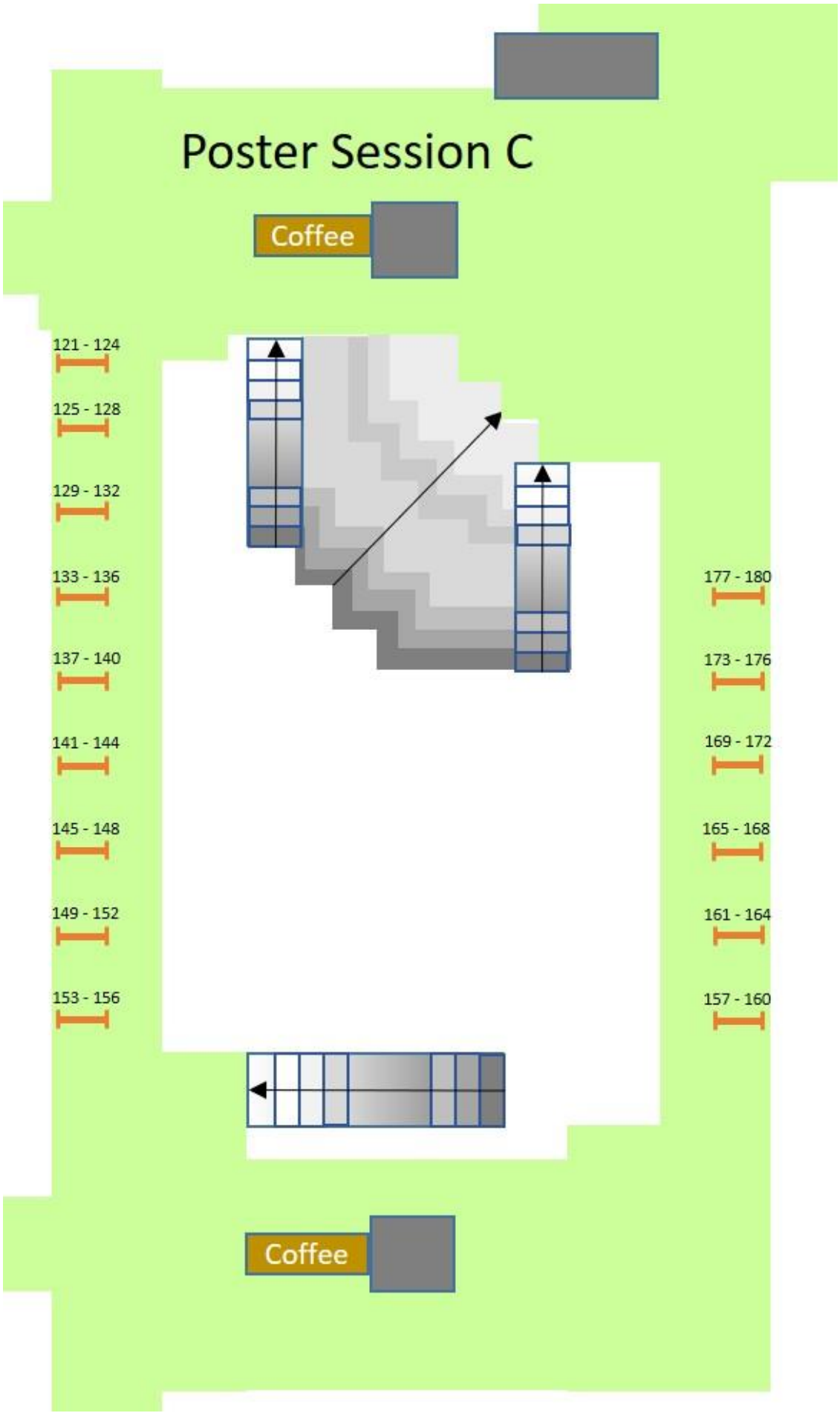

Abstracts to poster session C on Thursday 28.07.2022

121. A hybrid design approach revealed critical roles of ER and HER2 in regulating circadian pathways in breast cancer

Main author: Shiyang Li

Affiliation: Shi-Yang Li[1], Jan A Hammarlund[2], Gang Wu[3], Antony D Adamson[1], Charles H Streuli[1], John B. Hogenesch[3], Ron C. Anafi[4], Qing-Jun Meng
[1]Faculty of Biology, Medicine and Health. University of Manchester.
[2]Drexel University, School of Biomedical Engineering a and Health Systems, Philadelphia, United States. 3Cincinnati Children's Hospital, Division of Human Genetics, Cincinnati, United States. 4University of Pennsylvania, Division of Sleep Medicine, Philadelphia, United States.

Co-authors: -

Keywords: Circadian clocks, Breast cancer, Extracellular Matrix, ER, HER2, RNA-seq, CYCLOPS

Despite epidemiological and animal studies linking circadian rhythm disruptions to risks of developing breast cancer, how circadian clocks are regulated in subtypes of breast cancer is poorly understood. Here, we performed RNA-seq on 43 pairs of breast tumours and normal controls that were collected from the same individuals (with surgical resection time recorded). We also cultured primary breast tumour cells on 2D matrix or in 3D as organoids to investigate their intrinsic circadian clocks. RNA-seq revealed significant alterations in the expression of clock genes in tumour tissues as compared to normal controls. Such clock disruptions were clearly cell intrinsic as primary breast cancer cells demonstrated defective oscillations irrespective of their extracellular matrix microenvironment. Further analysis by Spearman's correlation co-efficient of breast cancer RNA-seq databases revealed clear cancer-subtype dependent molecular clock changes, with oestrogen receptor (ER) positive tumours displaying relatively preserved circadian rhythms, while human epidermal growth factor receptor 2 (HER2) positive tumours showing severely disrupted clocks. ER activation synchronised circadian oscillations of ER+ MCF-7 cells, while CRISPR-mediated ER deletion disturbed circadian rhythms. Additionally, over-expression of HER2 alone was sufficient to disrupt circadian rhythms in MCF-7 cells. CYCLOPS (Cyclic ordering by periodic structure) analysis of transcriptome data from our time-stamped breast samples and breast cancer databases identified rhythmic genes in human normal breast and revealed a shift of rhythmic pathways in Luminal A breast tumours. Molecular disruption of the cell intrinsic clocks by Bmal1 knockdown led to profound changes in proliferation and migration in breast cancer cells. Taken together, our hybrid approach provides insights into subtype-dependent changes of circadian regulation in breast cancer and reveals ER and HER2 as critical regulators for breast cancer clocks, laying the groundwork for further development of chronotherapy for breast cancer.

122. Effects of evening candlelight exposure in the home on the circadian melatonin rhythm

Main author: Katrina Rodheim

Affiliation: University of Colorado, Boulder

Co-authors: Katrina G. Rodheim, Sarila Ekin, Zofia Martinez-Lisowska, Kenneth P. Wright Jr.

Keywords: Circadian Rhythms, Light Exposure, Melatonin

Effects of evening candlelight exposure in the home on the circadian melatonin rhythm

Intro: Circadian rhythms are important for the optimal timing of physiology and behavior, including the timing of sleep and wakefulness. Later timed circadian rhythms and sleep are associated with adverse health outcomes and present a challenge for morning work and school start times. Exposure to electric lighting in the evening after sunset contributes to later circadian timing. The aim of the current study was to examine melatonin levels under typical electric lighting in the home environment and to determine if days of exposure to candlelight after sunset advances the circadian rhythm. Methods: Eighteen young healthy adults [6 males (24.2±6yr; mean±SD)] completed a weeklong protocol that included ambulatory monitoring (wrist-worn actigraph with light exposure, and thigh worn posture/activity monitors), and a daily sleep diary. After two baseline days, saliva samples were collected for melatonin assessment every hour in the home from 6pm until bedtime for one day under typical home electric lighting conditions and for four subsequent days under candlelight conditions. Participants were provided candles and three sturdy candlestick holders to use on each candlelight night. Results: Bedtimes and waketimes were similar across the study. On average, participants were exposed to ~ 23 lux during the electric lighting night and ~ 0.7 to 2.5 lux on candlelight nights. Melatonin levels were significantly lower on the electric lighting night versus all candlelight nights, and levels were lower on the first versus the last candlelight night (all $p<0.05$). The timing of the dim light melatonin onset was significantly earlier by ~1h on the last versus the first candlelight night ($p<0.05$). Conclusion: Our findings support the use of candlelight or perhaps electric lighting simulations of candlelight as a way to achieve earlier circadian timing. Future studies are needed to explore the health and performance benefits of such earlier circadian timing under real world conditions.

123. Mistimed sleep and waking activity in humans disrupt glucocorticoid signalling transcripts driven by SP1, but not plasma cortisol rhythms

Main author: Simon Archer
Affiliation: Surrey Sleep Research Centre, University of Surrey, UK
Co-authors: Carla Moller-Levet, Emma E Laing, Derk-Jan Dijk
Keywords: -

Cortisol is a robust circadian signal that synchronises peripheral circadian clocks with the central clock in the suprachiasmatic nucleus via glucocorticoid receptors that regulate peripheral gene expression. Misalignment of the cortisol rhythm with the sleep-wake cycle, as occurs in shift work, is associated with negative health outcomes, but underlying molecular mechanisms remain largely unknown. We experimentally induced misalignment between the sleep-wake cycle and melatonin and cortisol rhythms in humans and measured time series blood transcriptomics while participants slept in-phase and out-of-phase with the central clock. The cortisol rhythm remained unchanged, but many glucocorticoid signalling transcripts were disrupted by mistimed sleep. To investigate which factors drive this dissociation between cortisol and its signalling pathways, we conducted bioinformatic and circadian rhythm coherence analyses. We found that glucocorticoid signalling transcripts affected by mistimed sleep were enriched for binding sites for the transcription factor SP1. Changes in the timing of rhythms of SP1 transcripts and affected glucocorticoid signalling transcripts were closely associated. Furthermore, changes in the timing of the rhythms of SP1 transcripts, a major regulator of transcription, and changes in the timing of rhythms in transcripts of the glucocorticoid signalling pathways were closely associated. Associations between the rhythmic changes in factors that affect SP1 expression and its activity, such as STAT3, EP300, HSP90AA1 and MAPK1, were also observed. We conclude that plasma cortisol rhythms incompletely reflect the impact of mistimed sleep on glucocorticoid signalling pathways and that sleep-wake driven changes in SP1 may mediate disruption of these pathways. These results aid understanding of mechanisms by which mistimed sleep affects health.

124. Social jet-lag as a main trigger of hepatic clock disruption

Main author: Antonia Tomas-Loba
Affiliation: Chronodisruption and Cancer Unit, Chronobiology Lab, Department of Physiology, Faculty of Biology, University of Murcia, Mare Nostrum Campus, IUIE, IMIB-Arrixaca, Murcia, Spain.
Co-authors: García Cobarro C, Sánchez-Rodrigo C, Fernández-Silvente MT, Rol MA, Madrid JA and Tomás-Loba A.
Keywords: Circadian rhythm, chronodisruption, social jet-lag, liver cancer, hepatosomes

The circadian system is an evolutive adaptation of our molecular biology and physiology to external rhythmic fluctuations that occurs within ~24 hours like the day/night dichotomy. This adaptation give us adaptative advantages to obtain the maximum benefit from the ecosystem. To that end, our molecular program precedes the changes in a homeostasis perfectly geared with the outside. Thus, shift work, chronic jet lag, or any other chronodisruptive conditions directly impact health status triggering a wide spectrum of diseases. Several epidemiological studies have linked chronodisruption to an increased incidence of specific human malignancies such as prostate and breast cancer. Based on GLOBOCAN predictions, liver cancer is expected to increase more than 50% by 2040 probably due to alcohol consumption and hepatitis virus infection, but also to the current lifestyle. In our lab, we are interested in understanding whether chronodisruption is able to modify the hepatic molecular clock and/or downstream pathways to change cellular landscape for malignancies´ growth. Our preliminary data show that a mild stress induced by simulating social jet-lag induces a shift in the expression of hepatic Bmal1 and Clock genes inducing downstream changes in key pathways for cancer biology. Moreover, we are describing the hepatosomes profile of jet-lagged mice for further use it as a biomarker in liquid biopsy of chronodisruption-induced liver cancer.

125. Rhythmic fruit fly species in constant light – what are cause and purpose?

Main author: Peter Deppisch
Affiliation: University of Würzburg
Co-authors: Charlotte Helfrich-Förster
Keywords: Drosophila, constant light (LL), phase shifts, Timeless, Cryptochrome

Light is not only the most important Zeitgeber for most circadian clocks - constant light (LL) affects the clocks' free-running periods (parametric effects of light) and light-pulses shift their phases (non-parametric effects of light). Constant high light-intensities may even stop the circadian clocks making animals arrhythmic. Especially the clock of fruit flies is highly light-sensitive due to photoreceptive CRYPTOCHROME (CRY) that interacts with the core clock protein TIMELESS (TIM) and provokes its degradation as soon as activated by light. Fruit flies become arrhythmic in LL already at intensities of less than 10 lux and they show virtually no jetlag after phase-shifts of the light-dark cycle. While fast shifts of the clock might be of advantage, the high light-sensitivity to constant light appears maladaptive under very long summer days at high latitudes. Indeed, we recently found that certain wild D. melanogaster lines caught at higher latitudes remain rhythmic at medium LL intensities. These lines carry a natural isoform of TIM (L-TIM) that is more stable under LL due to a reduced interaction with CRY.
In addition, we found that certain fruit fly species from high latitudes such as D. funebris remain rhythmic under LL of high light-intensities (500 lux). Most interestingly, D. funebris flies can quickly phase shift their rhythms indicating that neither the function of CRY nor that of TIM is impaired. Indeed, we could not yet identify substantial alterations in the cry, jetlag, tim genes of D. funebris that could account for their observed reduced LL sensitivity. Currently, we are about to analyze additional genes

and factors that have previously been found to be involved in behavioral rhythmicity under constant light. Furthermore, we monitor the parametric effects of light in D. funebris flies in more detail by measuring their free-running periods under different intensities of LL.

126. Differences in annual rhythms of spadefoot from different populations: A common garden experiment

Main author: Maya Eldar
Affiliation: Tel Aviv University
Co-authors: Maya Eldar, Sarig Gafni, Yael Ballon and Noga Kronfeld-Schor
Keywords: -

The Eastern Spadefoot (Pelobates syriacus) is a nocturnal amphibian native to an area extending from Eastern Europe to Western Asia, with Israel on the south border of its distribution. In arid areas including Israel, where it depends on seasonal winter ponds, its populations are susceptible to local extinction. Climatic conditions and characteristics of winter ponds differ greatly from northern to southern Israel, and in accordance, populations differ in their morphology, activity patterns, metamorphosis, aestivation and breeding times. Those differences could be a direct response to environmental conditions, however, in previous experiments, we found that eastern spadefoot maintained in the laboratory under constant photoperiod and temperature for 4 years exhibited circannual rhythms in aestivation timing.
In this study, we hypothesized that the differences in annual rhythms and morphology documented in free-living populations may, at least in part, result from differences in characteristics of the internal circannual clock between populations.
We performed a common garden experiment, measuring metamorphosis dates, morphological characteristics, activity and estivation patterns of three eastern spadefoot populations for 2 years, starting at their tadpoles phase: a Northern population from Zaura winter pond, a southernmost population worldwide from Roberts winter pond, and tadpoles from our breeding colony, originally from the costal Check Post winter pond. It total 52 spadefoot toads were followed. In addition, we also followed activity patterns of 10 tadpoles. Individuals were held in 35 liter tanks with suitable habitat, unlimited food, controlled temperature and natural light. Activity levels was measured continuously by night vision cameras (IR) connected to a data logging system.
We found that tadpoles are diurnal and become nocturnal after metamorphosis. Moreover, even though the animals were held under the same conditions from early stages, there were significant differences between populations in dates of metamorphosis, weight at metamorphosis, and estivation patterns.

