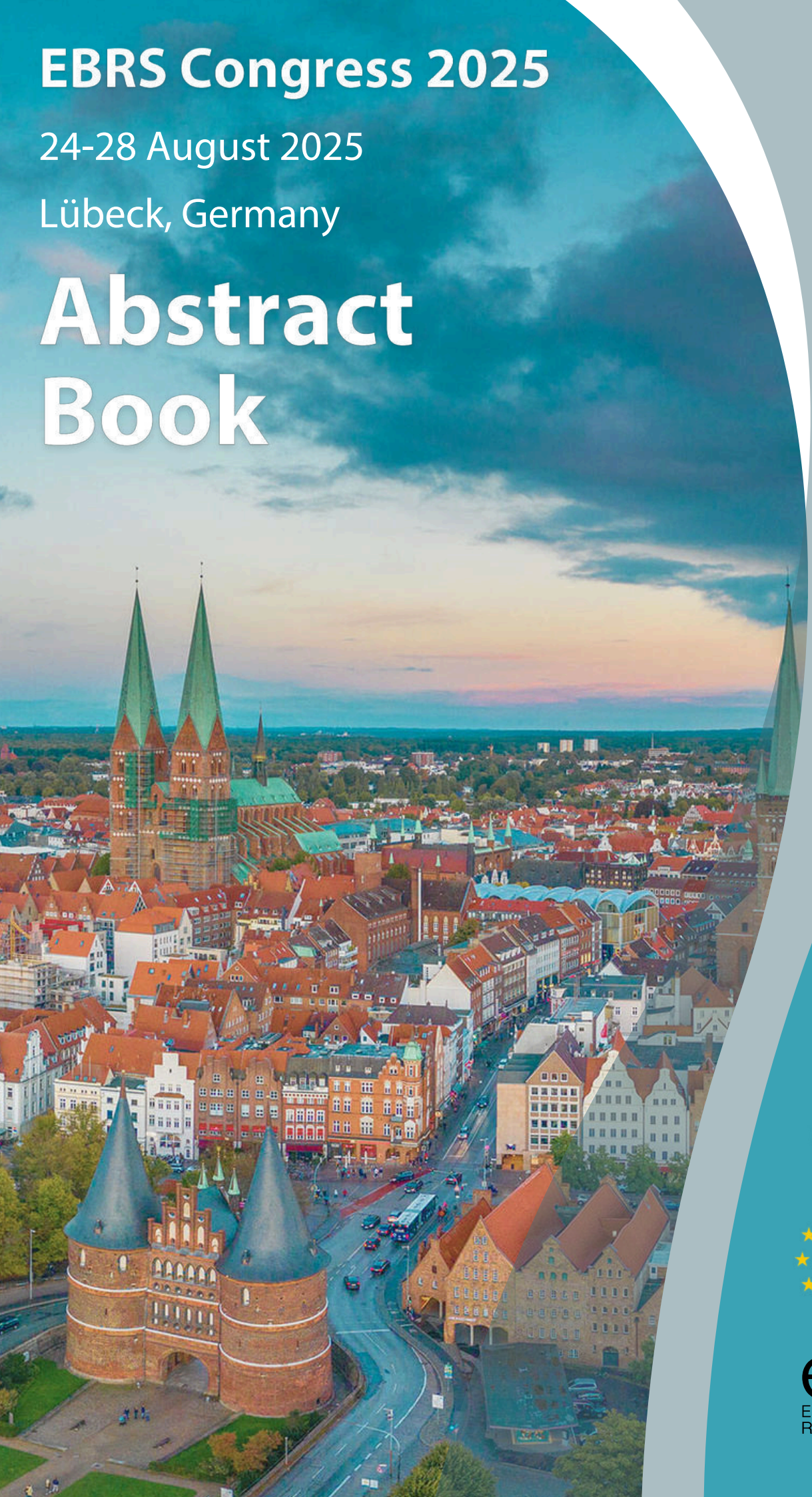
EBRS Congress 2025

24-28 August 2025

Lübeck, Germany

Abstract Book



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Acknowledgement

The EBRS Congress 2025 was funded by:

- the Deutsche Forschungsgemeinschaft (DFG, German Research Foundation) – project number 564929969.
- the Company of Biologists – Scientific Meeting Grant EA1430
- Leopoldina (Nationale Akademie der Wissenschaften) – Symposium „Sleep and Clocks in Health and Disease“.

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Keynote Lecture

Neuroendocrine mechanisms of seasonal adaptation and maladaptation

Valerie Simonneaux[1*]

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Annual changes in environmental factors had led organisms to develop adaptive biological and behavioural strategies. In mammals, the seasonal change in the nocturnal production of the pineal hormone melatonin is pivotal for metabolic and reproductive adaptations.

The discovery that melatonin acts on the pars tuberalis to control the synthesis of TSH, which in turn act on the hypothalamic tanycytes to modulate local thyroid hormone metabolism, has been a breakthrough in our understanding of the neuroendocrine mechanisms underlying seasonal adaptation. In this lecture, I will discuss how this melatonin/thyroid hormone signal regulates hypothalamic circuits to synchronize physiological functions with the seasons. I will also report how exposure to environmental disruptors may affect seasonal adaptation.

Keynote Lecture

Chrononutrition to Optimize Health

Frank A.J.L. Scheer
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Nutrition, sleep, physical activity, metabolism, and circadian rhythms are tightly intertwined and have an important impact on health and disease. There is convincing evidence that uncoupling their timing, e.g., occurring during shift work, light at night, or inverted activity rhythms, has adverse health consequences. Recent findings indicate that the normalization of the eating rhythm, by itself, can decrease or even prevent these adverse health consequences. These insights have ignited broad interest in the potency of chrononutrition to improve health.

In this keynote, I will present evidence for the role of the endogenous circadian system in regulating various aspects of metabolism, including glucoregulation, energy expenditure, and energy intake; will provide examples of the impact of circadian misalignment on cardiometabolic risk, mood, and inflammatory markers; will present findings on the influence of diurnal and circadian food timing on metabolism and their relationship with obesity and weight loss success; and will discuss the promise for levering chrononutrition for optimizing health, in the general population and in individuals experiencing circadian misalignment such as shift workers.

Keynote Lecture

Melatonin and Metabolomics: Tracking central and peripheral clock rhythms in humans

Debra J. Skene
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The endogenous melatonin rhythm is routinely used as a phase marker of the light-entrainable circadian clock in the hypothalamic suprachiasmatic nuclei (SCN), providing important information about the human circadian timing system. For example, melatonin measurements have characterised the phase-shifting effects of light (phase response curve), the intrinsic human period (tau), spectral sensitivity of circadian photoreception and desynchrony between the environment, behaviour and circadian timing when working night shifts.

With the discovery of peripheral clocks, tracking their timing in humans has proved more challenging. Early work measured clock gene expression in human tissue that could easily be obtained such as white blood cells, buccal tissue, adipose tissue, hair, skin and muscle biopsies. More recently high-throughput transcriptomics of human tissue has significantly increased sensitivity and coverage of the transcriptome. However, tracking human peripheral clocks in organs involved in digestion and metabolism, such as the liver, pancreas and gut remains a challenge.

In this lecture I will discuss the potential of metabolic profiling (metabolomics), using targeted UPLC-MS/MS technology, to track human peripheral clock timing in entrained and misaligned conditions. Experiments showing time-of-day and circadian variation in the human metabolome, the effects of sleep deprivation, mistimed food and simulated shift work on metabolite rhythms will be presented. After 3 nights of working shifts, endogenous circadian rhythms of many plasma metabolites were misaligned from the central SCN clock timing (melatonin and cortisol) by ~8-12 h (internal desynchrony) likely reflecting the peripheral clocks' response to cues driven by mistimed behaviours. Melatonin measurement combined with metabolic profiling will thus be useful to measure circadian misalignment in shift work and test management strategies. The ambulatory U-RHYTHM device offers a novel and practical way of measuring high-resolution melatonin and metabolite rhythms in real-life.

Keynote Lecture

Discovering the Ins and Outs of the SCN based on its prototypical neurotransmitter vasopressin

Andries Kalsbeek
Department of Endocrinology and Metabolism of the Amsterdam UMC, University of Amsterdam / Netherlands Institute for Neuroscience (NIN).

This lecture will concentrate on the significance of the vasopressin neurons in the SCN for the functional output of the biological clock that is contained within it. The vasopressin-containing subpopulation is a characteristic feature of the SCN in many species, including humans.

The activity of the vasopressin neurons in the SCN shows a pronounced daily variation in its activity that has also been demonstrated in human post-mortem brains. In the early years, animal experiments revealed an important role for SCN-derived vasopressin as an output signal in the control of neuroendocrine day/night rhythms, such as that of the hypothalamic-pituitary-adrenal (HPA) and hypothalamic-pituitary-gonadal (HPG) axes. More recently studies using vasopressin receptor knockout animals highlighted its importance in the intrinsic synchrony of the SCN as well.