127. BIG regulates the circadian clock and development

Main author: Dora Luz Cano Ramirez
Affiliation: Department of Plant Sciences, University of Cambridge, Downing Street, Cambridge CB2 3EA, UK
Co-authors: Elena Bidash, Sally Ward, Ottoline Leyser, Alex Webb
Keywords: Plants, Arabidopsis, Splicing

Due to Earth’s rotation on its axis, life exists in day-night cycles of approximately 24 hours. Circadian clocks are timing mechanisms that help organisms to organize and coordinate their activities, and to predict and adapt to fluctuations in their environment. It has been discovered that BIG (AT3G02260), functions in the establishment of a correct phase relationship between the internal timing of a plant and the environment and regulates development through strigolactone and auxin signalling. Our aim

is to identify how BIG exerts regulatory control over the synchronisation of the circadian clock and strigolactone signalling through RNAseq, genetic, physiological, and protein-protein interaction studies. Results show that strigolactone treatment has no effect on the circadian rhythm in wild type Arabidopsis thaliana, however, three different alleles of BIG mutants have different degrees of splicing defects possibly explained by interaction of BIG with proteins from the splicing machinery found by yeast two-hybrid essays. Our results suggest a new role of BIG in splicing in plants.

128. Time restricted feeding enhances leukocyte rhythm and reduces inflammatory potential

Main author: Krisztina Ella
Affiliation: Semmelweis University Department of Physiology
Co-authors: Ágnes Réka Sűdy, Zsófia Búr, Bence Koós, Ármin Szabolcs Kisiczki, Krisztina Káldi

Keywords: inflammation, leukocyte, metabolism, arthritis

Metabolism has a major impact on immune responses, and metabolic disturbances are linked to alterations of immune functions and increased infection incidence. The circadian clock controls both the metabolism and the immune system. Food intake and changes in serum nutrient levels are important entrainment signals for the circadian rhythm, particularly in tissues involved in metabolic regulation. Time restricted eating is considered beneficial in metabolic dysfunctions, such as obesity and diabetes, but its impact on immune responses is unspecified. Our aim was to investigate the effect of time restricted feeding (TRF) on inflammatory responsiveness including the progression of autoimmune arthritis. Mice were conditioned to either TRF by limiting the food intake to the first 10 hours of the active phase or ad libitum feeding. Timely changes in the abundance of peripheral leukocyte subsets and rhythmic behavior and inflammatory potential of leukocytes were examined. Progression of autoimmune inflammation was followed in the KBxN serum-transfer arthritis model. Marginated and tissue resident immune cells were quantified to assess the inflammatory potential of the lung. Symptoms of the autoimmune arthritis were slackened in the TRF group compared to the control. Moreover, TRF enhanced the oscillation of peripheral leukocyte counts and reduced the cell surface expression of adhesion molecules in neutrophils and monocytes which correlated with a substantial decrease of the tissue leukocyte pool of the lung. Our results suggest that TRF substantially modifies the inflammatory potential of leukocytes which may contribute to the milder reactivity of the immune system and thus serve as an effective complementary tool in the therapy of autoinflammatory processes.

129. Modification of the human's sleep/wake cycle in free running condition – Deep Time mission

Main author: Virginie Gabel
Affiliation: Université de Normandie, Inserm U1075 COMETE, Caen, France
Co-authors: Pierre-Louis Delaunay, Christian Clot, Stéphane Besnard, Corentin Hingrand, Benoit Mauvieux

Keywords: Sleep/wake cycle, circadian rhtyhm, free-running, DeepTime, Temperature

Introduction: In the 1960’s, multiple solitary isolation experiments achieved by Michel Siffre and his teams highlighted the endogenous rhythm of the circadian clock, estimated to be around 24.5h in the absence of any time giver. In our study, the objectives were to determine whether a group of people living out of time will exhibit the same endogenous rhythm as previously observed and whether their activity ratio will be modulated.

Methods: Through the Human Adaptation Institute, 14 individuals (7 ♀ and 7 ♂) isolated themselves from any time giver in a cave (Lombrives, France) for a period of 40 days between March and April 2021. Their sleep/wake cycle were monitored by wrist actimetry (MotionWatch R, CamNTech) for 14 days outside the cave ("Pre"), during the 40 days in the cave ("Per") and 14 days after exiting the cave ("Post"). A sleep diary was completed each cycle and core body temperature (CBT) was measured every 5 cycles for several hours.
Results: Within the 40 days, we recorded 24 to 31 sleep/wake cycles per subject (mean ± sd: 29.25 ± 2.56) with a mean duration of 31.7h ± 8.0 per cycle. The ratio "time awake" to "time asleep" stayed stable across all conditions ("Pre": 64.63% ± 5.9, "Per": 63.64% ± 8.78 and "Post": 62.36% ± 8.27, p=0.201). Preliminary results of CBT reveal a desynchrony between their sleep/wake cycle and their circadian rhythm (more analyses are underway to confirm these results).
Conclusion: We show that people spontaneously extended their sleep/wake cycle over the course of the 40 days, and not necessarily following their circadian rhythm. Interestingly, the ratio rest/activity stay the same in and outside the cave, even though their internal rhythms tend to be longer than what were previously shown by other studies, leaving a field open to other hypotheses concerning human adaptations.

130. It's time we talk about sex – sexual dimorphism of circadian-regulated metabolites in humans

Main author: Thomas Hancox
Affiliation: University of Birmingham
Co-authors/Supervisors: Dr Aneika Leney, Dr Robert Dallmann, Prof Debra J. Skene, Prof Warwick Dunn
Keywords: Untargeted Metabolomics, Circadian, Constant routine, Human

Introduction: It is increasingly apparent that the circadian system has a central role in the regulation of human metabolism and physiological processes relating to health and disease. Epidemiological evidence has significantly linked circadian disruption (e.g., by shift work) to the onset of numerous chronic conditions including type 2 diabetes, obesity and associated metabolic changes. The understanding of circadian metabolic regulation in health and deregulation in disease could elucidate why diseases develop, define how diseases can be treated with/without drugs and the best optimised time of the day for effective interventions. However, males have been preferentially studied in prior work investigating circadian regulation of metabolism and the extent of sex-dependent differences remains poorly understood.
Objective: To characterise metabolites modulated by the circadian system in a constant routine study and determine differences between healthy males and females.
Methods: Participants (n = 32, 16 females), age- and BMI-matched, were subject to a constant routine protocol with a blood sampling period of 33 h and 2-hourly untargeted metabolomics performed using four UHPLC-MS assays. Rhythm analysis was performed, applying a range of methods (Cosinor, JTK, ARS, ECHO, RAIN and BioCycle) to cross validate results.
Results and conclusions: In total 906/1139 rhythmic metabolite features were detected in males/females of which 268/501 (~30%/44%) were unique to either group, whilst acrophase differences of +/- 4 h were observed in 30 common rhythmic features between groups. Metabolite classes that demonstrate the greatest sexual dimorphism, with regards to rhythmicity, include glycerolipids and glycerophospholipids. Furthermore, individual level analysis revealed large inter-individual variation of detected rhythmic features, ranging from 10's-100's, across the cohort. Our data highlight sexual dimorphism and inter-individual variation in circadian-regulated metabolites and emphasise the need for balanced female:male representation within participant cohorts investigating circadian regulation.

131. Peripheral clocks gate-keep external signals to ensure continued tissue homeostasis

Main author: Thomas Mortimer
Affiliation: IRB Barcelona
Co-authors: Valentina M. Zinna, Paul Petrus, Carmelo Laudanna, Oleg Deryagin, Mireia Vaca-Dempere, Jacob G. Smith, Elisa García-Lara, Arun Kumar, Kevin B. Koronowski, Carolina M. Greco, Stephen Forrow, Paolo Sassone-Corsi, Patrick-Simon Welz, Pura Muñoz-Cánoves and Salvador Aznar Benitah
Keywords: Epidermis, Clock network, Mouse model, Communication, DNA damage

Integration by circadian clocks of rhythmic changes in the milieu of systemic, micro-environmental and extra-corporeal signals is essential to execute correctly homeostasis-maintaining programmes of daily physiology in bodily tissues. Many such signals originate as outputs from other niche and distal circadian clocks, implying that a given tissue clock must receive and then parse diverse external inputs from the bodily clock network to coherently drive daily physiology. Despite the increasingly acknowledged importance of this inter-clock communication, critical elements and properties of this network, such as key signalling nodes and the mechanisms of communication, remain undefined. To dissect this system, we developed a mouse model that allows the construction of minimal clock networks comprising only two nodes, enabling the isolation and interrogation of individual clock-driven interactions between signalling nodes for the first time. Using this system, we begin by dissecting the communication between the peripheral epidermal clock and the central brain clock. In doing so, we define in unprecedented detail those rhythmic epidermal processes requiring direct input from the central clock and those in which the broader network of peripheral clocks are necessary. Surprisingly, we also identify that alongside their integrative roles, peripheral clocks are able to suppress or correct external signals to ensure coherence of homeostatic processes. Specifically, in the epidermis, this gatekeeper function of the local clock acts to phase correct signals originating from the brain clock that would lead to DNA replication during peak DNA damaging conditions. Together, we present a novel approach for dissecting clock-network interactions, and in turn reveal new functionalities for peripheral circadian clocks in controlling tissue homeostasis.

132. Chronodisruption of immune and metabolic response to endotoxin by light at night exposure

Main author: Monika Okuliarova
Affiliation: Department of Animal Physiology and Ethology, Faculty of Natural Sciences, Comenius University, Bratislava, Slovakia
Co-authors: Viera Jerigova, Beata Benedikova, Michal Zeman
Keywords: circadian rhythms, ALAN, immune function, adipokines, clock genes

Metabolic adaptations during inflammation significantly affect the strength of the immune response and both immunity and metabolism are extensively regulated by the circadian clock. Hence, an impaired crosstalk among all three systems can participate in mechanisms leading from circadian disruption to chronic diseases. Our studies have shown that artificial light at night (ALAN) compromises circadian organization, including central clocks and peripheral rhythms in immune and metabolic parameters. Here, we addressed ALAN effects on daily variability in systemic endocrine changes and response of molecular clockwork in metabolically active tissues during acute inflammation. We used model of lipopolysaccharide (LPS)-induced inflammation in rats exposed to low-intensity ALAN (~2 lx). Endotoxin applied at the beginning of the light phase induced more pronounced increase of plasma corticosterone and TNF-α than during the night. ALAN-exposed rats

also showed increased corticosterone and TNF-α levels but day-night variability in response to LPS was abolished. Plasma adiponectin and leptin decreased post-LPS, while adiponectin reduction was attenuated when rats were injected during the night. ALAN eliminated LPS-induced adipokine response during the light phase. In the visceral fat and kidney, the up-regulation of NF-κB inflammatory pathway was more intense following daytime than night-time LPS injection. Importantly, suppressed day-night variability in NF-κB response was found in ALAN-exposed rats. Moreover, ALAN inversely changed the peripheral expression of clock genes Bmal1 and Nr1d1 in the light and night phase, indicating a shift of this additional circadian loop. Acute response of clock genes to endotoxin was strongly time-dependent, showing Bmal1 up-regulation and Nr1d1 down-regulation upon night-time LPS challenge. ALAN diminished the timing effect of LPS on hepatic expression of both clock genes and eliminated Nr1d1 response to LPS in the kidney. Together, our data demonstrate that ALAN disrupted time-of-day-dependent variability in immune and metabolic adaptations during inflammation. Supported by APVV-17-0178 and VEGA 1/0565/22.

133. Attenuated master clock and disrupted daily rhythms in neuroendocrine axis and cardiovascular system by dim light at night in rats

Main author: Michal Zeman
Affiliation: Department of Animal Physiology and Ethology, Faculty of Natural Sciences, Comenius University, Bratislava, Slovakia
Co-authors: Zuzana Dzirbikova, Lubos Molcan, Valentina Sophia Rumanova, Monika Okuliarova
Keywords: brain, clock genes, vasopressin, corticosterone, cardiovascular rhythms

Artificial light at night (ALAN) is a new environmental risk factor associated with negative health effects. Underlying mechanisms are not well understood, but might be attributed to disruption of the circadian timing system. The aim of our studies was to identify circadian output pathways targeted by ALAN, responsible for transmitting compromised information about the light/dark cycle from the master hypothalamic clock in the suprachiasmatic nuclei (SCN) to the periphery and the cardiovascular system. Using a protocol with low-intensity (~2 lx) ALAN in rats and in situ hybridisation, we found that 2 weeks of ALAN strongly attenuated the molecular clockwork in the SCN, as indicated by the damped and lost daily rhythmicity in the clock genes Per1 and Per2, respectively. Moreover, ALAN damped rhythmic expression of Nr1d1 and arginine vasopressin (Avp) in the SCN and disturbed rhythmic clock gene expression in the paraventricular and dorsomedial hypothalamic nuclei that convey the circadian signals from the SCN to endocrine and behavioural rhythms. Disruption of these output pathways in ALAN-exposed rats was manifested by lost daily variations in plasma melatonin, testosterone and AVP concentrations and by the damped and phase-advanced plasma corticosterone rhythm. Daily rhythms of heart rate and blood pressure had lower circadian power in normotensive and spontaneously hypertensive animals and the attenuated circadian control was reflected in exaggerated response of blood pressure to norepinephrine administration after 5 weeks of ALAN. Observed changes in corticosterone and AVP rhythmicity suggest a disturbed circadian control of stress response with a negative impact on the control of the cardiovascular system. Our results illustrate a disruption of multiple circadian output pathways, which are compromised by ALAN and can mediate a negative impact of light pollution on health. Supported by APVV-17-0178 and VEGA 1/0492/19.

134. The plastic shift in the endogenous rhythm in tidal adaptation of the freshwater snail

Main author: Takumi Yokomizo
Affiliation: Graduate School of Science and Engineering, Chiba University
Co-authors: Yuma Takahashi
Keywords: Biological clock, circatidal rhythm, circadian rhythm, transcriptome, river

Organisms have an endogenous time-keeping system to coordinate their biological processes with environmental cycles. The coordination of biological clocks and external environmental cycles is suggested to affect fitness and play an important role in the adaptation to rhythmic environments. The lower reaches of a river are a system with complicated rhythmic environments where organisms are exposed to both the tidal (12.4 h) and daily (24 h) cycle. In the freshwater snail, Semisulcospira reiniana, we have revealed that individuals inhabiting a non-tidal area have the circadian rhythm in their locomotion activity and gene expression, while those inhabiting a tidal area have the circatidal rhythm. Here, we investigated whether the circatidal rhythm of individuals of a tidal area are determined genetically or induced by environmental cycles. We exposed snails to a simulated tidal environment with a 12-h period for a month and then measured their activity and gene expression rhythms under the constant condition. Individuals in both tidal and non-tidal populations exhibited the circatidal rhythm, suggesting that tidal stimulations can induce the circatidal rhythm even in individuals of a non-tidal population. In a non-tidal population, we found that 186 genes showed the circatidal rhythm in their expression only in snails which experienced the simulated tidal cycle, indicating that these genes were affected by the tide. Additionally, transcriptome-wide population genetic analyses suggested that there is quite low differentiation between the two populations. Our findings suggest that the populations in a tidal and a non-tidal area were genetically almost identical and the ability of phenotypic plasticity of the endogenous rhythm underlie the expansion of the habitat of the freshwater snail.

135. Rhythms in the life of the marine diatoms

Main author: Angela Falciatore
Affiliation: Laboratoire de Biologie du Chloroplaste et perception de la lumiére chez les microalgues, UMR7141, 10 CNRS, Sorbonne Université, Institut de Biologie Physico-Chimique, Paris, 75005, France
Co-authors: Alessandro Manzotti, Raphael Monteil, Antoin Hoguin, Cheminant Navarro, Marianne Jaubert, Jean-Pierre Bouly and Angela Falciatore
Keywords: Diatoms, phytoplankton, bHLH-PAS Transcription Factor, marine rhythms

Periodic light-dark cycles govern the timing of basic biological processes in organisms inhabiting land as well as the sea, where life evolved. Although a variety of ecologically crucial biological rhythms have been described in prominent photosynthetic microalgae, known as phytoplankton, far less information is available on the underlying timekeepers. Our major interest is in understanding the biology of diatoms, the world's most diverse group of algae, standing at the crossroads of several evolutionary lineages (Falciatore et al., Plant Cell. 2020). Contributing around 20% of annual global carbon fixation, diatoms underpin major aquatic food webs and drive global biogeochemical cycles. Basic biological processes such as the cell cycle, gene expression and pigment biosynthesis show daily oscillations in various diatom species. In Phaeodactylum tricornutum, these rhythms persist under constant light conditions, providing evidence for the existence of an endogenous circadian clock in this alga. By characterizing a P. tricornutum bHLH-PAS protein, hereby named RITMO1, we are beginning to shed light on the regulation of the daily life of diatoms. RITMO1 is nuclear-localized and the alteration of its expression timing and levels by ectopic overexpression, or its obliteration by

CRISPR/Cas9, result in lines with altered rhythmicity in continuous light (Annunziata et al., PNAS 2019). By analysing the expression of selected genes, we also showed that RITMO1 plays a wide role in the circadian transcriptional regulation, likely acting as a component of the circadian central oscillator. Other bHLH-PAS proteins interacting with RITMO1 have been identified as additional candidate components of the diatom clock. Phylogenetic analysis revealed a wide distribution of RITMO1-like in diatoms, as well as in other marine algae, which may indicate a common function in these phototrophs. These findings unveil critical features of diatom biology and pave the way for a deeper understanding of marine rhythms and their evolutionary and ecological relevance.

136. Light-induced c-FOS in retinal and SCN clocks: distinct role of rods

Main author: Antonin Jandot
Affiliation: Univ Lyon, Université Lyon 1, Inserm, Institut Cellule Souche et Cerveau U1208, 69500 Bron, France
Co-authors: Hugo Calligaro, Christine Coutanson, Ouria Dkhissi-Benyahya

Keywords: Light, rod, retina, SCN, cFOS

Our recent study based on photoreceptor transgenic mice suggests a dichotomy of the light response between retinal and SCN clocks, involving rods (Calligaro et al., 2018). Indeed, a light stimulation (530nm, 10^14 photons/cm²/s) does not shift the phase of the retinal clock, but induces a behavioral phase shift in rod-deficient mice (Nrl-/-), while « rod-only » mice (Opn4-/-::TrB-/-) exhibit a light-induced phase change of the retinal clock without affecting the behavioral response. To go further in the analysis of the light integration mechanisms by both structures, our strategy is based on the quantification of c-FOS positive cells in SCN and retinal sections of 1- and 5-months old wildtype and Nrl-/- mice exposed to a monochromatic light stimulation (530nm, 10^14 photons/cm²/s, 30min) at CT16. In the SCN, our results showed that the light-induced c-FOS response is not altered by the absence of rods: an increase of c-FOS positive cell density is observed in wildtype and both 1- and 5-months old Nrl-/- mice compared to dark controls, with no difference between wildtype and 1-month old Nrl-/- stimulated animals. However, c-FOS density is reduced by 50% in the 5-months old Nrl-/- mice. Interestingly, the density of c-FOS positive cells is already reduced in both the inner and the ganglion cell layers of the Nrl-/- retinas at 1 month compared to light-stimulated wildtype mice. The number of c-FOS positive cells continues to decrease only in the ganglion cell layer of Nrl-/- at 5 months. We demonstrate for the first time 1) the dichotomy of the light response between retinal and SCN clocks using a cellular approach and 2) the indispensable role of rods in the light response of the retina.

137. Changes in reindeer sleep regulation across the year: A central role for rumination?

Main author: Melanie Furrer
Affiliation: Child Development Center and Children's Research Center, University Children's Hospital Zurich, University of Zurich, Zurich, Switzerland
Co-authors: Sara Meier, Maxime Jan, Paul Franken, Monica A. Sundset, Reto Huber, Steven A. Brown, Gabriela C. Wagner

Keywords: -

Most non-hibernating mammals maintain circadian sleep rhythms across the year in addition to homeostatic sleep-wake patterns in which increasing time awake is followed by increased sleep amount or intensity. Strikingly, reindeer in the Arctic show 24-hour rhythmicity around the equinoxes, but not around the solstices; and summertime activity greatly exceeds wintertime activity. So far, though, nothing is known about their sleep or how it might be seasonally modulated. We therefore

recorded non-invasive electroencephalography (EEG) in four adult, female reindeer (Rangifer tarandus tarandus) for four days at The Arctic University of Norway (69.65° N) in July, September, and December. We identified rapid eye movement (REM) sleep, non-REM (NREM) sleep, and rumination and calculated slow-wave activity (SWA, EEG power 1 - 4.5 Hz) during NREM sleep, the classic marker for homeostatic changes in sleep pressure. While reindeer sleep generally resembled that of other mammals, we additionally observed novel adaptations. As expected, sleep-wake distribution paralleled daily activity during seasonally changing light-dark conditions and SWA during NREM sleep was increased after prolonged wake periods ($t = 5.9$, $p < 0.01$). Surprisingly, prolonged waking produced a lower SWA response in summer than winter ($t = -2.54$, $p = 0.02$), possibly indicating increased baseline sleep pressure. Moreover, SWA decreased across rumination ($t = 4.4$, $p < 0.01$), and total rumination and NREM sleep durations were negatively correlated ($r = -0.63$, $p = 0.04$). It has previously been reported for some domestic ruminants that EEG during rumination sometimes shows characteristics of NREM sleep. Homeostatic modelling of our own data for SWA in reindeer further suggested that rumination was equivalent to sleep. Our results indicate that rumination acts as a partial substitute for conventional sleep, thus permitting near-constant feeding in the arctic summer while compensating for increased sleep pressure.

138. Fractal regulation of human motor activity, hypothalamic integrity and napping during ageing

Main author: Grégory Hammad
Affiliation: GIGA-CRC In Vivo Imaging, Sleep and Chronobiology lab, University of Liège
Co-authors: Reyt M. (1,2), Baillet M. (1), Deantoni M. (1), De Haan S. (1), Lesoinne, A. (1), Laloux S. (1), Lambot E. (1), Muto V. (1), Schmidt C. (1,2)
1: GIGA-CRC In Vivo Imaging, Sleep and Chronobiology Lab, University of Liège, Liège, Belgium
2: Psychology and Neuroscience of Cognition Research Unit, Faculty of Psychology and Educational Sciences, University of Liège, Liège, Belgium

Keywords: Fractality, Hypothalamus, Napping, Actigraphy, MRI, Constant routine

Human activity exhibits a fractal behaviour, characterised by scale-invariant patterns over time scales ranging from minutes to 24 hours. Animal studies have shown that alterations of the suprachiasmatic nucleus (SCN), the circadian pacemaker located in the anterior part of the hypothalamus, are associated with a reduced scale-invariant correlation. Such reduction is also observed in ageing and Alzheimer's disease, both marked by a loss of SCN integrity. Here, we aimed at assessing the association between fractal regulation and in-vivo hypothalamic integrity in healthy older nappers and no nappers differing in their 24-hour distribution of rest-activity patterns. 28 healthy elderly nappers and 31 age- and gender matched no-nappers (mean age (±SD): 69.0±5.3 years, 37% female) underwent a 40-h multiple nap constant routine (CR). Locomotor activity was recorded using actimetry during (13±2) days and fractal correlation, αcirca, was calculated using detrended fluctuation analysis. Macromolecular content of grey matter tissue in the anterior inferior region was quantified using MRI derived Magnetization Transfer saturation (MTsat) maps. Group comparison confirmed that the circadian modulation of sleep efficiency during the CR is reduced in nappers, compared to no-nappers ($p<0.05$). Furthermore, bayesian mixed-effects models indicated that fractal correlation, αcirca, was linked to MTsat values within the anterior inferior hypothalamic region, with napping acting as a modulating factor (MTsat : ß=0.074, HDI(95%)=[0.003, 0.114], MTsat*nap group,ß=-0.091,HDI(95%)=[-0.177,0.002]). Our results support the hypothesis that daytime rest is linked to circadian alteration. Furthermore, they reveal for the first time that fractal regulation of locomotor activity is linked to in-vivo assessed integrity of the anterior hypothalamus, encompassing the SCN.

Napping and associated circadian alteration seem to mediate this association. Together, these data put forward the functional relevance of fractal regulation as a potential health risk (circadian) indicator.

139. Diffusion enhanced oscillations in biochemical networks

Main author: Pablo Rojas
Affiliation: Theoretical Physics, University of Kassel, Germany
Co-authors: Claudia Arbeitman (University of Kassel, CONICET), Oreste Piro (University of Balearic Islands), Martin E. Garcia (University of Kassel)

-Keywords: molecular diffusion, mathematical modeling, biochemical networks

Biochemical networks are central components underlying oscillations in biology. At the cellular level, these networks produce periodic outputs that ensure a variable concentration of key proteins, thereby enabling important functions in living systems, such as circadian timing. Deterministic mathematical families of models, such as ordinary differential equations (ODEs), are widely used to study the working principles of biochemical oscillations by describing the time evolution of relevant especies. A well-known model ODE system, the Goodwin oscillator, consists of a negative feedback loop, in which the protein at the end of the loop represses its own transcription. We show in this work that molecular diffusion plays an important role in shaping and expanding the parameter space for oscillations in the Goodwin model. Along with deterministic numerical results, we present semi-analytical approaches and stochastic simulations that help us to understand the origin of these oscillatory solutions, and connect these results with potential applications.

140. The winter blue-greens: how cyanobacteria predict winter

Main author: Maria Luísa Jabbur
Affiliation: Vanderbilt University
Co-authors: Carl Hirschie Johnson

Keywords: photoperiodism, cyanobacteria, winter, cold responses

For the last three decades, cyanobacteria have become a prolific model organism for the study of circadian clocks. However, while we know much about how the clocks of cyanobacteria work on daily and circadian scales, less is known about how seasons might affect them. Here we discuss evidence that, much like has been demonstrated for diverse organisms across the eukaryotic tree of life, cyanobacteria are also capable of photoperiodic time-measurement, using day length as a cue of future temperatures and changing their physiology accordingly. We characterize how cyanobacteria respond to different day lengths and observe that cells that were previously exposed to short, winter-like days survive low temperatures 2-3x more than those that were exposed to long, summer-like days. We further see that this phenomenon is dependent upon the presence of a functional circadian clock, as well as on past photoperiodical exposure. It also appears to involve anticipatory changes in membrane composition and takes multiple cycles of light-dark to be formed. Finally, we see that it likely works synergistically with lowering environmental temperatures in order to ensure that cyanobacteria are capable of surviving winter in the wild.

141. Are social defeat stress-induced phase shifts of peripheral clocks mediated by glucocorticoids?

Main author: Roelof Hut
Affiliation: University of Groningen
Co-authors: Kong X, Luxwolda M, Hut RA, Meerlo P
Keywords: Peripheral rhythms, stress, phase shift, liver, lung, kidney, glucocorticoids

While stress does not affect the phase or period of the central pacemaker in the suprachiasmatic nucleus, it can shift clocks in peripheral tissues. Our previous studies showed significant delays of the PER2 rhythms in the lungs and kidneys following social defeat stress. The mechanism underlying these effects is not fully understood but might involve glucocorticoids (GC) released during the stressor. In this follow-up study, we performed social defeat stress in adrenalectomized (ADX) mice to see if the induction of endogenous GC is necessary for the stress-induced phase shifts of peripheral clocks. We used mice that carry a luciferase reporter gene fused to the circadian clock gene Period2 (PER2::LUC) to examine daily rhythms of PER2 expression in various peripheral tissues. Mice were exposed to 5 consecutive daily social defeat stress in the late dark phase (ZT21-22). Running wheel rotations were recorded during 7 baseline and 5 stress days, which showed that the social defeat stress suppressed locomotor activity without affecting the phase of the rhythm. This suppression of activity was not prevented by ADX. One hour after the last stressor, tissue samples from the liver, lung, and kidney cortex were collected and cultured for ex vivo bioluminescence recordings. In the liver, PER2 rhythms were not affected by social defeat stress, ADX, or the interaction of both. In the kidney, social defeat stress caused a >4h phase delay of the PER2 rhythms, which was prevented by ADX, supporting the hypothesis of a crucial role of GC in this stress effect. In the lung, social defeat stress caused an 8h phase delay, but, surprisingly, a similar phase delay was seen in ADX animals independent of defeat. The latter indicates the complex effects of stress and stress hormones on the lung clock. The findings show that social defeat stress in the dark phase can shift PER2 rhythms in some tissues (lung, kidney) and not others (liver). Moreover, the stress effect in some tissues appears to be mediated by glucocorticoids (kidney) whereas the mechanism in other tissues is more complex (lung).

142. TimeTeller for circadian clock analysis

Main author: Maria Veretennikova – poster presented by Robert Dallmann
Affiliation: University of Warwick
Co-authors: Robert Dallmann, David Rand, Vadim Vasilyev, Laura Usselmann
Keywords: Circadian clock, dysfunction, time prediction, confidence

In this talk I will discuss TimeTeller, which is a machine-learning technique, allowing to draw inference of circadian clock function or dysfunction. It has been shown to be suitable for analysing microarray and RNA-Seq data. An important output of TimeTeller is a confidence metric in the predicted biological time of a single biopsy. We will briefly go over the design and exemplar uses of TimeTeller.