Reducing vasopressin signaling within the SCN seems to reduce the robustness of the circadian clock and thereby facilitate resynchronization to a new L/D environment. The remarkable correlation between a diminished presence of vasopressin in the SCN and a deterioration of sleep-wake rhythms during ageing and depression indicate that, also in humans, the vasopressin neurons contribute considerably to the rhythmic output of the SCN, but also highlight its potential as a target for therapeutic interventions.

Keynote Lecture

Circadian temporal organization of behaviour and its benefits in insects

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Circadian rhythms are prevalent on Earth and temporally organize behaviour and physiology of organisms to occur in species specific 'temporal niches'. However, species differ in how strictly individuals are controlled by their circadian clock, suggesting that it may offer advantages to extend the temporal niche under certain circumstances, for example during stressful environmental conditions. In this lecture, I will discuss two insect species (the fruit fly Drosophila melanogaster and the red flour beetle Tribolium castaneum) as examples for tight and relaxed temporal niche control, respectively. A potential mechanism controlling temporal niche adherence involves the evolutionary capacitor and chaperon protein HSP90. Within a small subset of clock neurons in the fly brain, HSP90 mitigates inter-individual behavioural variation by regulating expression of the circadian neuropeptide Pigment Dispersing Factor (PDF), presumably restricting temporal niche extension to stressful environmental conditions.

Even though such temporal organization and circadian clocks in general are not essential for survival of an individual (at least under laboratory conditions), it is assumed that they contribute to the overall fitness of organisms. By applying a novel behavioural choice assay we could show that the fruit fly D. melanogaster actively chooses environmental conditions allowing it to live in a temporally organized manner. Flies become arrhythmic in constant light, and offering them the possibility to enter a dark area restored circadian rhythms. We show that this self-generated behavioural rhythmicity is correlated with an improvement of sleep quality, demonstrating a direct benefit for circadian clocks and temporal organisation.

Keynote Lecture

Neuronal feedback loop in the mammalian central circadian clock

Michihiro Mieda
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The suprachiasmatic nucleus (SCN), the central circadian pacemaker of mammals, is a heterogeneous structure composed of multiple types of GABAergic neurons and glial cells. Although individual cells have a cellular clock driven by the autoregulatory transcriptional/translational feedback loop of clock genes (TTFL), intercellular communication among SCN cells is essential for the SCN to generate a highly robust circadian rhythm. However, such network mechanisms of the SCN remain unclear.

We have used neuron type-specific recordings and manipulations of TTFLs and neuronal activity to dissect the SCN neural circuitry. Our recent studies focusing on the role of AVP (arginine vasopressin)-producing neurons in the SCN shell have suggested that these neurons may act as the primary circadian pacesetter cells to determine the ensemble period of the SCN network in vivo. Indeed, AVP neurons regulate the Ca^{2+} rhythm of VIP (vasoactive intestinal polypeptide) neurons in the SCN core. In addition to pacesetting the SCN ensemble rhythm, AVP neurons may also regulate the phase-setting of the evening and morning locomotor activities, at least in part through the GABAergic network from AVP neurons to VIP neurons. On the other hand, VIP has been known to be the most important contributor to the synchronization among SCN neurons. Simultaneous recordings of in vivo Ca^{2+} rhythms in AVP and VIP neurons in VIP-deficient mice suggested a critical role for the VIP peptide in regulating AVP neuronal Ca^{2+} rhythms by amplifying and delaying them. Thus, the neuronal feedback loop circuit composed of the AVP cellular oscillator and VIP peptide signaling may be a fundamental network mechanism underlying the mammalian central circadian clock for robust rhythm generation.

Keynote Lecture

On assumptions and hypotheses: two unlikely chronobiology stories, Bacillus and the Stonechat

Martha Merrow
Molecular Chronobiology Lab, Medical Faculty, LMU Munich

In 1990, Ebo Gwinner ublished (with John Dittami) experiments on annual rhythms in the Stonechat that spanned at least 7.5 years. In 2021, my group and collaborators published experiments on circadian rhythms in *Bacillus subtilis* that had started over a decade earlier.

In addition to describing both of these (disparate) stories, I will discuss the justification for such experimental work and how the foundations of chronobiology drive us forward whilst the structures of modern

Symposia

S 01-01

Suprachiasmatic circadian circuits

M.H. Hastings1, N.J. Smyllie1, E. del C. Gomez Garcia1, A.P. Patton1

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Circadian time-keeping in mammalian cells pivots around a transcriptional/translational feedback loop (TTFL) in which Per and Cry genes are transactivated by CLOCK/BMAL1 heterodimers at the start of circadian day. PER and CRY proteins accumulate, heterodimerise and enter the nucleus to oppose their transactivation. During circadian night PER and CRY are degraded, ultimately releasing the cycle to start anew, ~24 hours after its previous initiation. TTFL cellular clocks are active in all major organ systems and so to coordinate physiology and behaviour into adaptive daily cycles they are synchronised by the suprachiasmatic nucleus (SCN), the principal clock of the brain. Accordingly, the ~20,000 cells of the SCN are generate a robust and persistent self-sustaining circadian oscillation of neurophysiological and metabolic activity through which it signals daily time across the body.

This presentation will consider recent work that has examined how the cell-autonomous TTFLs and intercellular signalling bind the SCN circuit into such a powerful time-keeper. By real-time-imaging of fluorescently tagged PER2, CRY1 and BMAL1 proteins in SCN slices from knock-in mutant mice, allied with pharmacological perturbations, we reveal a rich and unanticipated complexity to the dynamic intracellular behaviours of the endogenous proteins that extends our perspective on the TTFL of the SCN. Second, the role of neurons expressing the neuropeptide gastrin-releasing peptide as a signalling hub for entrainment of SCN phase will be explored using intersectional genetic approaches based on a novel mouse line in which Cre-recombinase is expressed in these cells. Finally, the role of signalling between astrocytes and neurons in the SCN clock will be examined using a multi-omic approach. This has revealed a necessary role of astrocytic signalling via annexin A2 and S100a10 proteins in regulating astrocytic calcium levels and thereby SCN circuit coherence. Powerful SCN time-keeping is thus conferred by molecular, neuronal and neuro-astrocytic circadian circuits.

Symposia

S 01-03

Longitudinal Imaging of SCN Neuronal Behavior In Vivo

Alec J. Davidson, Ph.D.
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A comprehensive understanding of neural circuits requires cell-resolved and cell-type-specific observation of networks and their components within the context of receiving inputs and generating relevant outputs. Here we describe in vivo, longitudinal deep-brain Ca2+ imaging of large groups of AVP+ and NMS+ neurons in awake behaving mice. We observe single-cell Ca2+ waves indicative of burst firing across the day and night, stochastic circadian rhythms in single-cell parameters, rhythms in network dynamics, and, with some cell types, robust and diverse responses to sensory input. We demonstrate that such recordings are achievable in deep brain regions including mouse suprachiasmatic nucleus and are providing new insights into context-specific and longitudinal behavior of brain cells and circuits that modulate behavior.

Symposia

S 01-04

Relay and amplification of joint diffusible and neural signaling supports SCN global clock coordination.

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3. Pathology and Cell Biology Department, Columbia University Medical School, New York, NY, USA
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The brain clock, located in the suprachiasmatic nucleus (SCN) of the hypothalamus receives direct retinal input providing the entire body with information of local external solar time. The pathways by which the SCN signals so broadly involve co-occurring humoral and neural output signals, though the former are less understood. Portal pathways, such as the well-known pituitary portal pathway, provide a mechanism whereby signals of neural origin can reach local, specialized targets without suffering dilution in the systemic blood supply.

Three newly discovered portal pathways provide direct vascular connections between each of the sensory circumventricular nuclei at its point of attachment to the parenchyma. These nuclei line the brain's ventricles, and their leaky blood vessels and large perivascular spaces represent a route whereby secretions originating in the SCN can be relayed and then amplified, providing a pathway to achieve global coordination of circadian clocks. The finding of multiple portal pathways revises our understanding of SCN neural and diffusible output signals, with particular emphasis on the contribution of the brain's fluidic compartments and the fluids therein.

Symposia

S 01-05

Zinc finger homeobox-3 (ZFHX3) orchestrates genome-wide daily gene expression in the suprachiasmatic nucleus

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The mammalian suprachiasmatic nucleus (SCN), situated in the ventral hypothalamus, directs daily cellular and physiological rhythms across the body. The SCN clockwork is a self-sustaining transcriptional-translational feedback loop (TTFL) that in turn co-ordinates the expression of clock-controlled genes (CCGs) directing circadian programmes of SCN cellular activity. In the mouse, the transcription factor, ZFHX3 (zinc finger homeobox-3), is necessary for the development of the SCN and influences circadian behaviour in the adult. The molecular mechanisms by which ZFHX3 affects the SCN at transcriptomic and genomic levels are, however, poorly defined. Here, we used chromatin immunoprecipitation sequencing (ChIP-seq) to map the genomic localization of ZFHX3 binding sites in SCN chromatin. To test for function, we then conducted comprehensive RNA sequencing at six distinct times-of-day to compare the SCN transcriptional profiles of control and ZFHX3-conditional null mutants. We show that the genome-wide occupancy of ZFHX3 occurs predominantly around gene transcription start sites (TSS), co-localizing with known histone modifications, and preferentially partnering with clock transcription factors (CLOCK, BMAL1) to regulate clock gene(s) transcription. Correspondingly, we show that the conditional loss of ZFHX3 in the adult has a dramatic effect on the SCN transcriptome, including changes in the levels of transcripts encoding elements of numerous neuropeptide neurotransmitter systems while attenuating the daily oscillation of the clock TF Bmal1. Furthermore, various TTFL genes and CCGs exhibited altered circadian expression profiles, consistent with an advanced in daily behavioural rhythms under 12h light- 12h dark conditions. Together, these findings reveal the extensive genome-wide regulation mediated by ZFHX3 in the central clock that orchestrates daily timekeeping in mammals.