143. Effect of dim light at night on the locomotor activity of African pygmy mice (Mus minutoides)

Main author: Maria Oosthuizen
Affiliation: University of Pretoria
Co-authors: Arlin Viljoen
Keywords: African pygmy mouse, ALAN, LAN, locomotor activity, masking

Rodents are integral components of ecosystems as they provide several important ecosystem services. Despite their importance as prey, pollinators and seed distributers, African rodents are largely understudied. Furthermore, there is a paucity in studies on the effects of anthropogenic changes such as artificial light at night (ALAN) on African rodents. With the current trend in urbanisation and urban expansion, it becomes pertinent to investigate the impact of ALAN on African wildlife1. The effects of ALAN extend beyond¬ urban areas to peri-urban and rural habitats and can have profound effects on entire ecosystems and by extension, biodiversity2. We studied a small, strictly nocturnal rodent, the African pygmy mouse3, to consider the effect of light on their locomotor activity rhythms. We evaluated masking responses to light and dark pulses and assessed the effect of light at night with increasing intensities on the locomotor activity of pygmy mice. All the pygmy mice significantly reduced their activity during a light pulse during the night and half of the animals showed increased activity during a dark pulse during the day. This suggests that light has a strong masking effect on their activity, which is expected for nocturnal animals. In response to LAN, pygmy mice showed a drastic, intensity dependent reduction in their locomotor activity (Fig. 2 A, B), which was accompanied by a delay in the activity onset. These results show that pygmy mice are very sensitive to light and that LAN can have a large effect on their persistence and survival in nature. However, our laboratory setup represents a ‘worst case’ scenario with a very open ‘habitat’, whereas in their natural habitat, vegetation may provide some shelter against ALAN for pygmy mice. Other anthropogenic disturbances such as noise and habitat destruction could cause equally large disruptions in the behaviour and physiology of pygmy mice.

144. Time-dependent protecting role of heme oxygenase in the retina of Drosophila

Main author: Milena Damulewicz
Affiliation: Department of Cell Biology and Imaging, Jagiellonian University, Krakow, Poland
Co-authors: Elzbieta Pyza
Keywords: -

Heme oxygenase (HO) is an enzyme acting through degradation of heme to carbon monoxide (CO), ferrous ions, and biliverdin. Drosophila has only one gene encoding HO, which is cyclically expressed in the brain and in the retina. It is involved in the peripheral clock regulation in the retina, in cell protection against oxidative stress and DNA damage. In our study we focused on the role of HO in the retina. HO encoding gene (ho) shows bimodal rhythm in the expression with two peaks, in the morning and in the middle of the night, in LD12:12. This rhythm is maintained in constant darkness (DD), which suggests that it is clock-controlled. In addition, the morning peak of ho mRNA is enhanced by light, especially by UV and blue light, which helps to protect photoreceptors against the oxidative stress caused by light in the morning. Indeed, flies with inhibited HO activity have more DNA breaks in the retina than control flies, moreover enhanced HO activity protects photoreceptors against UV-induced DNA damage. Next, we focused on the role of HO during the night and its potential control of immune responses in the fly’s retina The microarray analysis showed that genes encoding Antimicrobial Peptides (AMPs) are expressed at higher level during the night than during the day. To check whether HO affects AMP level, we examined gene expression after ho silencing in the retina and in control

flies and found that in the experimental flies mRNA level of immune-related genes was higher during the night, but not during the day, which indicates that HO is involved in the regulation of innate immunity. To conclude, HO seems to affect many physiological processes in time-dependent manner. It protects the retina against light-induced damages during the day and suppresses immune response during the night.

145. Diurnal distributions of physical activity energy expenditure and effects on cardiometabolic health parameters in UK adults (The Fenland Study)

Main author: Philip Lewis
Affiliation: University Hospital of Cologne
Co-authors: Philip Lewis, Kate Westgate, Nick Wareham, Soren Brage
Keywords: accelerometry, physical activity energy expenditure, diurnal profile, cardiometabolic, compositional data analysis

Introduction: Response to an exposure can depend on circadian phase at time of exposure. The classic example is the circadian response to light but this principle is also demonstrated in cancer chronotherapy, chrononutrition, and timed-exercise. Exercise only contributes part of a person's overall physical activity energy expenditure (PAEE), similar to one high intensity light exposure among total diurnal light exposure. The diurnal distribution of light exposure is important in terms of its effects on physiology. The same may be true for PAEE.
Methods: First, we examined diurnal distributions of PAEE (relative to the overall total) in the Fenland cohort (n=12,435, 29-64 years old, male and female adults, born between 1950 and 1975). Second, we tested whether theoretical diurnal redistributions of PAEE (holding overall PAEE constant) affect associations with cardiometabolic parameters using a compositional data analysis. PAEE was determined using individually calibrated combined accelerometry and heart monitoring during 6-days of free-living.
Results: Diurnal profiles of PAEE were highly variable and associated with non-modifiable and sociodemographic characteristics. Compared to those less active, the most active had greater proportions of total PAEE in the evening. For the most part, a 5% redistribution of PAEE to earlier in the day was associated with an improvement in cardiometabolic parameters including levels of low-density lipoprotein (LDL), leptin, adiponectin, non-esterified fatty acids (NEFAs), insulin, glucose, c-reactive protein (CRP), body fat, and blood pressure. A conspicuous exception was redistribution to the noon-to-mid-afternoon time of day.
Discussion: Individual variability in diurnal distributions of PAEE would suggest that activity rhythm acrophase (or similar) in humans may not be an ideal estimate of 24-hour activity behaviour for association with cardiometabolic risk factors. Redistribution of PAEE to earlier times of day (except to noon-afternoon times) may provide health benefit for some individuals.
Further study is warranted.

146. The circadian REV-ERB nuclear receptors are essential for cardiac function and modulate NAMPT-dependent NAD+ biosynthesis via E4BP4

Main author: Pieterjan Dierickx
Affiliation: Max Planck Institute for Heart & Lung Research
Co-authors: Dierickx P*, Zhu K, Carpenter BJ, Jiang C, Vermunt MW, Xiao Y, Yamamoto T, Martí-Pàmies Í, Luongo TS, Mia S, Latimer M, Diwan A, Zhao J, Hauck AK, Krusen B, Blobel GA, Kelly DP, Pei L, Baur JA, Young ME, Lazar MA*
* Co-corresponding

Keywords: Circadian - Cardiovascular - Metabolism - NAD+ - REV-ERBs

The heart is a highly metabolic organ that uses multiple energy sources to meet its demand for ATP production. Diurnal feeding-fasting cycles result in substrate availability fluctuations which, together with increased energetic demand during the active period, impose a need for rhythmic cardiac metabolism. The nuclear receptors REV-ERB□ and □ are essential repressive components of the molecular circadian clock and major regulators of metabolism.
To investigate their role in the heart, we generated mice with cardiomyocyte (CM)-specific deletion of both Rev-erbs, which died prematurely due to dilated cardiomyopathy. Loss of Rev-erbs markedly downregulated fatty acid oxidation genes prior to overt pathology, which was mediated by induction of the transcriptional repressor E4BP4, a direct target of cardiac REV-ERBs. E4BP4 directly controls circadian expression of Nampt and its biosynthetic product NAD+ via distal cis-regulatory elements. Dietary supplementation of CM-RevDKO mice with an NAD+ precursor improved cardiac function and extended their lifespan. Thus, REV-ERB-mediated E4BP4 repression is required for Nampt expression and NAD+ production by the salvage pathway. Together, these results highlight the indispensable role of circadian REV-ERBs in cardiac gene expression, metabolic homeostasis and function.

147. CRISPR Base-editors: Highly precise and scar-proof genome-editing tool to generate unique point mutants and explore clock genes in Drosophila melanogaster

Main author: Nirav Thakkar
Affiliation: [1]Biology Centre AS CR, v.v.i., Institute of Entomology, Ceske Budejovice, Czech Republic
[2]Faculty of Science, University of South Bohemia, Ceske Budejovice, Czech Republic
Co-authors: Adela Hejzlarova[1,2], Vaclav Brabec[1,2], David Dolezel[1,2]

Keywords: Circadian Clock, CRISPR Base-editors, Nucleotide deaminase, Dead Cas9 (dCas9), Cytidine Base-editors, Adenine Base-editors, Drosophila melanogaster, Point mutation

Circadian clocks are defined by their ability; to retain the free-running period in constant conditions, to synchronize with daily light and temperature changes and to retain periodicity over a wide range of physiological temperatures (temperature compensation). Previous findings (resulting from mutants like perS, perL, timPL, timUL, timrit, timblind, timSL, dbtS, dbtL dbtar, dcoh, etc.) have made it evident that even a single nucleotide change is enough to destabilise the clock. Due to the limitations of a meticulous nucleotide editing tool, an array of point mutants of Drosophila clock genes is still very limited, and such mutants are a prerequisite to learn about the protein-protein interactions and overall regulatory complexity. CRISPR Base-editors help circumvent the complications of undesired indel mutations of the canonical CRISPR-Cas9 tool by employing the nucleotide deaminase fused to a catalytically dead Cas9. Consequently, CRISPR base-editors can specifically edit the target base in

the DNA without leaving a scar (insertion or deletion). In this study, we adapted four different base-editors (two cytosine base-editors, adenine base-editor and rainbow editor) in the Drosophila model system. In addition, using the multiple gRNA targeting strategy in combination with these base editors, we destroyed the splicing of the white gene to identify its editing efficiency.
Using the PmCDA cytidine base-editor, we successfully generated a functional timeless mutant with a clock slower by five hours compared to wild-type canton-s.

148. A minimal circadian clock network for glucose tolerance

Main author: Jacob Smith
Affiliation: Universitat Pompeu Fabra (UPF), PRBB
Co-authors: Kevin B. Koronowski, Tomoki Sato, Carolina Greco, Paul Petrus, Amandine Verlande, Siwei Chen, Muntaha Samad, Ekaterina Deyneka, Lavina Mathur, Ronnie Blazev, Jeffrey Molendijk, Thomas Mortimer, Arun Kumar, Oleg Deryagin, Mireia Vaca Dempere, Peng Liu, Valerio Orlando, Benjamin L. Parker, Pierre Baldi, Patrick-Simon Welz, Cholsoon Jang, Selma Masri, Salvador Aznar Benitah, Pura Muñoz-Cánoves and Paolo Sassone-Corsi

Keywords: clock network, metabolism, inter-organ signaling, glucose, muscle, liver

A network of molecular circadian clocks throughout the brain and periphery achieves homeostatic control of daily physiology in mammals. Clocks in peripheral organs regulate metabolism, though whether they do this in an independent or interdependent manner is unclear. Here, we use genetic reconstitution of Bmal1 in the liver and skeletal muscle of otherwise clockless mice, to assess their sufficiency and codependency. We report a subset of genes under control of the local clock in liver and muscle (15% and 4%), and find little evidence of transcriptional interdependency. However, glucose-tolerance tests and 13C-glucose tracing revealed a synergistic effect in mice containing clocks in both tissues, but not each tissue alone, on systemic glucose handling- pointing to a linking of metabolism across the two tissues downstream of glucose. Tolerance to pyruvate was restored to WT levels with both clocks present, indicative of a rescue of gluconeogenic control and/or pyruvate oxidation. Overall, our results demonstrate that liver and muscle clocks control local tissue metabolism in a transcriptionally independent manner, but the concerted action of both is required for the buffering of glycemia.

149. Impact of the rodent estrous cycle on liver transcriptomics

Main author: Terry Lin
Affiliation: Salk Institute
Co-authors: -

Keywords: female, estrous cycle, estrogen, sex, metabolism, liver, transcriptomics, uterus, rhythmicity

C57BL6 mice are considered as the gold standard pre-clinical mouse model for diet-induced obesity. However, female C57BL6 mice are resistant to weight gain and metabolic disease, owing to the protective effect of estrogens. Circulating hormones associated with the estrous cycle impact core circadian rhythms in body temperature and activity. The sexual dimorphism behind liver health and disease is widely studied but no circadian analysis of the liver incorporating the estrous cycle has been shown. Our goal was to assess the impact of the estrous cycle and circulating hormones on liver metabolism. We fed young C57BL6 female mice a standard rodent chow and determined the estrous cycle stage by vaginal lavage at ZT14 daily for 6 weeks. These animals were sacrificed at the peak (proestrus) and trough (metestrus) of the estrous cycle and collected with 3 replicates, every 3 hours

for 24 hours. Frozen liver and uterus tissues were processed for RNA sequencing. Principal component analysis revealed in the uterus, samples in proestrus and metestrus were clearly separated with over 30% of variance while there was high overlap in most of the liver samples across both stages. A time-independent differential gene expression analysis of all samples across both stages found 42% and 5.2% of expressed genes in the uterus/ liver significantly changing between proestrus and metestrus. In the liver, pathway enrichment suggested that genes involved in translation and fatty acid metabolism were upregulated in proestrus and genes involved in steroid biosynthesis and coagulation/ complement cascade were upregulated in metestrus. Periodicity analysis with MetaCycle (R v1.2.0) revealed 1280/486 and 2756/2068 genes rhythmic in proestrus/metestrus in the uterus and liver respectively. These data suggest that while the liver transcriptome is generally homeostatic, key metabolic pathways and rhythmic processes may be uniquely affected by circulating hormones in different estrus stages.

150. Circadian rhythm and physical activity fragmentation behaviours among normal and obese adolescents

Main author: Jeffrey Pagaduan
Affiliation: Faculty of Physical Culture, Institute of Active Lifestyle, Palacký University, Olomouc, Czechia
Co-authors: Jan Dygrýn, Vincent Van Hees, Xiaoling Wang, Aleš Gába

Keywords: adiposity, physical activity, adolescence, obesity

Adolescent obesity has been found to influence maladaptive changes in circadian rhythms. With the advancement of technology, objective and non-invasive quantification of motor activity rhythm (MAR) in humans is now available. The purpose of this activity was to examine the MAR and physical activity fragmentation (PAF) behaviours in normal and obese/overweight adolescent girls and boys. 156 girls and 111 boys from four high school in Czech Republic volunteered to undergo actigraphy (wGT3x-BT/GT9X, Actigraph, Pensacola, USA) for seven consecutive days during the spring and fall seasons between 2018 and 2019. Participants were categorized as normal or obese/overweight based on the WHO guidelines. Data (.gt3x) from the accelerometers were extracted and analysed using an open source R-package (GGIR ver 2.7.1) to derive MAR and PAF metrics. Mann-Whitney U test was carried out to determine any significant difference in relation to sex. Results revealed the following significant differences between obese and normal girls: 1) intradaily stability (IS), $U = 1705$, $p = 0.038$; 2) cosinor midline estimating statistic of rhythm (Cos_MESOR) between groups, $U = 1679.0$, $p = 0.029$; 3) number of fragments from inactivity to light physical activity (N_Frag_IN_to_LIPA), $U = 1726$, $p = 0.048$; 4) number of blocks during IN (N_Blocks_IN), $U = 1716$, $p = 0.043$; 5) number of blocks during LIPA (N_Blocks_LIPA), $U = 1692$, $p = 0.038$; and, 6) transition probability from inactivity to light physical activity (TP_IN_to_LIPA), $U = 1729$, $p = 0.049$. On the other hand, the mean of the number of LIPA fragments ($U = 780.0$, $p = 0.037$) were found to be significantly different between obese and normal boys. In conclusion, it seems that girls are susceptible to alterations in motor activity rhythm under adolescent obesity, and such changes may be supported by physical activity fragmentation behaviours.

151. A circadian clock in the fly retina: deCRYpting new roles

Main author: Gabriella Mazzotta
Affiliation: Department of Biology, University of Padova, Italy
Co-authors: Milena Damulewicz, Francesco Gregoris, Giovanni Minervini
Keywords: -

In Drosophila, time-keeping mechanisms reside in a multitude of peripheral cells, including retinal photoreceptors, in which a functional circadian clock has been shown to contribute to visual system health and function. In this context, the circadian photoreceptor CRYPTOCHROME (CRY) modulates synaptic plasticity and circadian visual sensitivity and is involved in the ability of flies to entrain their locomotor behaviour to red-light cycles.
We have unveiled new roles for this photoreceptor in the retinal clock. First, an interaction with mitochondrial dynamics proteins suggests its possible involvement in the circadian modulation of mitochondria functioning. Second, the interaction with proteins participating in the proteasomal pathway indicates its contribution to the regulation of protein homeostasis. Very importantly, we have observed that CRY in the photoreceptor cells is able to influence sleep. This is the first observation of a direct contribution of the clock in the photoreceptors to the behavioural sleep/activity rhythms, a function so far ascribed to the pacemaker and glia neurons.

152. Exploring the role of gastrin-releasing peptide neurons in circadian time-keeping by the suprachiasmatic nucleus

Main author: Elena del Carmen Gomez Garcia
Affiliation: Medical Research Council, Laboratory of Molecular Biology, Cambridge U.K.
Co-authors: Elena d.C. Gomez Garcia, Emma L. Morris, Antony Adamson*, Andrew P. Patton, Michael H. Hastings
* Faculty of Biology, Medicine and Health, University of Manchester, U.K.
Keywords: SCN, circadian, neuropeptides, network, phase, GRP, VIP, transcriptomics, intersectional genetics

The suprachiasmatic nucleus (SCN) is the master clock in mammals, providing daily time cues to subordinate clocks across the body that control our behaviour and physiology. SCN clock function is encoded at two levels: the intra-cellular, cell-autonomous transcriptional /translational feedback loop (TTFL) and the intercellular network. Cell-autonomous circadian rhythms are coupled at the network level by paracrine signalling mediated by neuropeptides that confer the SCN with emergent properties: synchrony, ensemble period, ensemble phase and spatiotemporal complexity. This project aims to determine the contribution of gastrin-releasing peptide (GRP) neurons to SCN time-keeping. Confirming its relevance, application of exogenous GRP phase-shifted the circadian TTFL (PER2::Luciferase bioluminescence) of organotypic SCN slices. The effect was phase- and dose-dependent (a delay of -1.5 ±0.6h (mean ±SEM) at CT16-17, 100 nM GRP). Grp-neurons were defined computationally using a single cell RNA-seq dataset (Morris et al. 2021, EMBO J.), revealing that ~54.7% of Grp-neurons also express Vip, suggesting the existence of three distinct populations that may have independent, shared and/or collaborative functions: Grp-only, Vip-only and Grp/Vip-neurons. To obtain genetic access to these populations, I have characterised a novel Grp-Cre recombinase knock-in mouse to be used in combination with an existing Vip-Flp recombinase knock-in mouse. Cre recombinase is expressed in a sub-population of cells in the central SCN, confirmed by release of a Cre-dependent AAV-encoded fluorescent reporter. The circadian properties of heterozygous and homozygous Grp-Cre SCN (period and waveform of PER2::Luciferase bioluminescence) and mice (period and period stability of wheel-running behaviour under light:dark

and constant darkness conditions) are no different from wild-type. Understanding the function and interaction between neuropeptidergic cell populations in the SCN will help us understand the SCN emergent properties that distinguish it from any other subordinate clocks in the brain and make it the dominant pacemaker of our daily life. Funding. MRC MC_U105170643.

153. Nogo-A is a melatonin-driven regulator of circadian memory dynamics and learning

Main author: Jörg Stehle
Affiliation: University Frankfurt, Theodor-Stern-Kai 7, D-69590 Frankfurt, Germany
Co-authors: Laura Hausmann, Ajmal Zemmar
Keywords: Hippocampus, learning, Nogo-A, melatonin, time-of-day, plasticitry, LTP, ganglionectomy

Synaptic plasticity and subsequent memory formation are time-of-day-dependent processes, with molecular mechanisms unclear. We identified in mouse hippocampus by immunochemistry a circadian oscillation of the neurite-outgrowth-inhibitor Nogo-A, with lowest levels occurring at Zeitgeber Time 2 (ZT2) and highest abundance at ZT14. Low Nogo-A levels at ZT2 coincided with better learning in a food-rewarded spatial memory test, while at highest Nogo-A levels at ZT14 learning was diminished. As in both, melatonin-deficient C57BL mice and in melatonin receptor deficient MT-/- mice hippocampal Nogo-A levels were constantly elevated, we suggested a role of melatonin for the hippocampal Nogo-A rhythm. We extirpated the superior cervical ganglia (SCG) in mice, rendering a knockdown of rhythmic melatonin synthesis. Indeed, in SCGX mice Nogo-A was constantly elevated, and learning showed no day-night differences. When we substituted melatonin at nighttime only in WT-SCGX mice the circadian Nogo-A rhythm re-appeared and the day-night differences in learning was re-established. Our results grant Nogo-A a fundamental and novel function in regulating daily homeostasis of mouse hippocampal signaling, with the rhythm determined by melatonin that temporarily removes constraints on neuronal plasticity, merging into shaping time-of-day-dependent cognitive performance.

154. Within-subject and between-subject variability of urinary creatinine excretion over a two-month period

Main author: Lennart Seizer
Affiliation: Medical University Innsbruck, Austria
Co-authors: Johanna Gostner, Dietmar Fuchs, Harald R Bliem, Christian Schubert
Keywords: creatinine, urine, time series, variability, normalization, circadian rhythm

Urinary creatinine is commonly used to determine glomerular filtration rate and normalize urinary analyte concentrations to control variations in the urinary flow rate. However, the value of urinary creatinine as a reference parameter has been questioned due to inter- and intra-individual variability in its excretion rate, possibly due to everyday influences such as physical activity or psychosocial stress. In this study, four healthy women (two of which were breast cancer patients in remission) collected their entire urine over a period lasting between 28 and 63 days in consecutive 12-h collection intervals with subsequent analysis of urinary creatinine (HPLC) to study both diurnal (12–12h) and day-to-day (24–24h) variability. To determine within-subject variability in urinary excretion rates, coefficients of variation (CV) were calculated for consecutive 12-h- and 24-h-collection intervals. We found the within-subject variability in urinary excretion rate to be high compared to previous studies with shorter observation periods (12-h-CV: 18.6 to 25.2%; 24-h-CV: 12.5 to 18.9%). Additionally, creatinine excretion was significantly higher during the night than during the day in three of four subjects; however, this diurnal difference was not present consistently throughout the study period.

Finally, by combining our data with literature, we provide an update to previous recommendations on creatinine sampling and analyte normalization.

155. Theta and alpha EEG oscillations homeostatically increase in amplitude with time awake – except during the wake maintenance zone

Main author: Sophia Snipes
Affiliation: University Children's Hospital of Zurich
Co-authors: Elias Meier, Sarah Meissner, Hans-Peter Landolt, Reto Huber

Keywords: wake maintenance zone eeg oscillations 2 process model theta alpha

Electroencephalographic (EEG) oscillations are typically quantified using power analyses such as the Fast Fourier Transform. This has led to the observation that theta (4-8 Hz) and alpha (8-10 Hz) power are affected by sleep homeostasis, with theta increasing and alpha decreasing with time spent awake, and both are further affected by circadian rhythms. However, power values are dependent simultaneously by the quantity of oscillatory bursts and their amplitudes. We wished to investigate whether the quantity and amplitude of EEG oscillations followed circadian and homeostatic trajectories, respectively. Data was obtained from 18 young healthy adults undergoing 24h sleep deprivation, with wake EEG recordings collected approximately every 3h. The 24h wake interval began at the midpoint of their habitual sleep period (4:00, normalized clock time), so they were also sleep restricted. We applied cycle-by-cycle analysis to identify oscillatory bursts between 4 and 12 Hz. We found that both the quantity of theta bursts and their amplitude significantly increased over the 24h period. Notably, the average amplitude of theta increased along a saturating exponential function (R-squared=.96) until 18:00, then significantly decreased during the hours prior to their habitual bedtime (21:00, 23:30), before returning to the previous trajectory for the final recording at 3:00. The quantity of alpha bursts did not significantly change until the end of the sleep deprivation period (3:00), during which they decreased. Conversely, the amplitudes of alpha bursts, like theta, followed an increasing saturating exponential function (R-squared=.83), then decreased from 21:00-23:30, and reached the overall maximum at 3:00. In conclusion, when controlling for the quantity of oscillatory bursts, the amplitudes of both theta and alpha oscillations in the waking rest EEG follow the trajectory predicted by the homeostatic component of the two-process model of sleep, except during the wake maintenance zone just prior to habitual sleep onset.

156. Inter-layer and inter-subject variability of circadian gene expression in human skin

Main author: Marta del Olmo
Affiliation: Institute for Theoretical Biology, Charite University Medical Hospital and Humboldt University Berlin
Co-authors: Marta del Olmo, Florian Spörl, Sandra Korge, Karsten Jürchott, Matthias Felten, Astrid Grudziecki, Jan de Zeeuw, Claudia Nowozin, Hendrik Reuter, Thomas Blatt, Hanspeter Herzel, Dieter Kunz, Achim Kramer, Bharath Ananthasubramaniam

Keywords: skin, human, gene expression, variability, clocks, biomarkers, internal time

The skin is the largest human organ with a circadian clock that regulates its function. Although circadian rhythms in specific functions are known, rhythms in the proximal clock output, gene expression, in human skin have not been thoroughly explored. This work reports circadian gene expression in two skin layers, epidermis and dermis, in a cohort of young, healthy adults, who maintained natural, regular sleep schedules. 10% of the expressed genes showed rhythms at the

population level, of which only a third differed between the two layers. Broadly, expression magnitudes of circadian genes were consistent across subjects in each layer. Amplitude and phases of circadian gene expression, however, varied more across subjects than layers, with amplitude being more variable than phases. Expression amplitudes in the epidermis were larger and more subject-variable, while they were smaller and more consistent in the dermis. Core clock gene expression was similar across layers at the population-level, but were heterogeneous in the their variability across subjects. We used this data to identify small sets of biomarkers for internal clock phase in each layer, which consisted of layer-specific non-core clock genes. This work provides a valuable resource to advance our understanding of human skin to realize the potential of circadian medicine as well as a novel methodology to quantify sources of variability in human circadian rhythms.

157. Targeted plasma metabolomics of endocannabinoids and acylethanolamides in response to combined sleep restriction and circadian misalignment

Main author: Dana Withrow
Affiliation: University of Colorado Boulder
Co-authors: Alivia B. Blumenstein, Shannon M. Lanza, Kate E. Sprecher, Michael L. Armstrong, Christopher M. Depner, Nichole Reisdorph, Kenneth P Wright Jr.
Keywords: -

Introduction: Insufficient sleep and circadian misalignment are common in shift-workers and are associated with negative physiological and cognitive health outcomes, yet the underlying mechanisms are not fully understood. Endocannabinoids and acylethanolamides can impact physiological and behavioral function and the influence of combined sleep restriction and circadian misalignment on these bioactive lipids is unknown.
Methods: Following rigorous health screening and 2 weeks of at-home monitoring with 8h sleep opportunities verified by actigraphy, healthy participants underwent experimentally induced sleep restriction and circadian misalignment in the laboratory. The first night participants were given an 8h sleep opportunity, followed by a 3h sleep opportunity on night 2, then 3h sleep opportunities occurring during the afternoons on days 3 and 4. Blood draws occurred at baseline following the 8h sleep opportunity and during combined sleep restriction and circadian misalignment approximately 69h later. Blood samples from 12(6F) participants, mean age of 25.75y were analyzed for targeted plasma metabolomics including 14 endocannabinoids/acylethanolamides. Linear mixed effects models to analyze the impact of combined sleep restriction and circadian misalignment, with subject as a random factor were utilized followed by a false discovery rate correction for multiple comparisons.
Results: The endocannabinoid AEA (FDR $P < 0.01$) and eight acylethanolamides were decreased (all FDR $P < 0.02$) after combined sleep restriction and circadian misalignment compared to baseline.
Discussion: Combined sleep restriction and circadian misalignment leads to alterations in bioactive lipids in the plasma. Additional research is necessary to explore the effects of reduced circulating concentrations of this endocannabinoid and acylethanolamides on physiological and behavioral functions during sleep and circadian disruptions.

158. High-fat diet (HFD)-induced obesity (DIO) is secondary to HFD-induced circadian disruptions in the pattern and length (tau) of endogenous circadian rhythms

Main author: Roee Gutman
Affiliation: Department of Animal Sciences, Tel-Hai College; and Department of Nutrition and Natural Products, MIGAL – Galilee Research Institute, Israel
Co-authors: -
Keywords: circadian rhythms; aftereffect, locomotor activity, obesity, circadian disruption.

Obesity in mice is postponed under a photic cycle oscillating at a period length similar to or shorter than their endogenous circadian rhythm period length
Cumulative data shows that T-cycle's deviation from tau correlates with weight gain, suggesting that energy balance is most tightly regulated under near-tau T-cycles. Indeed, we have recently demonstrated that DIO in mice under a 24-h light-dark cycle (T-cycle) is also secondary to the entrainment process to this near-tau T-cycle, as suggested by the DIO-prevention under a T-cycle oscillating at their expected tau of 23.7-h. We herein further examined this hypothesis by studying energy homeostasis of low-fat diet (LFD) and HFD-fed mice held under a T-cycle oscillating at the tau of same-age LFD-fed mice, compared with a 24-h T-cycle, a T-cycle with a period faster than tau by the tau-24-h deviation (∆tau), and under constant darkness (DD). The energy balance of LFD-fed mice was unaffected by photic regimes. DIO onset under the 24-h T-cycle resembled that under DD, preceded by HFD-induced prevention of the age-related shortening in tau. Compared to 24-h T-cycle, DIO onset under the tau-like and ∆tau T-cycles was similarly delayed, underlined by a difference in energy expenditure rather than intake, and accompanied by a T-cycle-related tau-shortening (i.e., 'aftereffect'). These results highlight the centrality of the HFD-induced deceleration in tau in predisposing to DIO under a circadian desynchrony due to T-cycle with a slower-than-tau period length. Correspondingly, they suggest that enforcing a zeitgeber resonating with or slightly faster than tau, attenuating the HFD-induced deceleration, postpones DIO onset without caloric restriction. Hence, it is suggested that pharmacological, nutritional, or environmental zeitgebers that slightly shorten or enhance endogenous circadian rhythms' robustness may attenuate DIO, at least in mice, even while feeding on HFD ad-libitum.

159. Diet diurnally regulates small intestinal microbiome-epithelial-immune homeostasis and enteritis

Main author: Karina Ratiner
Affiliation: Weizman Institute
Co-authors: Tuganbaev T, Mor U, Bashiardes S, Liwinski T, Nobs SP, Leshem A, Dori-Bachash M, Thaiss CA, Pinker EY, Ratiner K, Adlung L, Federici S, Kleimeyer C, Moresi C, Yamada T, Cohen Y, Zhang X, Massalha H, Massasa E, Kuperman Y, Koni PA, Harmelin A, Gao N, Itzkovitz S, Honda K, Shapiro H, Elinav E.
Keywords: Crohn disease; HFD; IBD; IL-10; MHC class II; SFB; circadian clock; epithelial cell; high-fat diet; inflammatory bowel disease; interleukin-10; major histocompatibility complex; microbiome; segmented filamentous bacteria; small intestine.