Symposia

S 02-01

Rhythms in the gut – a novel link between diet and metabolism?

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[4] Faculty of Health and Medical Sciences, University of Surrey, Stag Hill Campus, GU27XH, Guildford, UK

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3. Pathology and Cell Biology Department, Columbia University Medical School, New York, NY, USA

4. Zuckerman Institute Affiliate, Columbia University, New York, NY, USA

Emerging evidence reveals that the gut microbiota exhibits intrinsic 24-hour rhythms, tightly regulated by the host's intestinal circadian clock and influenced by dietary composition and feeding schedule. Disruption of these rhythms, which is common in modern lifestyles, has been linked to metabolic and inflammatory diseases.

Our research integrates longitudinal human infant studies, chemostat cultures, and mechanistic mouse models, including intestinal clock-deficient (Bmal1$^{IEC-/-}$) and inflammatory bowel disease models (IL-10-/-, Bmal1$^{IEC-/-}$xIL-10-/-), to determine how microbial oscillations are established and modulated. Using 16S rRNA sequencing, metabolomics, and transcriptomic profiling, we demonstrate that breastfeeding and galacto-oligosaccharide supplementation enhance microbial rhythmicity early in life. Strikingly, rhythmicity in dominant taxa and functional pathways was maintained ex vivo in chemostat cultures, indicating an intrinsic microbial clock influenced by diet.

In mice, disruption of the intestinal clock abolished microbial oscillations and impaired carbohydrate and lipid metabolism. Fecal microbiota transfer from arrhythmic donors into germ-free mice induced obesity, establishing a causal role for microbiota rhythmicity in host metabolic regulation. While purified diets including high fat diet, westernized diet and control diet (low fat/fiber) suppressed microbial oscillations, fiber supplementation restored microbial rhythmicity and improved metabolic outcomes.
In inflammatory models, we observed intestinal clock dysfunction, which led to disrupted microbial oscillations, exacerbated colitis and reduced survival. Remarkably, time-restricted feeding restored circadian gene expression and microbiota oscillations, improved immune function, and alleviated inflammation. These effects depended on an intact intestinal clock, suggesting the intestinal circadian oscillator as potential target to influence IBD development.
Together, these findings highlight microbial rhythmicity as a critical mediator linking diet, feeding patterns, and host physiology. Chrononutrition strategies, including dietary fiber and feeding-time interventions, offer promising avenues for preventing and managing metabolic and inflammatory disorders.

Symposia

S 02-02

Hepatic BMAL1 and HIF1a regulate a time-dependent hypoxic response and prevent Hepatopulmonary like Syndrome

*Vaishnavi Dandavate[1#], Nityanand Bolshette[1#], Rachel Van Drunen[1], Gal Manella[1], Hanna Bueno-Levy[2], Mirie Zerbib[2], Ippei Kawano[1], Marina Golik[1], Yaarit Adamovich[1], and Gad Asher[1]**

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[2]Department of the Veterinary Resources, Weizmann Institute of Science, 7610001, Rehovot, Israel.

The transcriptional response to hypoxia is temporally regulated yet the molecular underpinnings and physiological implications are unknown. We examined the roles of hepatic Bmal1 and Hif1a in the circadian response to hypoxia in mice. We found that the majority of the transcriptional response to hypoxia is dependent on either Bmal1 or Hif1a, through shared and distinct roles that are daytime determined. We further show that HIF1a accumulation upon hypoxia is temporally regulated and Bmal1-dependent. Unexpectedly, mice lacking both hepatic Bmal1 and Hif1a are hypoxemic and exhibit increased mortality upon hypoxic exposure in a daytime-dependent manner.

These mice display mild liver dysfunction with pulmonary vasodilation likely due to ERK activation, endothelial nitric oxide synthase and nitric oxide accumulation in lungs, suggestive of hepatopulmonary syndrome. Our findings indicate that hepatic BMAL1 and HIF1a are key time-dependent regulators of the hypoxic response and can provide molecular insight on the pathophysiology of hepatopulmonary syndrome.

Symposia

S 02-03

Human meal anticipation

Cheryl M. Isherwood[1], Debra J. Skene[1], Hana Hassanin[2], Jonathan D. Johnston[1] and Daan R. Van der Veen[1]

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While substantial evidence in rodents points to food anticipation and food entrainment being driven by a circadian food entrainable oscillator (FEO), evidence for similar impacts of meal timing on human circadian metabolic physiology remains limited.

Two of our recent, highly controlled laboratory studies[1,2] (Study 1: 5-h delay of 3-meal schedule; Study 2: two large meals (7.5h and 14.5h after lights-on) vs hourly snacks, both followed by constant routines), enabled us to test the hypothesis that human circadian metabolic physiology is altered by meal-timing, and advance our understanding of circadian food entrainment in humans.

Targeted UPLC-MS/MS metabolomics analysis of plasma samples (every 30-120 min) shows that a 5h delay in meal timing resulted in a 5.7h delay in glucose rhythms[1]. Among 127 detected metabolites, 16 out of 31 rhythmic metabolites exhibited phase shifts that were intermediate to delayed meal-timing and the unchanged light-dark cycle. When contrasting timing of rhythms between the participants receiving 2 large meals or hourly small meals, glucose rhythms were 11.8h (interstitial) and 7.7h (plasma) later in large meals, and 32 out of 52 rhythmic metabolites had a significantly different phase between both meal patterns. These results strongly support entrainment by meal timing.

In terms of food anticipation in human physiology, results are less clear and depend on metabolite class. Comparing groups with different mealtimes before a constant routine showed that concentration of some amino acids, acylcarnitines and lysophosphatidylcholines increased prior to expected meals and some phosphatidylcholines decreased, reflecting food anticipation rather than circadian effects.

In conclusion, delaying meal timing whilst keeping the light/dark and sleep/wake constant, results in large phase delays of glucose rhythms, and smaller phase shifts in some plasma metabolites. Metabolic profiling of meal anticipation showed a differential effect dependent on metabolite class.

[1]Wehrens et al. Curr. Biol 2017; [2]Isherwood et al. Curr. Biol 2023

Symposia

S 02-04

A sexually dimorphic hepatic cycle of periportal VLDL generation and subsequent pericentral VLDLR-mediated re-uptake

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2 Institute of Bioengineering, School of Life Sciences, Ecole Polytechnique Fédérale de Lausanne, Lausanne, Switzerland

We recently performed a scRNA-seq analysis of the liver from female and male mice, using hepatic cells collected at distinct time-points within a day, and developed a new method of analyzing scRNA-seq data. This allowed us to digitally re-position the dissociated hepatocytes back onto the hepatic pericentral to periportal axis and, for the first time, obtain a comprehensive overview of how lobular position, time of day, and sex shape the lobular transcriptome. These data revealed that the very low density lipoprotein (VLDL) receptor (Vldlr), crucial for VLDL uptake, and previously reported to be very lowly expressed in the liver, is restricted to the hepatic pericentral zone, with significantly higher mRNA levels in female mice, which was confirmed on the protein level with immunostaining.

Additionally, we demonstrated a periportal bias in VLDL assembly, both with transcriptomics and electron microscopy, revealing a previously unknown sexually dimorphic hepatic cycle of periportal formation and pericentral uptake of VLDL. This spatial separation of VLDL production and lipoprotein re-uptake is additionally enhanced in time, as lipoprotein production is boosted postprandially, enabling systemic circulation of triglyceride-rich lipoproteins during the fasting phase in mammals. Conversely, VLDLR-mediated lipoprotein uptake occurs at the fasting-feeding transition, in anticipation of feeding, leading to rhythmic changes in the hepatic lipidome. VLDLR's sexual dimorphism is conserved in humans, with significantly higher VLDLR expression in premenopausal women than in age-matched men. We also found a strong association between low hepatic VLDLR and an incidence of progressive atherosclerotic lesions and a medical history of myocardial infarction. These findings suggest that sex-specific VLDLR regulation may underlie observed differences in coronary heart disease risk between young men and women. Our current research builds on these insights to refine diagnostic tools and therapeutic strategies in humans, while also probing the molecular mechanisms governing hepatic lipoprotein homeostasis

Symposia

S 02-05

Protein Secretion Ex Vivo Reveals Regulation of Hepatokines by the Liver Circadian Clock

Christopher Litwin[1,2], Qing Zhang[1,2], Zhihong Li[1,2], Sophia Hernandez[1,2], Mallory Keating[3], Tomoki Sato[4], Paolo Sassone-Corsi[5], Kevin F. Bieniek[3,6], and Kevin B. Koronowski[1,2,]*

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Symposia

S 02-05

Protein Secretion Ex Vivo Reveals Regulation of Hepatokines by the Liver Circadian Clock

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[1]Wehrens et al. Curr. Biol 2017; [2]Isherwood et al. Curr. Biol 2023

Symposia

S 02-06

Re-scoping ultradian metabolism

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Ultradian rhythms are rhythms with periods shorter than 24 hours that are observed across behavioural, physiological and molecular domains. Yet, these rapid rhythms remain poorly understood and are often considered secondary to circadian rhythms, or even dismissed as erratic dynamics. This oversight stems, in part, from the expectation that ultradian oscillators and rhythms should conform to the same criteria used to define circadian clocks and rhythms. Such a view risks obscuring the biological significance of ultradian rhythms, and may cause us to overlook features that are unique, and potentially critical, to the function and mechanism of ultradian regulation.

Circadian rhythms are tightly coupled to stable environmental cycles (e.g., the light-dark cycle) driven by defined oscillators. In contrast, behaviour-related ultradian rhythms are dynamic in period and amplitude. They persist in the absence of the circadian clocks even though they exhibit circadian-like phase-dependent synchronisation to light in voles, the gold-standard ultradian model. These rhythms are closely linked to metabolic processes across species, which is also evidenced in metabolomic and gene expression analyses. Moreover, accumulating evidence shows that ultradian rhythms can be highly plastic, emerging or intensifying in prominence in response to physiological changes, particularly those related to energy balance.

Here, we lay out the argument that variability of ultradian rhythms is not a sign of weakness, but an adaptive response to internal metabolic needs, allowing organisms to dynamically match behavioural and metabolic processing to energetic state. This model defines ultradian rhythmicity as an emergent, plastic timing strategy embedded within metabolic regulation, and actualises Aschoff's early observations that ultradian rhythms serve metabolic self-preservation. We now need to test key emerging questions about the mechanisms, functions, and health implications of ultradian rhythms; questions that remain underexplored, but are crucial for understanding how biological timekeeping systems combine ultradian and circadian rhythms to supports energy homeostasis.

Symposia

S 03-01

The light-dark cycle during development and later life affective disorders

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Investigating if and how perinatal light affects later life risk of, inter alia, psychiatric conditions is a union of many research frontiers. It stems from the enduring "season-of-birth" research frontier and runs all the way to modern-day epigenetics. In recent years, abundant studies demonstrated that longer vs shorter photoperiods or chronic phase shifting of light-dark cycles during development (i.e., modifying perinatal light) affects circadian biology, physiology, and behavior in various animal models. Imprinting of the circadian timing system has been postulated as a driving mechanism. Furthermore, the circadian biology, physiology, and behavior outcomes have links to affective disorders.

As perinatal light is both ubiquitous and readily modifiable for humans, answering the question as to whether what is observed in animal models translates to humans could be significant for individual and public health. Of course, observational studies in humans are not simple. Zoning in on perinatal time windows of sensitivity, considering the constant light-dark paradigms in the lab compared to the dynamic natural photoperiod across months, and identifying appropriate exposure metrics add to the complexity. Nonetheless, studies of combined perinatal time-of-year and latitude (determinants of natural perinatal photoperiods), of shiftwork during pregnancy, and of different light-dark paradigms in neonatal care units hold promise as starting points for research in humans. Experimental and observational research findings so far, limitations, translatability, and research opportunities for this research frontier will be presented.

Symposia

S 03-02

Sleep and circadian rhythm disruption in mental health: a role for functional brain networks?

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Evidence points to a bidirectional link between sleep and mental health. Sleep and circadian rhythm disruption (SCRD) is highly prevalent in depression, anxiety and psychosis, and may exacerbate symptoms or even precipitate acute episodes. Moreover, targeting sleep has been shown to improve clinical symptoms across disorders. Numerous potential mechanisms have been proposed to explain these links, although the details still remain elusive.

Here I will describe the links between specific brain neurotransmitter systems relevant to neuropsychiatric disease and SCRD. Many of these systems relate to specific brain networks – typically measured using resting state functional magnetic resonance imaging (fMRI) – and have been shown to be disrupted in both psychiatric disorders and SCRD. Recent work has shown that it is possible to measures these networks in animal models, providing a target for focused experimental studies at both the clinical and fundamental neuroscience level. This approach may provide insight into the mechanistic links between sleep and mental health disorders.

Symposia

S 03-03

Delayed sleep timing and mental disorder: implications and interventions

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Circadian rhythm sleep-wake disorders (CRSWD), encompassing the clinical diagnoses of delayed sleep-wake phase disorder (DSWPD) and non-24 hour sleep wake rhythm disorder (N24SWD), commonly co-occur with a range of psychiatric disorders. Delayed sleep timing and psychiatric symptoms have a bidirectional relationship, and interventions which target the CRSWD may be an important strategy for treating the mental disorder.

A delay in endogenous circadian rhythm has traditionally been assumed to underlie the delay in sleep timing. It has recently been reported, however, that some individuals with DSWPD do not demonstrate a delay in the melatonin rhythm. This suggests that other mechanisms such as a dissociation between sleep timing and the circadian clock, driven by differences in sleep homeostasis, psychological and behavioural factors, may contribute to both the pathophysiology of these CRSWDs. We review the latest evidence for sub-groups in DSWPD pathophysiology, their relationship with mental disorder, the importance of circadian phase measurement in improving our understanding of sleep timing disorders, and implications for personalised interventions.

Symposia

S 03-04

Relaxation of social time pressure extends fasting and reveals a tight coupling between sleep/wake and fast/eat daily behaviors.

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As a day-active species, humans predominantly fast and sleep at night. Due to industrialization, the social clock diverged from the natural light-dark clock with far-stretching consequences for both fast/eat and sleep/wake daily cycles in modern societies. During the COVID-19 pandemic, prolonged social restrictions (SR) offered a quasi-experimental protocol to directly test the impact of the relaxed social clock on mealsand sleep timing and the coupling between them. Using data from a global survey of 5,747 adults (mean age 37.2±13.7, 67.1% females, 100% worked/studied), we show that relaxation of the social time pressure during the COVID-19 pandemic's social restrictions, led on average to a 42 min increase in the fasting duration (FD) (from 12:16±2:09 to 12.57±2:04) and a 34 min delay in the fasting window. FD was extended by lengthening both the pre-sleep fasting and sleep durations. Pre-SR breakfast eaters delayed sleep and fasting, while breakfast skippers delayed sleep and advanced meals. Stopping alarm use on workdays was associated with a larger increase in FD. The correlations between chronotype, FD and the mid-fast time became more robust during SR. We conclude that relaxed social time pressure promotes co-alignment of daily fasting and sleeping and extends FD. Given the finding that the sleep-fast phase relationship during social restrictions remained stable, we suggest that a "daily sleep-fast structure" may be a novel circadian marker. Relaxation of social time pressure extends fasting and reveals a tight coupling between sleep/wake and fast/eat daily behaviors. These results may inform strategies of public circadian health management.

Symposia

S 03-05

Evening light reduction for improving depressive and insomnia symptoms in people taking an SSRI

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Antidepressant medication is the most popular treatment for depression but only a third of patients benefit. Selective serotonin reuptake-inhibitors (SSRIs), a common type of antidepressants, profoundly increase sensitivity to light, which may amplify the disruptive effects of evening light. However, it is unclear whether changing evening home lighting can improve SSRI treatment outcomes. We investigated whether an at-home intervention focused on reducing evening light improved depressive and insomnia symptoms for people experiencing depression while taking an SSRI. A total of 25 participants aged between 18-40 were regularly taking an SSRI, and reported at least a moderate level of depression, according to the Beck Depression Inventory. Participants completed a baseline week of at-home monitoring of sleep, mood, and light exposure before they were randomly allocated to either the control group (n = 11) or the smart light intervention group (n = 12) for four weeks. The intervention conditions involved replacing regular evening lighting with dim, orange smart lights.

The intervention successfully achieved a change in evening light with those in the intervention group demonstrating significantly less evening light at one, two, and three hours before sleep compared to the control group. While depressive and insomnia symptoms did not differ between groups at baseline, those in the intervention group showed a significant reduction in both depressive and insomnia symptom severity. Patients in the intervention group went from reporting severe depressive symptoms to mild symptoms. No changes in symptom severity were observed for those in the control group. However, a subset of participants in the control condition subsequently underwent the same intervention and experienced similar improvements. These findings suggest that reducing evening light adjunct with SSRIs is effective in improving treatment symptom severity. Simply changing an individual's lights at home could therefore address the low efficacy of antidepressants.

Symposia

S 03-06

Impact of night shifts on cognitive, psychological, and cardiovascular health: findings from the OPTI-SHIFT observational study

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Symposia

S 03-06

Background: While laboratory and epidemiological studies have shown that shift work negatively affects cognitive, psychological, and cardiovascular health, prospective data from real-life occupational settings remain limited.