Throughout a 24-h period, the small intestine (SI) is exposed to diurnally varying food- and microbiome-derived antigenic burdens but maintains a strict immune homeostasis, which when perturbed in genetically susceptible individuals, may lead to Crohn disease. Herein, we demonstrate that dietary content and rhythmicity regulate the diurnally shifting SI epithelial cell (SIEC)

transcriptional landscape through modulation of the SI microbiome. We exemplify this concept with SIEC major histocompatibility complex (MHC) class II, which is diurnally modulated by distinct mucosal-adherent SI commensals, while supporting downstream diurnal activity of intra-epithelial IL-10+ lymphocytes regulating the SI barrier function. Disruption of this diurnally regulated diet-microbiome-MHC class II-IL-10-epithelial barrier axis by circadian clock disarrangement, alterations in feeding time or content, or epithelial-specific MHC class II depletion leads to an extensive microbial product influx, driving Crohn-like enteritis. Collectively, we highlight nutritional features that modulate SI microbiome, immunity, and barrier function and identify dietary, epithelial, and immune checkpoints along this axis to be potentially exploitable in future Crohn disease interventions.

160. Bioelectronic Zeitgebers: towards neuromodulation of neurological disorders synchronized to biological rhythms

Main author: Alceste Deli
Affiliation: Nuffield Department of Surgical Science and Nuffield Department of Clinical Neurosciences, University of Oxford
Co-authors: Alceste Deli, John E. Fleming, Mayela Zamora, Amir Divanbeighi Zand, Sean Martin, Moaad Benjaber, Victoria Revell, Russell G. Foster, Derk-Jan Dijk, Alexander L. Green, Timothy Denison

Keywords: implanted chronotherapy, circadian neuromodulation, subcortical arousal networks

Implantable bioelectronic systems that stimulate the nervous system have been shown as an effective adjunct therapy for neurological disorders such as Parkinson's disease and epilepsy. Symptoms of these disorders vary with time of day. Most devices, however, run a fixed stimulation without consideration of circadian/diurnal rhythms. The diagnostic capabilities of device-based therapies have been limited until now; few studies have investigated the impact of rhythms on therapy efficacy and therapy's influence on rhythm-related symptoms and physiology. To maximize therapeutic benefits, bioelectronic devices should integrate chronobiology by detecting time-related variations in disease symptoms and altering therapy output. The aim is to respect, and potentially promote, healthy biological rhythms.

We developed an implantable bioelectronic device with digital algorithms that adjusts stimulation based on both time and sensor-based physiological biomarkers. To illustrate the "digital chronotherapy" concept, we present preliminary data from two patients with Multiple System Atrophy. The bioelectronic device interfaces with the pedunculopontine nucleus. Patient symptoms followed a diurnal rhythm, which motivated exploration of anticipatory, time-based stimulation adjustments where parameter adjustments were aligned to the sleep-wake rhythm. Incorporating night-time settings that did not promote wakefulness preserved sleep while maintaining daytime therapy efficacy. In addition, a feedforward motion-adaptive (sensor-based) detector provided acute stimulation boosts to avoid suboptimal therapy during transient, night-time ambulatory periods. The time and motion-based adjustments ensured that therapeutic stimulation aligned to the requirements for both sleep and wakefulness.

Preliminary evidence from the algorithms' first use in a clinical study supports its impact on sleep-wake pathology. We noted a decrease in excessive daytime sleepiness accompanied by longitudinal modulation of wake-related oscillations. Bioelectronic devices capable of data recording and adaptive algorithms provide insight into rhythmic disease processes and how bioelectronic chronotherapy might optimize treatment. We also demonstrate that multiple biomarkers in closed-loop algorithms might enhance therapy beyond use of single physiological signals.

161. The role of RNA methylation in the crosstalk of circadian clock and neuroinflammation in suprachiasmatic nuclei

Main author: Eva Filipovská
Affiliation: Department of Physiology, Faculty of Science, Charles University, Czech Republic
Co-authors: Zuzana Čočková, Zdeňka Bendová
Keywords: SCN, RNA methylation, m6A, inflammation

Neuroinflammation and circadian rhythm dysfunction are critical risk factors in a variety of neuropsychiatric disorders, but their mutual interplay is poorly understood. Both systems are influenced by N6-methyladenosine (m6A), the most abundant epitranscriptomic mark in mammalian mRNA. Our objective was to elucidate the mechanisms of the immune-circadian crosstalk by investigating the role of the m6A modification in rat suprachiasmatic nuclei (SCN) under normal and LPS-induced inflammatory conditions.
To assess the expression of critical m6A regulatory enzymes, primary cultures obtained from Wistar rats' SCN were analyzed using the RT-qPCR technique. We also evaluated the levels of m6A in RNA during 24h and determined the relative production of reactive oxygen species (ROS) by ROS-sensitive fluorescent probe.
Our experiments show that the RNA methylation of N6-adenosine in primary cultures of rat SCN is driven by the circadian system. Inflammatory conditions induced by LPS treatment lead to increased RNA methylation probably by decreasing expression of Fto demethylase. Fto silencing also diminishes oxidative stress induced by LPS, suggesting that decreased expression of Fto after LPS treatment may have protective effect. Overall, our work suggests a potent role of m6A in sensitivity of SCN cells to immune challenges and in circadian-immune interplay.

162. Effect of Cyclic versus Continuous Enteral Nutrition on Circadian Rhythms in Critical Illness: Protocol for a Randomized Controlled Trial

Main author: Hiemstra Floor
Affiliation: Department of Intensive Care & Department of Neurophysiology, Leiden University Medical Center, The Netherlands
Co-authors: Evert de Jonge, Joke Meijer, David van Westerloo, Laura Kervezee
Keywords: circadian rhythms; 24-h rhythms; critical illness; intensive care unit; ICU; enteral nutrition; time-restricted feeding

Background - Circadian rhythms are severely disrupted in patients in the intensive care unit (ICU), which has been associated with poor clinical outcomes. A potential contributor to this disruption is the enteral nutrition, usually administered 24 hours a day. Rhythmic feeding-fasting cycles can provide a potent synchronizing cue for the circadian clock, suggesting that optimalization of feeding-fasting cycles in the ICU might strengthen circadian rhythms.
Objective - The aim of this study is to evaluate whether cyclic daytime enteral nutrition can improve 24-h rhythms in critically ill patients compared to continuous enteral nutrition.

Methods - This study is an investigator-initiated randomized controlled trial in a tertiary care ICU in

the Netherlands. Critically ill patients (aged ≥ 18 years) with an expected ICU stay ≥ 48 hours receiving or with intention to start enteral nutrition are eligible for inclusion. Patients (n=60) will be randomized to the continuous enteral nutrition or cyclic daytime enteral nutrition group. In the continuous enteral nutrition group (control), nutrition will be administered 24 hours a day. Patients in the cyclic daytime enteral nutrition group will receive nutrition in a 12-hour period during daytime hours, between 8 AM to 8 PM. In both groups, similar daily nutritional goals are prescribed. On day 3 after the start of the enteral nutrition, seven 4-hourly blood samples will be drawn and a 24-hour electroencephalography recording will be done. Additional study data will be collected from the monitors and electronical patient record. The primary outcome is the amplitude of 24-h rhythms in melatonin, vital signs (including core body temperature), and heart rate variability. Secondary outcomes include clock gene expression, sleep quality, glucose regulation, insulin administration, caloric intake, feeding intolerance and clinically relevant outcome measures.
Conclusion – This study will help to gain more insight into strategies to optimize circadian rhythms in ICU patients.

163. Impact of Nuclear Magnetic Resonance therapy (tNMR) on the circadian clock and the associated Hypoxia Inducible Factor-1 (HIF1-α) in the mouse fibroblast cell line NIH 3T3 under normoxic and hypoxic conditions.

Main author: Mauracher David
Affiliation: Leopold Franzens University, Innsbruck
Co-authors: Viktoria Thöni, Margit Egg
Keywords: -

The retinal clock protein Cryptochrome of migrating animals is known to respond to changes in the geomagnetic field of the earth via the radical pair mechanism. Accordingly, low intensity electromagnetic fields (EMFs) are suspected to possibly influence the circadian clocks of cells, tissues and organisms. Nuclear Magnetic Resonance (NMR) is a specific type of radiofrequency magnetic field, which forms the basis for several clinical applications like Magnetic Resonance Imaging (MRI), Magnetic Resonance Spectroscopy (MRS) and, in its low intensity form, therapeutic Nuclear Magnetic Resonance (tNMR). In the present study we want to assess and characterize the effects of tNMR, which has been shown to influence the circadian clock in mammalian cells at the level of mRNA, on core clock proteins of mammalian NIH 3T3 cells. For this purpose, we treated Dexamethasone (Dex) synchronized NIH 3T3 cells for 6h with tNMR (MBST® Open System 350) in the morning (8:00 to 14:00) and quantified the core clock proteins BMAL1, CRY1, CRY2 and the clock the associated HIF1-α using Western Blots. We found that BMAL1 was upregulated under normoxic conditions, while it was reduced after tNMR treatment in combination with hypoxia of 1% O2. CRY2, in turn, appeared to be downregulated under normoxic conditions. Counterintuitively, HIF1-α protein levels were reduced after hypoxic treatment, and even more after the combined hypoxia-tNMR treatment. According to literature, we assign these reduced levels of HIF1-α protein to the treatment with the corticosteroid derivative Dex. Furthermore, HIF1-α showed distinct circadian oscillations under all experimental conditions. To understand the functional significance of the observed tNMR induced alterations in clock protein levels, Per2 reporter assays will be performed.

Since HIF1-α and the circadian clock play key roles in ischemic diseases like infarct, stroke or cancer, tNMR might represent a potential treatment option and needs further investigation.

164. Sorghum circadian system and its role in resilience to environmental stresses

Main author:	Or Silberstein
Affiliation:	Department of Plant and Environmental Sciences, Institute for Life Sciences, The Hebrew University, Jerusalem
Co-authors:	Arlin Viljoen
Keywords:	Nitzan Weisman, Yuri Dakhiya and Rachel Green

Endogenous circadian time-keeping systems enable plants to anticipate and adapt to day/night and seasonal changes in their environment. Amongst numerous biological processes regulated by the circadian oscillator in plants are defense responses, photosynthetic capacity, stress-related genes, hormone production and the control of development. Circadian rhythmic traits (such as period, phase, and amplitude) vary between and within species. Studies have suggested that having a robust circadian system that is correctly synced to the plant's environment confers a significant advantage for growth and vitality. In crops, the circadian system affects every developmental stage from germination to flowering time and harvest and heavily influences production, biomass and yield. We predict that a better understanding of the circadian mechanism can be exploited to optimize growth and resilience in agricultural crops to cope with stresses such as climate change.
Sorghum (Sorghum bicolor) is widely grown in the developing world and about half a billion of the world's poorest people rely on it as a staple food. Although sorghum is more resistant to heat and drought than other cereals, extreme and frequent weather events are making the need to develop more stress-resistant varieties urgent. However, the circadian system of this orphan crop is poorly understood.
The main objective of this project is to understand the sorghum circadian system and its role in resilience to environmental stresses. To approach the question of adaptation we have a panel of sorghum accessions originating in diverse habitats across Africa, America and Asia. Using chlorophyll fluorescence imaging to measure circadian traits and available eco-geographic data about the sites of origins of the plants we are investigating interactions between circadian traits and environmental characteristics in stress and non-stress conditions. Our goals are to identify sorghum accessions with circadian rhythm traits that sustainably enhance their resilience to a range of stresses.

165. Circadian influence on intrusive re-experiencing in trauma survivors' daily lives

Main author: Laura Meister
Affiliation: Department of Psychology, University of Zurich, Zurich, Switzerland; Department of Psychiatry, Psychotherapy and Psychosomatics, Psychiatric University Hospital Zurich, Zurich, Switzerland;
Co-authors: Alex Rosi-Andersen*, Belinda Graham, Steven Brown, Richard Bryant, Anke Ehlers and Birgit Kleim

Keywords: circadian; trauma memory; chronobiology; rhythm dysregulation; Posttraumatic Stress disorder; intrusive re-experiencing;

Background: The core clinical feature of posttraumatic stress disorder (PTSD) is recurrent reexperiencing
in form of intrusive memories. While a great number of biological processes are regulated by sleep and internal biological clocks, the effect of 24-hour biological cycles, named circadian rhythm, has not been investigated in the context of intrusive memories.
Objective: Here we examined effects of time of day on frequency and characteristics of intrusive re-experiencing.
Methods: Fifty trauma survivors reported intrusive memories for 7 consecutive days using ecological momentary assessment in their daily life. We investigated (i) time-of-day dependent effects on frequency and distribution of intrusive re-experiencing in the overall sample as well as in PTSD versus non-PTSD and (ii) time-of-day dependent effects on the memory characteristics intrusiveness, vividness, nowness and fear.
Results: Intrusive memories showed a curvilinear pattern that peaked at 2pm. Intrusive memories in the PTSD group showed a constant level of intrusive re-experiencing in the afternoon and evening, whereas a descending slope was present in the non-PTSD group. In PTSD, intrusive memories might thus be experienced in a more time-scattered fashion throughout the day, indicating chronodisruption. Intrusion characteristics did not follow this pattern.
Conclusion: Although preliminary and based on a small sample size, these findings contribute to a better understanding of the everyday occurrence and characteristics of intrusive memories, and point to the added value of examining time-dependent effects, which can directly inform prevention and intervention science.

166. Time-memory consolidation within the engram

Main author: Alex Rosi-Andersen
Affiliation: Brown's lab, University of Zürich
Co-authors: -

Keywords: -

Performance in encoding and retrieving memories varies with the time of day. Daily phenomena are typically governed by the suprachiasmatic nucleus (SCN), which coordinate peripheral clocks in the body. However, recent research has questioned this role in memory formation, and hinted at the

presence of a semi-independent oscillator for memory.

First, we reconfirmed the existence of time-dependent memory in mice using the IntelliCage system. Next, we demonstrated that mice lacking a functional molecular clock in the SCN can fully recapitulate a time-dependent memory phenotype. Finally, as a tool to study non-SCN dependent time-memory, we captured active hippocampal memory engrams (the neuronal networks encoding specific memories) using the TRAP2 technology. This method allows us to retrieve or manipulate memory engrams. Using this tool, we were able to reactivate memories at unexpected time-points via DREADD expression and activation or manipulate engram clocks.

167. The circadian clock and G-protein-coupled receptor signaling: RGS16 and how it controls Chronotype

Main author: Tanja Schwarzmeier
Affiliation: LMU
Co-authors: Martha Merrow
Keywords: RGS16, cAMP, GPCR

The circadian clock is a reaction and an adaptation to the 24h rhythm of the world. It gates different physiological functions from behaviour to the level of single cells. One possible measurable output is Chronotype, which describes the phase of entrainment of the individual to the 24h rhythm of the environment.
G-protein-coupled receptors (GPCRs) are a class of receptors that play a major role in relaying signals from the outside world to the organism. They are involved in many different pathways and functions, but little is yet known of the interplay between GPCRs and the circadian clock.
RGS16 is a regulator of G-protein signaling which negatively regulates GPCR signaling by increasing the GTPase activity of G-proteins. Several studies have linked RGS16 with Chronotype: It has been shown as a top hit in GWAS studies looking and the genetics of Chronotype and has been shown to be critical for cAMP rhythmicity in the Suprachiasmatic Nucleus (SCN), the so-called central pacemaker of the brain and the body.
The circadian clock as well as GPCRs are considered ubiquitous in the mammalian body, so we hypothesize that the function of RGS16 in interacting with the clock is not restricted to the SCN, but is also important in non-SCN cells. Therefore my work is focused on the influence of RGS16 on circadian properties such as free-running period, temperature compensation and phase of entrainment, as well as the influence of RGS16 levels on cAMP levels, which in turn feed back to the clock. In my experiments, a knockdown of RGS16 in U2OS cells lead to an alteration in the circadian properties, and RGS16 levels impacted the cAMP response after GPCR stimulation. Therefore, RGS16 seems to have an impact on the circadian clock in peripheral cells in addition to the previously reported SCN.