Methods: Seventy-two shift workers (82% female; median age 31 [IQR 21 to 35] years; body mass index 22.7 [20.4 to 26.2] kg/m^2) underwent three consecutive night and day shifts. The Standard Shiftwork Index, Sustained Attention to Response Test (SART), Stanford Sleepiness Score (SSS), International Physical Activity Questionnaire, WHO-5 Well-being Index, Patient Health Questionnaire-9, blood pressure, biochemical markers, and 24-hour heart rate (CamNTech® Actiheart) were compared between shift periods.

Results: Of all participants, 44% reported increased emotional stress, while 60% experienced less time pressure during night compared to day shifts. Night shifts resulted in poorer sleep quality and greater fatigue (all $p<0.001$). Post-night shifts, the SART showed lower mean accuracy (Δ: -0.4 [-2.2 to 0.4] %, $p<0.001$) and higher commission errors (Δ: 4 [-4 to 16] %, $p=0.001$) than post-day shifts, with no differences in omission errors, reaction time, and inverse efficiency. This impaired cognitive performance was due to higher sleepiness (SSSΔ: 2 [0 to 2], $p<0.001$) during the test. No differences in physical activity levels, mental health, and well-being between shifts periods (all $p>0.05$). Higher morning systolic (Δ: 1.9 ± SD 6.9 mmHg, $p=0.03$) and diastolic blood pressure (Δ: 1.4 ± 6.0 mmHg, $p=0.05$), but lower heart rate (Δ: -3.5 ± 10.0 bpm, $p=0.005$) and plasma cortisol (Δ: -108.5 [-216 to 36] nmol/l, $p=0.007$) were observed post-night shifts. Additionally, night shifts led to higher nighttime and lower daytime 24-hour heart rate compared to day shifts, while the heart rate variability was higher around midday on night shifts compared to day shifts, indicating perturbed 24-hour parasympathetic activation (all $p<0.05$).

Conclusion: Night shift work is linked to impairments in sleep, cognitive performance, and cardiovascular health.

Symposia

S 04-01

Lunar effects on the behavior of humans and other primates

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Throughout evolution and history, humans have progressively isolated themselves from natural cycles in built environments that shield them from the external environment. Key to this isolation is our ability to manipulate artificial light and extend our activity into the nighttime. Recent studies from our laboratory suggest that moonlight not only had a similar effect on activity in ancestral times, but also that the phases of the moon continue to shape our daily sleep in highly urbanized communities. I will present data from human and non-human primates that provide evidence for the synchronization of sleep by lunar gravity and for the mechanisms by which the moon may regulate sleep physiology.

Symposia

S 04-02

Neuronal mechanisms controlling photoperiodic diapause in flesh fly larvae

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Abstract: For seasonal adaptation, many insects inhabiting temperate zones alter their physiological states for development or diapause according to photoperiod. The mechanisms underlying insect photoperiodic responses include photoreceptors, photoperiodic clocks, photoperiodic counters, and endocrine systems. The circadian clock system is considered to be involved in the photoperiodic clock and counter, and it has been shown that the expression of different clock genes is a prerequisite for photoperiodism in many species. However, little is known about how the circadian clock cells integrate day-length information. To address this, we use larvae of the flesh fly, Sarcophaga similis exhibiting photoperiodic responses to control pupal diapause. We first examined the neural circuitry involving circadian clock lateral neurons (LNs) and prothoracicotropic hormone (PTTH) neurons possibly controlling the ecdysteroid production, and then the photoperiodic effects on LN-fiber patterns in third-instar S. similis larvae.

PERIOD and a neuropeptide pigment-dispersing factor (PDF) were immunohistochemically co-localized in four cells per hemisphere (PDF-LNs). Single-cell PCR of backfilled neurons from the ring gland showed that two pairs of pars lateralis neurons with contralateral axons (PL-c neurons) expressed ptth. Spontaneous electrical activity of ptth-expressing PL-c neurons were strongly suppressed by short neuropeptide F and glutamate but not by PDF. Double labeling revealed that PDF-immunoreactive varicose fibers projected in the proximity of fibers from PL-c neurons. The number of PDF-immunoreactive varicosities of PDF-LNs in the dorsal protocerebrum was significantly higher under short days than that under long days in a time-dependent manner. These suggest that PDF-LNs and PTTH neurons form a potential neural circuity, and that photoperiod modifies the connectivity strength between PDF-LNs and their post- or pre-neurons in the circuitry to change ecdysteroid production.

Symposia

S 04-03

Tidal timekeepers of the abyss: Circatidal gene expression in hydrothermal vent shrimps and methane seep mussels reveals deep conservation of ~12-hour rhythms

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Biological rhythms in the dark, deep-sea biosphere remain enigmatic, with tidal cycles emerging as key drivers of adaptation in chemosynthetic ecosystems. Here, we present two complementary studies illuminating the role of circatidal (~12-hour) gene expression in organisms from hydrothermal vents and methane seeps. First, in the hydrothermal vent shrimp Rimicaris leurokolos, free-running experiments under constant conditions revealed endogenous circatidal transcriptional rhythms dominating the temporal transcriptome. These oscillations, synchronized to tidal cycles rather than circadian cues, regulate processes spanning DNA repair, stress responses, and metabolic coordination.

Strikingly, these tidal genes show evolutionary parallels to ~12-hour ultradian rhythms in terrestrial models (e.g., mice, fruit flies), suggesting deep conservation of circatidal timing mechanisms. Second, in the methane seep mussel Gigantidas haimaensis, we uncovered analogous circatidal gene expression patterns tied to fluctuating seep environments as monitored by in situ sensors. Both species, despite inhabiting distinct chemosynthetic ecosystems, exhibit convergent reliance on endogenous circatidal clocks to navigate extreme, aphotic habitats. These findings position deep-sea vent and seep organisms as pivotal models for unraveling the evolution and molecular basis of ~12-hour rhythms, bridging marine chronobiology with terrestrial ultradian research. Our work highlights the tide's universal influence across biospheres and reshapes our understanding of temporal adaptation in Earth's largest yet least-explored ecosystems.

Symposia

S 04-04

Faster clock in mice and cells lacking mRNA cap methylation

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Abstract: The 5' cap of eukaryotic mRNA is the focus of several methyltransferases, including CMTR1 that targets the first transcribed nucleotide at the 2'-O ribose position. Cap methylation by CMTR1 has been shown to enhance transcript stability, export and translation and has important roles in protecting transcripts from degradation by the intracellular immune system.

Despite increasing research on the molecular function of CMTR1 little is known about its physiological function in adult animals. Here, we investigated the circadian phenotype associated with the lack of CMTR1 in GABAergic neurons in mice. Circadian locomotor activity recordings showed CMTR1 KO mice to have a significantly shorter circadian period compared to WT litter mates. This short period was replicated in PER2::LUC MEFs lacking a functional CMTR1. RNA sequencing revealed the lack of CMTR1 to downregulate the expression of transcripts encoding ribosomal proteins and translation factors and ribosome profiling analyses indeed revealed a deficiency in translation. We propose that a deficit in ribosome biogenesis is associated with the short period in CMTR1 KO cells and animals.

Symposia

S 04-05

Circadian clock-independent ultradian rhythms in lipid metabolism in the Drosophila fat body

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The role of circadian clocks in regulating metabolic processes is well known; however, their impact on metabolic states across species and life stages remains largely unexplored. This study investigates the relationship between circadian rhythms and metabolic regulation in the Drosophila larval fat body, a metabolic hub analogous to the mammalian liver and adipose tissue. Surprisingly, the fat body of period null mutants, which lack a functional circadian clock in all tissues, exhibited 12-hour rhythms in gene expression, particularly those involved in peroxisome function, lipid metabolism, and oxidative stress response. These transcriptomic rhythms were aligned with 12-hour oscillations in peroxisome biogenesis and activity, reactive oxygen species levels, and lipid peroxidation. Furthermore, period mutants exhibited 12-hour rhythms in body fat storage, ultimately leading to a net reduction in body fat levels.

Collectively, our results identify clock-independent ultradian rhythms in lipid metabolism that are essential for larval survival and development.

Symposia

S 04-06

Platynereis dumerilii as a functional molecular model organism for circalunar clock analyses

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Symposia

S 04-06

Since its beginnings, life has been exposed to the regular environmental cycles caused by sun and moon, requiring adjustments of physiology and behavior. Especially in the marine environment – considered to be the cradle of evolution – a rich body of literature describes endogenous organismal rhythms that are not linked to the solar, but to the lunar cycle. These rhythms have considerable impact, ranging from behavioral and physiological control to ecosystem organization. As the origin of circalunar timing mechanisms likely predates the conquest of land, the underlying molecular oscillators might still be involved in physiological and behavioral cycles of several terrestrial animals, including birds and humans.

However, as no prominent, reproducible circalunar oscillator phenomenon has so far been uncovered in “conventional” molecular model species (mouse, zebrafish, Drosophila, C.elegans), disentangling the molecular and cellular mechanisms of moon-controlled rhythms and oscillators requires the establishment of new model species.