168. Transcriptome profiling, diurnal species, circadian organisation
Circadian organisation in the diurnal African striped mouse, Rhabdomys pumilio

Main author: Rose Richardson
Affiliation: The University of Manchester
Co-authors: Beatriz Bano-Otalora, Robert J. Lucas
Keywords: -

Circadian clocks are essential for generating and coordinating rhythms in animals' physiology, behaviour, and metabolism. These activities are regulated by intracellular molecular clocks that operate with a ~24 hour periodicity. The African striped mouse, Rhabdomys pumilio (R. pumilio) is notable for undergoing temporal niche switching from ancestrally nocturnal to diurnal, although the molecular components of its' circadian organisation remain unknown.
Firstly, RNA data generated from peripheral tissues was used as evidence for gene prediction and de novo genome annotation. We then used our annotation to undertake transcriptome profiling of daily rhythms in the Suprachiasmatic nucleus (SCN) and in the lung, liver and retina of R. pumilio stably entrained to a 12:12 light-dark cycle. Tissues were collected at two time points: two hours after lights on (Zeitgeber Time (ZT2)) coinciding with high behavioural activity, or two hours after lights off (ZT14) during the animal's resting/sleep period, and RNA-sequencing performed. Interrogation of differentially expressed genes (DEGs) between ZT2 and 14 highlighted rhythmic transcripts including core clock genes. For R. pumilio, phasing of clock genes in peripheral tissues resembled that of the SCN, revealing a fundamental change in circadian organisation compared to that reported in laboratory mice and rats whose peripheral clocks cycle out of phase with the SCN. Functional enrichment analysis of DEGs confirmed rhythmicity in cellular function and tissue specific networks.
Our data suggest that the R. pumilio switch to daytime activity is associated with realignment of circadian rhythms in peripheral tissues with respect to the light-dark cycle and the SCN clock.

169. Stress-Inducible Circadian Rhythms in Gymnosperms

Main author: Nitzan Weisman
Affiliation: Rachel Green Lab, Department of Plant and Environmental Sciences, The Alexander Silberman Institute of Life Science, The Hebrew University of Jerusalem
Co-authors: Yuri Dakhiya, Miriam Hassidim, Rakefet David-Schwartz, Rachel Green
Keywords: Gymnosperms, Aleppo pine, Stress-Inducible Circadian Rhythms

Circadian systems that regulate ~24 hour innate free-running rhythms are almost ubiquitous amongst eukaryotic organisms from bacteria and fungi, to plants and animals. In plants, the circadian system controls the expression of about one third of the genome and regulates key metabolic, developmental,
hormonal and abiotic and biotic stress signaling pathways. Studies have suggested that a robust circadian system that is correctly synched to the plant's environment contributes to the reproductive success, growth and vitality of the plant. The circadian system - the oscillator that generates rhythms

and the pathways by which it is entrained and in turn controls circadian outputs - has been extensively explored in Arabidopsis and several other angiosperm models.
By contrast, almost nothing is known about the circadian system in gymnosperms. Gymnosperms unlike angiosperms, produce seeds without fruits. Gymnosperms originate about 390 million years ago, it precedes the other major group of plants, angiosperms, by 90 million years. Among gymnosperms are conifers, cycads, gnetophytes and gynkgophyta, in it the single living species- Ginkgo biloba.
Surprisingly, given the ubiquity of circadian rhythms, a recent study reported that the model gymnosperm, Norway spruce, lacked innate free-running rhythms. However, in preliminary experiments carried out in our lab, we observed circadian rhythms under heat stress conditions in Aleppo pines, another gymnosperm, leading us to hypothesize that gymnosperms have the capacity for circadian rhythmicity but that rhythmicity might be conditional.
We hypothesize that having a robust high amplitude rhythm may be adaptive for gymnosperms growing under stressful conditions but less necessary when conditions are optimal. In this project we are examining the circadian responses to different stress conditions in Aleppo pines and a range of other gymnosperms. We are also using available databases to identify genes that may be involved in the circadian oscillator mechanism and examine their regulation.

170. The muscle stem cell clock regulates muscle regeneration

Main author: Valentina Sica
Affiliation: Department of Medicine and Life Sciences, Universitat Pompeu Fabra, CIBER on Neurodegenerative Diseases (CIBERNED), 08003, Barcelona, Spain.
Co-authors: Oleg Deryagin, Pura Muñoz-Cánoves

Keywords: -

Organisms have evolved timekeeping mechanisms to adapt to daily environmental changes that depend on the 24 h cycle generated by the rotation of the earth. In mammals, the central clock, located in the brain's suprachiasmatic nucleus (SCN), and peripheral tissue clocks allow the synchronization of physiological and metabolic processes to appropriate daytimes. Satellite cells (SCs), the adult muscle stem cells, also have daily rhythmic oscillations. In our studies, we aim to understand how the SCs circadian clock interacts with the other clock of the body. We observe that arrhythmic SCs, which lack the master regulator of circadian rhythms Bmal1, show signs of premature activation and MyoD upregulation in basal conditions, indicating that the quiescent state is perturbed. Accordingly, these Bmal1-depleted SCs lose their capacity to properly regenerate injured muscle tissue. Their circadian transcriptome shows a set of genes that switch to an ultradian regulation and that detrimental pathways implicated in cellular damage acquire de novo rhythmicity. These data shed light on the impact of the circadian regulation on SCs homeostasis, on the synchronization of SCs biological rhythms with the SCN and its role in regeneration.

171. Diurnality versus nocturnality at the level of suprachiasmatic nucleus neuronal activity.

Main author: Patrycja Orlowska-Feuer
Affiliation: University of Manchester
Co-authors: Beatriz Bano-Otalora, Riccardo Storchi, Robert Lucas
Keywords: SCN, diurnality, nocturnality, Rhabdomys pumilio, electrophysiology, light

The suprachiasmatic nucleus (SCN) plays a central role in coordinating 24-h rhythms in metabolism, physiology and behaviour by detecting day/night changes in environmental light intensity and translating that into changes in the phase, waveform and amplitude of circadian rhythms. Previous electrophysiological studies on nocturnal rodents have shown that neuronal activity in the SCN is not only strongly influenced by light, but also directly depends on stimulus irradiance. That type of 'irradiance response' is considered to reflect the SCN's use of retinal input to track the time of the day. Here, we ask whether this basic SCN function is retained in day-active animals. To this end, we performed comparative in vivo electrophysiological study on nocturnal mice and the diurnal murid rodent, Rhabdomys pumilio. The visual response properties of SCN cells were overall very similar between species, with most neurons excited by light stimulation and responding in either sustained or transient manner. In both species, SCN neurons tracked irradiance increments and decrements presented either as steady light steps or when higher frequency modulations (such as temporal white noise or chirp) were superimposed. In R. pumilio however, neurons responded faster to light stimuli, had higher firing rate and more effectively encoded the white noise stimulus. A strong species difference was observed in the peri-SCN region though, with visual responses much more common in R. pumilio (70% vs 10%). These peri-SCN neurons carried comparable amounts of information to SCN neurons, however, were biased towards transient responses to light increments/decrements and were not reliably coding irradiance. Our findings show that regardless of the temporal niche, many SCN neurons code irradiance in their maintained firing rate. On the contrary, the high percentage and sensory diversity of light-responsive neurons outside the SCN in R. pumilio suggest widespread processing of more sophisticated visual information in the peri-SCN region.

172. The circadian clock during development: an in vivo imaging study

Main author: Miho Sato
Affiliation: University of Zurich, Institute of pharmacology and toxicology
Co-authors: Jun Kajimoto, Urs Hofmann, Weiye Li, Daniel. Razansky, Makoto Akashi, Steven Brown
Keywords: Circadian oscillation, in vivo imaging, Development

The circadian clock is an endogenous molecular oscillator that programs daily rhythmicity in behavior and physiology. The clock consists of transcription/translation-based autoregulatory feedback loops of clock genes, such as Period2 (Per2), expressed in almost all cells in mammals. Here, we employed a highly sensitive CCD camera to monitor bioluminescence of transgenic animals, with the goal of examining how the molecular clock in peripheral organs emerges from

neonate to adult. Period2::Luciferase mice were monitored throughout their development on postnatal day (P) 10, 20, 30, 46 and 56 to track their oscillatory characteristics in the same animals. Under 12:12 light dark (LD) conditions, luminescence was detected in kidney, liver and submandibular gland. We saw dramatic change in their daily variation from postnatal day 10 (P10) to P20 followed by gradual shift in their circadian phase along their development. By contrast, animals kept in constant darkness (DD) from the time of fertilisation (E1) showed highly dispersed phases among animals, indicating the importance of photic conditions during embryogenesis. Further, to find how the clock initiates in utero, the potential of optoacoustic imaging for deep tissue imaging was investigated using the chromoprotein AausFP2, a cyan-blue pigment discovered in a jellyfish A. cf. australis.

173. The effect of continuous enteral feeding on 24-h rhythms in blood glucose control in patients in the Intensive Care Unit: a retrospective observational study

Main author: Floor Hiemstra
Affiliation: Department of Intensive Care & Department of Neurophysiology, Leiden University Medical Center, Leiden, The Netherlands
Co-authors: K. Nieboer, J.H. Meijer, D.J. van Westerloo, L. Kervezee

Keywords: circadian medicine, intensive care unit, critical care, nutrition, glucose metabolism, electronic health records, clinical settings

Critically ill patients in the Intensive Care Unit (ICU) typically receive continuous enteral tube feeding over the entire 24-hour period. However, how continuous enteral nutrition impacts daily cycles in glucose control in ICU patients is unknown. Therefore, the goal of this study was to characterize 24-h variation in blood glucose values in critically ill patients during continuous enteral feeding in electronic health records data and to explore the influence of confounding factors and potential sources of bias. To this end, all time-stamped glucose measurements and other relevant clinical variables from adult ICU patients who stayed in the ICU for at least 4 days, received enteral feeding during their stay, and had at least one glucose measurement were extracted from the MIMIC-IV database. Linear mixed-effects modelling was used to determine the effect of time of day on blood glucose values during continuous enteral feeding, adjusted for relevant confounders. Sensitivity and subgroup analyses were carried out to explore potential sources of bias. In total, 6,957 ICU patients were included, who had all together 208,560 glucose measurements while they received enteral nutrition. Blood glucose levels were significantly affected by time of day ($p < 0.0001$), with a peak of 167 [166 - 168; estimated marginal means, 95% CI] mg/dL at 10:00 in the morning and a trough of 149 [148 – 150] mg/dL at 03:00 at night. Subgroup analyses showed that the 24-h variation in glucose levels was present regardless of diabetes diagnosis, hospital mortality, sedation status, or ventilation mode. Likewise, sample frequency or sample type had no influence on the observed 24-h rhythm. These results indicate that glucose levels in critically ill patients show marked 24-h variation. This daily pattern persists when controlling for potential sources of bias and confounding factors, suggesting that the observed 24-h variation is due to biological processes.

174. Investigating diurnal variation in the electrophysiology of retinorecipient cells in the visual thalamus

Main author: Abigail Pienaar
Affiliation: University of Manchester
Co-authors: Abigail Pienaar, Annette Allen
Keywords: vision, visual thalamus, optogenetics, electrophysiology, mouse, diurnal

One of the most dramatic yet predictable changes accompanying the transition from day to night is the change in the visual environment, which varies hugely in both spectral composition and intensity across the day. Circadian clocks can regulate aspects of early visual coding within the retina, with previous studies demonstrating daily rhythms in retinal physiology and function.
The dorsolateral geniculate nucleus (dLGN) of the visual thalamus is the first output target of the retina, with some evidence of circadian rhythmicity in its molecular and neuronal activity. However, it remains unclear how the clock might modulate visual processing in this region. Circadian variations in signals generated by the retina may simply be inherited, or signals may be processed through clock-controlled mechanisms independent of the retinal clock.
Our work aims to determine how early visual processing within the dLGN is regulated by the clock. Using both electroretinograms and in vivo electrophysiology, visual responses were examined in mouse retina and dLGN at mid-day and mid-night to investigate their circadian modulation. Optogenetic techniques were also used to activate retinal ganglion cell (RGC) terminals within the dLGN, bypassing the retinal clock, to further characterize how the dLGN responds to retinal inputs at different times of day.
Our investigations demonstrate that light-responsive cells in the dLGN have higher spontaneous firing activity at mid-day compared to mid-night. We also show that whilst retinal bipolar cells demonstrate diurnal variation in their response to mesopic visual stimuli, light responses within the dLGN do not show diurnal variation to the same stimulus. We find optogenetic activation of RGC terminals drives robust activation of dLGN units, with direct and indirect responses detectable at each timepoint. Current work is exploring the extent to which this activity is under circadian control. These results indicate there may be diurnal modulation of visual processing within the visual thalamus, independent from the circadian modulation of signals generated by the retinal clock.

175. Finding new clock modifiers, a proximity-labelling approach

Main author: Manon Torres
Affiliation: AG Kramer institute of Chronobiology, Charité - Universitätsmedizin Berlin
Co-authors: Manon Torres, Achim Kramer
Keywords: circadian transcription, proteomic, proximity labelling

Circadian rhythmicity relies on molecular oscillators present in most mammalian cells and drive circadian (~24 h) of a wide range of molecular, physiological and behavioral functions. One central aspect of molecular oscillator function is the tight regulation of circadian transcription. Over the years, several proteins (i.e. BMAL1, CLOCK, PERs, CRYs) and cis-regulatory enhancer elements (i.e. specific sequences located around the promoter region, e.g. E-box, RRE, D-box) have been shown to

be essential for circadian transcription. Additionally, studies suggest that the circadian transcription is more complex and further components contribute to its regulation. What are these factors? What is their role for transcriptional regulation and circadian rhythm generation? When do they bind to chromatin?
With a focus on proteins biding to E-box elements, we used the newly developed CRISPR/Cas9-APEX proximity labelling method to identify new clock modifiers.
In this system, a catalytically inactive RNA-guided nuclease Cas9 (dCas9) is fused to the engineered ascorbate peroxidase APEX2 (dCAS9-APEX2). By selecting defined guide-RNAs, this complex was brought to the proximity of the Dbp-gene's E-box. There, APEX2 was activated by adding chemicals to the cell culture medium, and biotinylated proteins in close proximity. Biotinylated proteins could then be purified for subsequent LC-MS/MS analysis.