The marine bristle worm Platynereis dumerilii possesses a circalunar oscillator that is set by dim nocturnal light. Building on work from the 1960ies, the KTR/FR labs have been pushing to establish this species as a model for circalunar clock analyses. Besides the published work on transgenesis, conditional cell ablations, reverse genetic mutants via TALENs and Cas9/Crispr, transcriptomic and proteomic resources, long-term and high resolution measurements of sun- and moonlight at the natural habitat, establishment of illumination devices mimicking sun- and moonlight, automated behavioral recordings and tracking analyses, immunohistochemistry, TUNEL, HCR-based RNA in situ hybridization (optionally with EdU labeling), tissue clearing, we here report on our recent (unpublished) advancements on larval deep-freezing for long-term strain storage, the design of outside tank systems (mesocosms), genome editing by Cas9-deaminase, as well as a chromosome-scale genome for a highly inbred lab strain, as well as its comparison to an animal recently isolated from its natural habitat (Bay of Naples, Italy).

Symposia

S 05-01

Allele-Specific Expression in Circadian Clock Genes Across Multiple Tissues in Baboons

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Circadian rhythm research has traditionally relied on homozygous inbred lines. However, this approach contrasts with the genetic diversity of humans, who are heterozygous at~2million loci. At heterozygous sites, the two alleles of a gene may not express equally. Instead, they can display allele-specific expression (ASE)—a phenomenon where one allele is preferentially expressed over the other in certain tissues or contexts. ASE plays a crucial role in modulating the penetrance of both pathogenic and beneficial alleles and influences a wide array of biological processes. Notably, ASE has been linked to disease susceptibility, and in conditions like cancer, it can be both a marker and a mechanism of disease progression.

Symposia

S 05-01

Despite its importance, comprehensive studies assessing the extent of ASE across multiple tissues in healthy, young individuals remain scarce. To address this gap, we conducted a genome-wide ASE analysis using 11 distinct tissue types collected from 12 age-matched healthy olive baboons (Papio anubis). Each genome was sequenced to a minimum depth of 30X, resulting in the identification of over 16 million single nucleotide variants (SNVs). Additionally, we generated long-read sequencing data, allowing us to phase coding region variants into single haplotype blocks for 96.5% of assayable protein-coding genes.

Owing to the greater heterozygosity in baboons compared to humans, we were able to assess ASE for 72% of all annotated protein-coding genes. Strikingly, we found that core circadian clock genes exhibited widespread ASE. Furthermore, the allele preference for expression was not fixed but often varied across different tissues, suggesting a dynamic, tissue-specific regulatory landscape. These findings have significant implications for understanding how genetic variation influences physiological outcomes. In particular, they enhance our ability to interpret the phenotypic effects of mutant alleles—especially those involved in circadian regulation—and underscore the importance of considering ASE in functional genomic analyses.

Symposia

S 05-02

Investigating the mechanisms driving TRE-induced fat loss

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Today, we have unprecedented access to food and, with that, unprecedented rates of obesity. Time-restricted eating (TRE) is an effective dietary regimen for fat loss that focuses not on limiting calories themselves but on limiting caloric intake to a specific window of time during the active phase. Because many of the physiological mechanisms underlying metabolism and aging are conserved between Drosophila and humans, we developed a TRE protocol for Drosophila to investigate the effects of timed eating on aging and metabolism. We found that our TRE protocol delayed aging and extended Drosophila lifespan; we also found that, similar to humans, our TRE protocol causes significant fat loss (decreased triacylglycerides). This is not due to decreased caloric intake; flies on TRE eat more overall than animals on ad lib diet.

Moreover, TRE flies maintain and even increase their protein levels. This is in sharp contrast with GLP-1 agonists, which sharply reduce caloric intake; GLP-1 agonists cause not only fat loss but also muscle loss and sometimes nutritional deficiencies. There is currently no such drug that can mimic the weight-loss effects of TRE because the underlying molecular mechanism(s) remain largely unknown. We performed RNA seq analysis to identify genes that are differentially regulated between ad lib and iTRF flies and have identified candidate genes, pathways, and behaviors as possible drivers of TRE-induced fat loss. We are currently testing these hypotheses; the longterm goal is to identify new and critical therapeutic targets for obesity.

Symposia

S 05-04

Linking the establishment of circadian rhythm with the establishment of long-term epigenetic silencing

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X chromosome inactivation (XCI) is the mammalian epigenetic mechanism that ensures equal gene dosage between XX females and XY males, resulting in near complete silencing of one female X chromosome. XCI is setup in the early post-implantation period in vivo or the in vitro equivalent in the early stages of mouse embryonic stem cell (mESC) differentiation, and then is mitotically heritable for the lifetime of the organism, representing an excellent model system to study the processes of long-lived . The XCI process is multilayered and not fully understood despite significant research effort. Moreover, many of the mechanisms of epigenetic silencing discovered by studying XCI have borne out for autosomal silencing as well.

Therefore, we were interested to discover more about the mechanisms of XCI, using unbiased screens in female embryonic stem cell differentiation. We discovered that the establishment of XCI is controlled by core circadian rhythm regulators. mESC have not yet undergone XCI, and similarly, they also do not possess circadian rhythm; we find that circadian rhythm and XCI are temporally co-established during early differentiation. Our data show that the processes are also functionally linked, as depletion of circadian rhythm regulators accelerates the rate at which XCI proceeds, suggesting that circadian rhythm tempers the establishment of XCI. To ask whether the same is true in vivo, we took advantage of the ability for the mother's circadian rhythm to enforce an altered rhythm in her embryos. We found that mothers with an altered day night cycle accelerated the establishment of XCI in blastocyst outgrowths. Taken together our data reveal a novel role for the establishment of circadian rhythm in influencing the major epigenetic reprogramming that occurs concurrently in the developing embryo which may have widespread consequences for XCI but also potentially for autosomal gene silencing.

Symposia

S 05-05

Title: Role of GRIP1 as a Coactivator in Bmal1 Promoter Rhythm Formation

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GRIP1 is a transcriptional coactivator that enhances Per2 transcription via the BMAL1:CLOCK complex, contributing to the amplitude of the Per2 promoter rhythm and the robustness of the core negative feedback loop. While a secondary feedback loop involving RORα and REV-ERBα regulates Bmal1 transcription and forms an interlocked system with the core loop, the role of coactivators in this minor loop remains unclear.

To address this, we utilized NIH3T3 cells, which exhibit circadian oscillations, and generated GRIP1-deficient cells via CRISPR-Cas9. We assessed Bmal1 promoter activity and its regulation via RORα in the presence or absence of GRIP1. GRIP1 knockout cells showed significantly reduced amplitude of the Bmal1 promoter rhythm, suggesting its involvement in rhythm generation.

We then investigated the interaction between GRIP1 and RORα using a mammalian two-hybrid system. Consistent with previous findings, transient overexpression of GRIP1 increased reporter activity driven by RORα, indicating a physical interaction. However, this RORα-mediated reporter activation was lost in GRIP1-deficient cells, confirming the necessity of GRIP1 for functional interaction.

These findings indicate that GRIP1 contributes to the amplification of circadian transcriptional rhythms not only in the core Per2 loop but also in the secondary Bmal1 loop, likely via coactivation of RORα-mediated transcription. Our results highlight a broader regulatory role for GRIP1 in circadian gene expression.

Symposia

S 06-01

A Mechanism for Circadian Rest Time Allocation

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How long do you sleep every night? The amount of time spent for rest per night differs between individuals and animal species—multiple genetic and environmental factors influence this trait. From both molecular and physiological perspectives, when to sleep (i.e., timing) and how long to sleep (duration, or allocation) are different traits; the former has been well elucidated by the state of art science in chronobiology, while the latter still remains an enigma.

The unique G-protein subclass Gz (also initially referred to as Gx) is an evolutionarily conserved vertebrate G-protein that belongs to the family of canonical Gi/o. Gz possesses the activity to reduce cAMP synthesis. However, Gz is not just an “ersatz” Gi. As its name suggests, Gz displays a range of unique properties: First, in contrast to the ubiquitous expression of Gi, tissue distribution of Gz is specific to the CNS including the SCN. Secondly, the Gi/o inhibitor PTX does not inhibit Gz, posing hurdles in pharmacological approach to this protein. Thirdly, Gz bears approximately 100 times slower GTPase activity than Gi. Thus, once GTP binds to it, Gz does not readily return to its inactive form, resulting in a prolonged signal in terms of temporal resolution, which suggest differential roles for Gz and Gi in brain signal processing.

Consequently, only scanty information is currently available for the function of Gz in CNS—In this research background, I report, here, the identification of a crucial functional contribution of Gz to the mechanism of rest time allocation. Our data demonstrate that the SCN is not simply a 24-h metronome. Besides providing periodicity (τ) to the organism, the central clock has a role in directing timing and duration of rest behavior through a mechanism employing Gz. A detailed molecular/neuronal mechanism will be discussed.

Symposia

S 06-02

Clock in the choroid plexus is a gatekeeper for the brain

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Choroid plexus (ChP), which is located in all four ventricles of the mammalian brain, actively produces cerebrospinal fluid (CSF) by filtering blood from fenestrated capillaries. In addition, the ChP plays an essential role in transporting nutrients and electrolytes from the blood and in removing neurotransmitter degradation products and toxic substances from the CSF into the bloodstream. It also secretes biologically active substances that may have nourishing and neuroprotective properties and thus plays an overlooked role in signaling in the brain. The ChP harbors a robust autonomous circadian clock that runs without any input from the SCN, as demonstrated ex vivo in organotypic explants from mPer2luc mice.