176. Arrhythmic Tumors become Rhythmic in Mice

Main author: Audrey Schult
Affiliation: 1University of Zurich, Institute of Pharmacology and Toxicology, Zurich, Switzerland
2University of Basel, Basel, Switzerland
Co-authors: Robert Dallmann1, Ermanno Moriggi1, Daniel Zumofen2, Steven Brown1

Keywords: circadian clock, cancer, tumors, chronopharmacology

The connection between the circadian clock and cancer has been evident for quite some time, and night shift work is even classified as a probable carcinogen to humans (1). Many pathways involved in cancer growth and development are regulated by the circadian clock, such as: cell cycle regulation, DNA damage response, apoptosis, and metabolism (2). Despite these discoveries, clinical cancer chronotherapeutics are still in their infancy. A more complete understanding of the interplay between the circadian clock and cancer is necessary to fully exploit the circadian toolbox for potential cancer therapies. It was previously shown that in arrhythmic mice, tumors grow three times as fast (3), so we wanted to investigate what happens when the tumors themselves are arrhythmic. We also know that when local clocks are turned off, locally-driven rhythmic genes lose rhythmicity, while systemically-driven rhythmic genes have their rhythmicity preserved (4), and we wanted to apply this to a cancer context. We demonstrate that even among cancer cells and tumors of the same class, some have circadian rhythms in vitro while others do not, as measured by Bmal1 bioluminescence using a lentiviral reporter system. Additionally, we show that rhythmic and two different types of arrhythmic tumor cells in vitro (Bmal1 KD or naturally clock-disrupted) all form rhythmic tumors in vivo via Bmal1 bioluminescence recordings in freely moving mice, both for circadian and cell-cycle reporters. Surprisingly, circadian physiology in rhythmic and rhythmicity-rescued tumors is completely different at the transcriptomic level. Irrespective of circadian state, tumors that show robust or nonexistent clocks in vitro, show a similar proportion of circadian genes in vivo. Surprisingly, the circadian phase and identity of these genes change, arguing against circadian rescue via uniform systemically driven circadian gene expression. Our data suggest that the endogenous clock state of a tumor does not change its ability to be circadian, but rather the physiological pathways under direct circadian control. Since many tumors are treated with fractionated radiotherapy that presumes DNA replication in tumor cells to be distributed over time,

our results may have significant implications for cancer chronopharmacology and personalized medicine.

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177. Hippocampal long-term synaptic plasticity control by histamine, orexins, and circadian clock gene Per1

Main author: Oliver Selbach
Affiliation: Institute of Anatomy II, Medical Faculty, Heinrich-Heine-University, 40001 Dusseldorf, Germany
Co-authors: -
Keywords: histamine, hypocretin, orexin, Per1, sleep-wake cycle, circadian, synaptic plasticity, bistability, LTP, LTD, eligibility trace, kinase, phosphorylation, transcription, RNA, translation, protein, macromolecular, phase assembly, hypothalamus, hippocampus

Wake-active histamine (HA) and hypocretin/orexin (OX) neurons located in the posterior and lateral hypothalamus of the mammalian brain project widely throughout the entire neuraxis, including the hippocampus, to control and synchronize complex brain functions linking food, mood, movement, sleep, and memory. Here, convergent electrophysiological, pharmacological and genetic evidence is provided for synergistic roles of HA, OX, and clock gene Per1 in the control of bi-stable molecular "switches" governing neuronal excitability (Fig.1), synchronized local circuit activity (Fig.2), neurotransmitter outflow (Fig.3), kinase- (Fig.4) and macromolecular synthesis-dependent transformation of "early" long-term potentiation/depression (LTP/LTD) eligibility traces into "late phase" LTP (L-LTP) (Fig.5). Consistently, mice deficient in Per1, a key regulator of transcription-translation feedback loops (TTFL) and molecular clock "re-set", exhibit selective deficits in the capture, maintenance, and reinforcement of L-LTP but not L-LTD (Fig.6). Thus, HA, OX, and Per1 co-operate in linking spike-timing-dependent (Hebbian) AND homeostatic (non-Hebbian) synaptic plasticity with neuronal transcription AND phospho-proteome dynamics to control the transformation of transient bi-stable eligibility traces into persistent synaptic weight gain. The combinatorial logic and mechanistic signature unifies multiple learning rules and is perfectly suited to optimize contextual "spacetime" memory storage across scales; eg imposing a value-based temporal structure on eligibility trace formation (during waking) and clustered synaptic memory engram re-play, (re)consolidation and homeostatic plasticity "re-set" (during rest and sleep). Convergent logical endpoints and key regulatory motifs of HA, OX, Per1 control:

1. Modulation of neuronal excitability AND network activity (ie spike-timing-dependent LTP/LTD eligibility trace "formation" AND kinase/phosphatase ("set/reset switch" bi-stability) -dependent metaplasticity and homeostatic synaptic weight up-/downscaling)
2. Transformation of "early-to-late phase" long-term potentiation (E-LTP -> L-LTP) by neuronal transcription AND phospho-proteome-dependent "covalent-to-macromolecular phase" transitions)
3. Reinforcement of LTP "tagged" synaptic weight gain "states" through Per1-dependent synaptic (re-)consolidation, (re-)scaling and "re-set"

178. Investigating the Metabolic Actions of Chrono-Modulator Lithium

Main author: Brooke Prakash
Affiliation: University of Oxford
Co-authors: Zeinab Wakaf, Simona Di Pretoro, Harshmeena Sanghani, Dorota Nawrot, Aarti Jagannath, Sridhar Vasudevan

Keywords: Metabolism, Chrono-modulation, Lithium

Sleep and circadian disruptions (SCRDs) are becoming more commonplace in the modern 24/7 world. In addition to this, symptoms of metabolic syndrome including weight gain and glucose intolerance are becoming more prominent with the mass consumption of energy dense food. There appears to be a link between SCRDs and metabolic disorders as shift work has been linked to increased body weight and worsened glucose tolerance. Furthermore, there is genetic evidence that links the disruption of circadian genes to symptoms of metabolic syndrome. Recent studies have suggested that increasing the robustness of the circadian clock could present a new paradigm for treating metabolic disorders. Lithium is a well characterised chrono-modulator that is largely used as a mood stabiliser in the treatment of bipolar disorder. It has been shown to increase slow wave sleep and to increase the period and amplitude of circadian rhythms. However, little is known about the metabolic effects of lithium. We therefore examined whether this chrono-modulator could improve symptoms of metabolic disorder in vivo. We used a diet-induced obesity (DIO) mouse model, administered lithium and monitored metabolic changes. We found that lithium significantly increased weight gain in the DIO mice while also improving insulin and glucose tolerance. This action was specific to the DIO mice as mice fed control chow did not gain weight and their insulin and glucose tolerance remained unchanged under lithium administration. We therefore shed light on the metabolic actions of this chrono-modulator and suggest that improving circadian rhythmicity does not have a uniform effect across all symptoms of metabolic syndrome.

179. Role of the Central Brain Clock in the Pathophysiology of Insulin Resistance - Study Protocol and Pilot Data

Main author: Esther Speksnijder

Affiliation: 1. Amsterdam UMC, University of Amsterdam, Department of Endocrinology & Metabolism, Amsterdam Gastroenterology Endocrinology Metabolism, Meibergdreef 9, Amsterdam, Netherlands.
2. Spinoza Centre for Neuroimaging, Meibergdreef 75, Amsterdam, Netherlands.
3. Netherlands Institute for Neuroscience, Royal Dutch Academy of Arts and Sciences, Amsterdam, Netherlands.

Co-authors: Esther M. Speksnijder, Marloes D.A. Bet, Jurjen Heij, Eus J.W. van Someren, Susanne la Fleur, Sarah E. Siegelaar, Peter H.L.T. Bisschop, Chun-Xia Yi, Andries Kalsbeek, Wietske van der Zwaag, Dirk Jan Stenvers

Keywords: Suprachiasmatic Nucleus, Type 2 Diabetes Mellitus, Insulin Resistance, Functional MRI

Introduction: Insulin resistance (IR) is a key process in the development of type 2 diabetes (T2DM). Disruption of circadian synchrony leads to IR. Animal studies and post-mortem human brain studies suggest involvement of the suprachiasmatic nucleus (SCN) in the development of IR. We hypothesize that the in vivo rhythm in light responsiveness of the SCN is disturbed in individuals with IR. Therefore, we will determine the daily rhythm of SCN-area light responsiveness in subjects with progressive stages of IR, using 7-Tesla functional magnetic resonance imaging (fMRI).
Methods: In this observational cohort study, we will include three groups of 10 participants, aged 25-65, with a BMI above 30. Participants in Group 1 have normal insulin sensitivity, participants in Group 2 have impaired insulin sensitivity and participants in Group 3 have T2DM. Participants will be scanned at 4 time points within 24 hours, based on their average wake-up time; At Zeitgeber time (ZT) 2:00, 8:00, 14:00 and 20:00. We will assess SCN-area response to light and functional connectivity by means of:

1. Task-based fMRI to assess the blood-oxygenation-level-dependent (BOLD) response of the SCN-area to white light (122 lux) enhanced with blue light (490nm).
2. Resting-state fMRI to assess SCN-area functional connectivity to regions of interest.

Outcomes are mean SCN-area light responsiveness, time point of highest and lowest SCN-area light response, and difference between highest and lowest SCN-area light response.
Results of Pilot Data: One healthy subject, aged 22 years, was included for a pilot. The subjects' average wake-up time was 08:00 AM. A pilot scan was performed at ZT 2:00. The first-level analysis demonstrated significant functional connectivity between the SCN-area and the hippocampus ($r=0.258$, $p=0.0031$).
Conclusion: We present a protocol of a novel study to determine the SCN-area rhythm of light responsiveness using 7T fMRI in subjects with progressive stages of IR.

180. Investigating the molecular role of transcription factor MAFG in circadian rhythm regulation

Main author: Sejal Kapoor
Affiliation: Nuffield Department of Clinical Neuroscience, University of Oxford
Co-authors: S. Kapoor, L. Taylor, N. Varga, A. Ashton, S. Walsh, A. Jagannath
Keywords: Mafg, AP-1, photo-entrainment

The molecular mechanisms underlying circadian photo-entrainment are complex, comprising several regulatory factors that regulate transcription in response to light. Our group has found differential chromatin accessibility in the suprachiasmatic nucleus of mouse, in response to nocturnal light, as profiled by ATAC-Seq. Chromatin at the promoter of the transcription factor Mafg closed following a nocturnal light pulse (LP), indicating repression of the gene. Additionally, the AP-1 motif was highly over-represented in the light regulated transcriptome, leading us to hypothesise that Mafg, that is part of the AP-1 transcription factor family, regulates photo-entrainment through the regulation of AP-1.

To investigate this, we conducted in vitro analyses to profile the regulation of Mafg and profiled the photic entrainment phenotype of Mafg knockout mice. We profiled the Mafg promoter using a reporter luciferase assay, where ections of the promoter were cloned into a luciferase reporter plasmid and transfected U2OS cells. The effect of Forskolin (which simulates a LP via the cAMP-CREB/AP-1 pathway) on the transcriptional activity of Mafg was measured. We found that the Mafg promoter region is indeed closed to input stimuli, and is itself further repressed in response to Mafg siRNA mediated knockdown. Furthermore, the baseline circadian phenotype, and photic entrainment phenotype of Mafg knockout mice was profiled. Periodogram analysis of circadian wheel running behaviour under constant dark showed reduced amplitude in knockout mice, suggesting that they have weak & fragmented rhythms. Photic entrainment was also found to be reduced; knockout animals entrained more slowly to a 6-hour phase-advancing jetlag protocol and showed smaller phase shifts following a nocturnal LP (CT 16). In summary, these findings indicate a role for the AP-1 related transcription factor, MAFG, in regulating transcriptional responses in response to light, and thus photic entrainment.

KEYWORDS

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CONTACT

University of Zurich
Institute of Pharmacology & Toxicology
Mrs. Jacqueline Vicario
Winterthurerstrasse 190
CH-8057 Zürich (Switzerland)

Email: ebrs2022@pharma.uzh.ch

Tel: +41 (0)44 635 59 59

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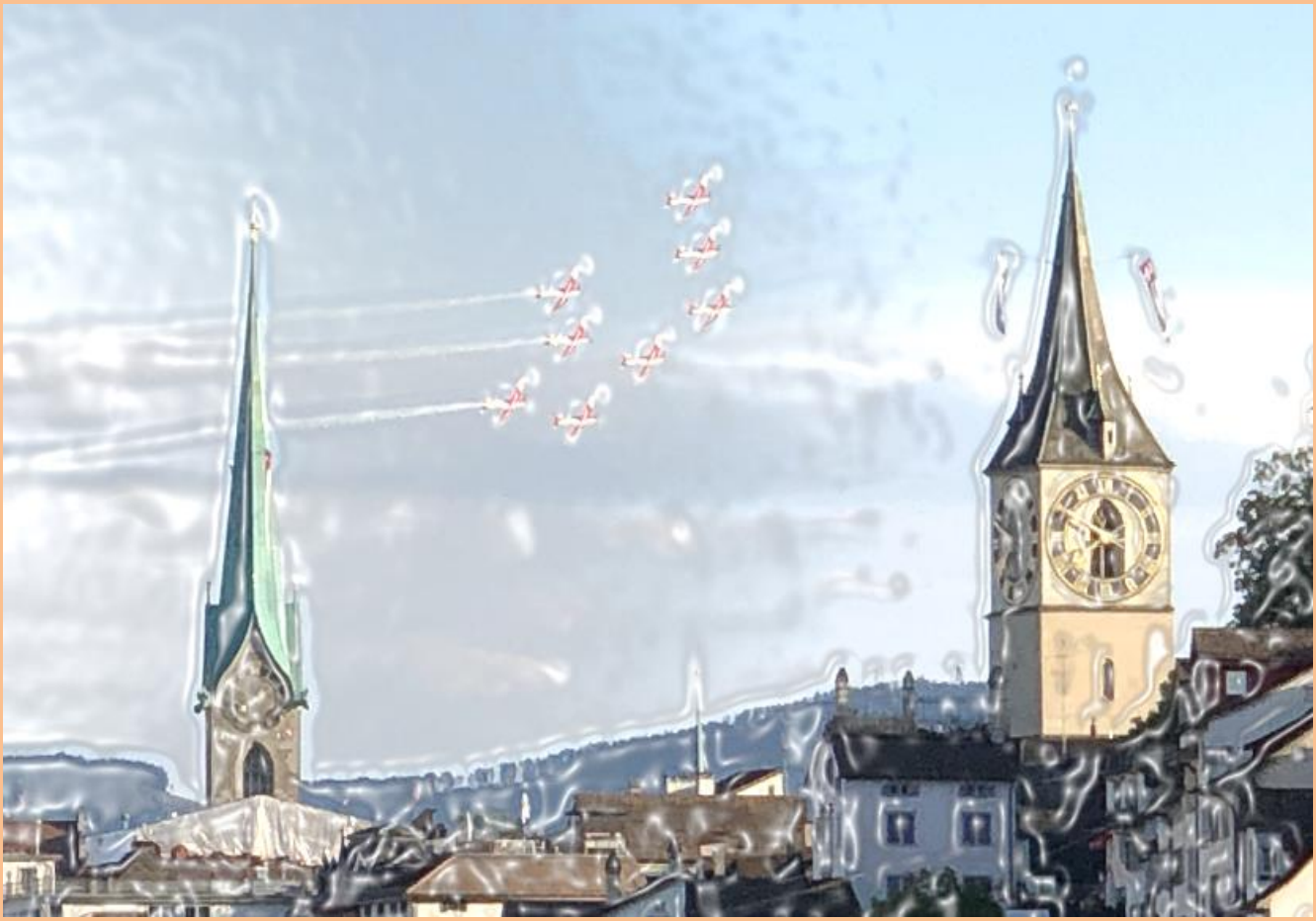

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