The aim of our study was to decipher the mechanisms of how the ChP clock is synchronized and what role it plays in the physiological functions of the tissue. We also investigated the sensitivity of the ChP clock to chronodisruption and inflammation.

Using in vivo and ex vivo approaches, we identified glucocorticoids as signals used by the central clock in the suprachiasmatic nuclei (SCN) to synchronize the ChP clock. Using time-resolved transcriptomics and single-cell luminescence microscopy, we identified rhythmically controlled cellular processes in the mouse ChP and assessed the role and nature of signals originating from the master clock in the SCN that control ChP rhythms. Our data show that the ChP clock controls tissue-specific gene expression and is strongly dependent on the presence of a functional connection with the SCN. In addition, our data demonstrate the high resistance of the ChP clock to inflammation, highlighting its role in protecting the brain from neuroinflammation. On the other hand, the ChP clock is highly sensitive to chronodisruption. The results contribute to the search for a new link between ChP clock dysfunction and impaired brain health.

Symposia

S 06-03

On the role of circadian dopamine rhythms in mammalian time memory

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Recalling the time of day that significant appetitive or aversive conditions are encountered is a feature of autobiographical memory that has been demonstrated broadly in animals including insects, birds, rodents and primates. On the days following an experience, goal directed behaviors may be expressed at the same time of day as the experience, suggesting that animals anticipate the recurrence of similar conditions using the setting of a circadian oscillator. We tested two hypothetical roles for circadian rhythmic dopamine (DA) signaling in either the formation and/or expression of an implicit time memory (ITM) regulating the timing of anticipatory behaviors in mice. Locomotor rhythms with periods of 27-30 hrs in constant dark were elicited by ad libitum D-amphetamine (AMPH) delivered through the drinking water.

These rhythms were superimposed on the 24 hr circadian patterns generated by the suprachiasmatic nucleus (SCN). Single-trial passive avoidance learning resulted in significant avoidance at 48 hrs and 72 hrs following a training trial but not at 58 hrs and 87 hrs, indicating that the aversive stimulus was anticipated at ca. 24 hr intervals but not at the AMPH-induced periodicity. Avoidance increased with higher locomotor activity, but this had no relationship to the time of conditioning. Therefore, whereas DA signaling likely coordinates the setting of ITM mechanisms among several DA-target tissues, the rhythm in DA activity per se is not required for the formation or retrieval of time memory.

Symposia

S 06-04

Elucidating The Mechanisms of Bright Light Therapyvia Per1

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Mood disorders represent a major toll on the health of people, severely deteriorating the quality of life. Among different therapies, bright light is used as a non-invasive option. 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 expression of the clock gene Period1 (Per1) in the lateral habenula, a regioninvolved in controlling mood-related behaviors, as a component required to mediate the benefits of light. Per1 is also the clock gene that is responsible for light mediated clock resetting.

Therefore, is the ability of the clock resetting important in mood regulation, or is it just the expression of the Period 1 in the lateral habenula that drives these responses, providing hence, a region-specific role of Per1?

To that extend, we have achieved a deletion of Per1 in the lateral habenula via stereotaxic surgeries, which led to suppression of the valuable effects of light applied at zeitgeber time (ZT) 22. Mice with a Per1 deletion did not show a reduced immobility score in the behavioral test of Tail Suspension Test (TST), following a light pulse, as compared to the control injected counterparts. Meanwhile, deletion of Per1 in the suprachiasmatic nuclei (SCN), abolished the phase advancing response of the mice, which did not lead to a worsening in the mood as per the TST results. The following behavioral changes are further consolidated at the molecular level, by observing changes in the enzymes, transporters and receptors involved in neurotransmitters regulation.

Taken together, these resultslet us conclude that the light induction of Per1 in the lateralhabenula is sufficient for the beneficial effects of light, whereas the presence or absence of phase advances mediated by the SCN are not altering the mood.

Symposia

S 06-05

Identification of the key diffusible output factor that regulates circadian behavioral rhythms

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In mammals, the suprachiasmatic nucleus (SCN) of the hypothalamus plays an important role in regulating circadian rhythms in physiology and behavior. Circadian information from the SCN is output to various brain regions, which in turn regulate physiological functions such as sleep and wakefulness. The SCN was identified as the central circadian clock, and the mechanisms underlying outputs of the SCN in the circadian system have been studied. Although previous studies have highlighted the importance of diffusible factors in regulating circadian behavioral rhythms, it still remains unclear which specific diffusible factors are critical.

To identify the key diffusible factor(s) that regulates circadian behavioral rhythms, we performed the isolation of the SCN (iSCN) using a micro-knife. This procedure truncates neuronal axons extending from the SCN to external regions. Importantly, iSCN mice exhibited circadian behavioral rhythms even under constant darkness (DD), indicating the involvement of diffusible factors from the SCN that regulate circadian behavioral rhythms.

We then focused on these diffusible factors. Brain-derived neurotrophic factor (BDNF) is known as a candidate molecule that outputs circadian behavioral rhythms. To investigate the role of BDNF in circadian behavioral rhythms, we generated BDNF conditional knockout (cKO) mice by crossing Vgat-cre mice with Bdnf-floxed mice. BDNF cKO (Vgat-cre$^{+/-}$; Bdnf$^{fl/fl}$) mice exhibited robust circadian behavioral rhythms, similar to the control (Vgat-cre$^{-/-}$; Bdnf$^{fl/fl}$) mice under light-dark and DD. We next performed iSCN surgery on BDNF cKO mice to investigate the possibility that circadian behavioral rhythms were maintained by neural connections of the SCN. We found that BDNF cKO mice with iSCN failed to exhibit circadian behavioral rhythms under DD. Moreover, when adeno-associated virus carrying BDNF was injected into the SCN, circadian behavioral rhythms were restored in BDNF cKO mice with iSCN. These results indicate that BDNF is a key diffusible factor for regulating circadian behavioral rhythms.

Symposia

S 06-06

Light intensity modulates thermoregulation in a cooling environment

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Introduction. The suprachiasmatic nucleus (SCN) is the pacemaker of our circadian clock[1] and receives its main input from light[2,3]. It regulates many processes, including the variation of core temperature [4]. The coordinating center for thermoregulation is the hypothalamic preoptic area[5] (POA), which receives input from the SCN and a subpopulation of ipRGCs[6], the latter playing a key role in non-visual responses to light[7–9]. In this experiment, we assessed the thermoregulatory effects of daytime light exposure. Given that both ipRGCs[10,11] and thermoregulatory[12] pathways involve excitatory glutamatergic projections, we hypothesize that increased light input will enhance thermoregulatory responses.

Methods. 24 participants took part in two experimental sessions: one in dim light condition (14 lux) and the other in bright light (5350 lux). They were placed in rooms set at 15°C, 25°C or 35°C, spending 30 minutes at each temperature before switching to another. The order of exposure to the different ambient temperature followed either a warming protocol (exposure to 15°C, then 25°C, then 35°C) or a cooling protocol (exposure to 35°C, then 25°C, then 15°C). Measurements of core and skin temperature were performed using swallowable telemetric sensors and temperature loggers respectively.

Results. Mean values after 20 minutes of exposure to each ambient temperature condition show: 1) higher core temperature and lower peripheral temperature at 15°, with the opposite pattern at 35°C, in both cooling and warming protocols ; 2) a light × temperature interaction in the cooling protocol only, showing that at 15°C, core temperature was higher in the dim light condition compared to the bright light, suggesting that in a cooling environment, dim light promotes better heat conservation than bright light.

Symposia

S 07-01

Circadian clock detection in humans: challenges and opportunities

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Application of circadian-biology-based approaches to medicine holds promise for improving public health. This is particularly relevant in modern societies in which environmental factors such as abundant availability of artificial light or shift work may negatively impact circadian physiology. Accurate and cost-effective approaches for assessing the status of circadian clocks that can be implemented at scale are often considered to be key enablers of the translation of circadian principles to clinical practice. The multi-oscillator, multiorgan and multi-tissue nature of the circadian timing system combined with the multitude of behavioral and environmental factors that shape overt rhythmicity in nearly all potential markers of circadian clocks imply that developing appropriately validated biomarkers is challenging. A first question is: what do we mean by 'monitoring the clock'? Are we satisfied that the phase of rhythms in clock gene expression represent the clock, are we interested in monitoring the status of the overall rhythmic program in a particular organ or do we want to know the phase of the output of the SCN? A second question concerns the clock parameter, e.g. period, phase, amplitude, that is to be monitored by the biomarker. Another question concerns the organ, tissue, or brain area that is to be monitored by the biomarker. Whereas in the classical circadian literature concepts such as 'endogenous' and 'masking' played an important role, more recently the phrase circadian rhythmicity is often used to mean 24-h rhythmicity as observed. Over the past decade we have explored rhythmicity in the human blood transcriptome, compared it to rhythmicity in multiple organs and tissues in the Baboon, and investigated the impact of training sets and feature selection algorithms on the performance of biomarkers. We conclude that there are significant challenges and opportunities in the development of biomarkers for monitoring the clock in real life.

Symposia

S 07-02

Decoding circadian clock state heterogeneity at single-cell resolution

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Circadian rhythms, fundamental to organismal physiology, originate from molecular clocks within individual cells. However, understanding the heterogeneity and biological relevance of these single-cell rhythms in vivo is challenging, as conventional bulk-tissue analyses obscure these dynamics.

To address this, we developed a probabilistic model to infer circadian parameters from single-cell RNA sequencing (scRNA-seq) data, specifically designed to navigate multiple cellular contexts. Our model is tailored to the specifics and key challenges in single-cell circadian analysis, particularly the low abundance of clock transcript counts and cell-type-specific geometric properties of circadian gene expression. Our approach improves upon and generalizes previous models, allowing us to obtain reliable quantification of harmonic gene parameters and single-cell circadian phases, including uncertainty measures. Our analysis and simulations show that with current technologies, circadian phases can typically be estimated with median absolute errors (MAE) between 2-3 hours, which is remarkable considering the sparsity of scRNA-seq data.

Applying our method to a compendium of scRNA-seq time-series datasets we identified context-dependent clock parameters. Notably, the observed variation in inferred phases consistently exceeds the predicted uncertainty, providing a handle into genuine biological heterogeneity, with cell-type-specific differences in phase coherence. We also extended our approach to single-molecule RNA FISH (smFISH) data post-synchronization, demonstrating the expected decrease in phase coherence over time.

Symposia

S 07-03

Temporal patterns in circadian alignment: insights from real-life behavioral data

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Current lifestyles are often characterized by low daytime light exposure, increased nighttime illumination, 24/7 food availability and reduced physical activity. Considering the role of light and food as zeitgebers, modifiable behaviors such as light exposure and nutrient intake can importantly affect health and well-being via their influence on circadian organization. Weak or inconsistent zeitgebers often delay internal clocks and/or lead to circadian misalignment, a discrepancy between internal time and social demands that frequently results in irregular sleep-wake timing. Thus, metrics that characterize exposure to zeitgebers and that quantify irregularity in sleep times have often been used in naturalistic studies as proxies for circadian misalignment. This is also a scenario where there is likely underlying misalignment of internal daily rhythms.

In this talk, I will present how we have leveraged longitudinal behavioral data to explore associations of such proxies with daily well-being, aiming to lay out a temporal framework for understanding the effects of light exposure and circadian misalignment in real-life settings. Furthermore, I will discuss how new tools for estimating circadian phase can offer insights into how behavioral irregularity (e.g., in activity, light exposure) may translate into changes in internal time.

Symposia

S 07-04

Telling time from intercellular communication in the blood-brain barrier

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The blood-brain barrier (BBB) is a neurovascular structure vital for the regulation of nutrient uptake by the brain as well as protection against harmful substances. As one of the tightest barriers in the human body, however, it also poses a significant hinderance for neurotherapeutics to reach the brain.

We show that BBB tightness is circadian in a tri-culture model containing mouse brain endothelial cells, astrocytes and pericytes. Claudin-5 is a key regulator of BBB tightness and rhythmically expressed in MBECs. Knock-out or overexpression of Cldn5 disrupts the rhythm, as does disruption of the clock by Bmal1 deletion. Moreover, co-transfection of Bmal1 and Clock in a transcriptional assay downregulates Cldn5 suggesting direct clock control.
Interestingly, the rhythm in clockless endothelial cells can be rescued by co-cultured clock containing astrocytes and pericytes suggesting inter-cellular communication between the BBB cell types. To investigate how the rhythmic regulation of the tightness of the barrier is restored by the other cell types, we examined the role of extracellular vesicles (EVs). Previous studies have suggested EVs can modulate BBB permeability and core clock gene expression in peripheral cells. Thus, we hypothesise that EVs are involved in communication between the cells of the BBB.
From astrocyte and pericytes conditioned media, we isolated EVs and characterise their size and classical marker expression using MACSPlex. We show differences between EVs produced by astrocytes and pericytes, and currently further investigate the content of the vesicles as well as their impact on endothelial cell clocks.
Future work will allow us to understand the role of EVs in the circadian cell-to-cell communication within the BBB. Ultimately, this might play a role in diseases such as Alzheimer's where BBB integrity is compromised, and robustness of the internal clock is reduced; but could also improve the delivery of therapeutics to treat such neurodegenerative diseases.

Symposia

S 07-05

Quantifying Circadian Rhythmicity in Vital Signs of Preterm Infants in the NICU: A Cosinor-Based Approach

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Background- The circadian timing system governs 24-hour rhythms in key physiological functions, including heart rate, core body temperature, and arterial pressure. At what point these rhythms emerge during early development is unknown. Therefore, the goal of this study is to characterize 24-hour rhythms in vital signs across a large cohort of preterm infants stratified by postnatal age.

Methods- Using a retrospective study design, we extracted high-resolution vital signs and clinical data from the electronic health records from preterm infants admitted to the NICU at UMC Utrecht between 2008 and 2024. Inclusion criteria included gestational age below 32 weeks and a length of stay of at least 5 days. To assess 24-hour rhythms in heart rate, linearized cosinor analysis with a fixed period of 24 hours and an autoregressive noise component was used on 72-hour segments of heart rate time series. The extracted cosinor parameters (mesor, amplitude, phase) and the significance of the cosinor fits were summarized.
Results- The available dataset comprised a total of 12,334 72-hour time series from 816 infants. Heart rate showed a significant 24-hour rhythm ($p < 0.05$) in 25% of the time series, with a median amplitude of 3.9 bpm (IQR: 2.84, 5.29). Acrophase showed a bimodal distribution, with most time series peaking around 08:00 or 21:00. Model predictions showed a high degree of heterogeneity within and between patients. Next steps include investigating to what extent this heterogeneity is explained by postnatal age and other relevant covariates, such as gestational age, sex, medication use, or ventilation support.

Conclusion –This study provides an analytical framework for quantifying 24-hour rhythmicity using cosinor modelling in vital signs of preterm infants in the NICU (and other clinical populations). Future analyses will further elucidate the developmental trajectory of circadian organization in the early postnatal period in this patient population.

Symposia

S 07-06

Little overlap in circadian phase biomarker predictors developed from different methods/datasets, but overlap in underlying biological processes

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There is currently a growing interest and need to establish approaches to develop human biomarkers for circadian phase that are non-invasive, economical, and easily applicable in clinical and field settings. These biomarkers should be evaluated against gold-standard markers (e.g., dim light melatonin onset) and should provide near-equivalent accuracy. Blood samples offer a convenient way to establish time series datasets and also provide a window on whole-organism biology across 24h. Several approaches have been used [e.g., partial least squares regression (PLSR), molecular timetable, zeitzeiger, elastic net] to develop blood-based transcriptomic biomarkers for circadian phase that exhibit varying accuracy. We have applied these approaches to transcriptomic time series datasets collected in multiple sleep/wake conditions; total sleep loss after one week of either sleep restriction or sufficient sleep, sleeping in and out of phase with the body clock (Laing et al., 2017), and during 60 days of constant bed rest (Moller-Levet et al., in revision).

Our analyses show that overall PLSR performs best but accuracy depends upon training sets used during development. Comparing different methodological approaches and different training sets, we find there is little overlap between the resultant predictor transcripts, although some features are consistently reported in different studies (e.g., PER1, NR1D2, DDIT4, FKBP5). More consistent is the finding that in many different approaches and methods, predictor features are enriched for transcripts associated with glucocorticoid signalling. Glucocorticoid is a peripheral circadian entrainment signal, and our findings show this underlies a robust predictor of phase in a variety of different sleep/wake conditions. We have also developed transcriptomic biomarkers for acute and chronic sleep loss (Laing et al., 2018). Here there is less overlap with circadian phase features, with predictors for sleep loss being more associated with processes related to cellular stress.

Symposia

S 08-01

Evolution of circadian clock genes

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As we live in a periodic environment, nearly all organisms possess internal time-keeping devices, known as circadian clocks, which orchestrate an organism's physiology, metabolism, and activity within the Earth's 24-hour cycle. These clocks are well studied at the molecular and genetic levels in model organisms such as the fruit fly Drosophila or the mouse. Although the majority of genes and underlying mechanisms are conserved among flies and mammals, there are several important differences. For example, various insect species contain different combinations of Drosophila and mammalian clock components. Furthermore, mammalian genomes have undergone several near-complete genome duplications, resulting in various levels of clock gene multiplication, further shaped by secondary gene losses.

I will present our recent data addressing the evolution of circadian clock genes across animals — from the earliest available single-cell organisms to humans — and describe some lineages of animals with unusual clock setups or remarkable gene modifications. I will highlight some interesting twists in the origin of mammalian clock genes. Finally, I would like to illustrate how phylogenetic comparisons inspire exciting experimental research directions and share our recent reverse genetic data on unique clock components in insects.

Symposia

S 08-02

Mechanisms underlying circadian plasticity loss in an equatorial endemic species

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Widely distributed species experience substantial environmental variation, which they often accommodate through behavioral plasticity. Although this ability is integral to fitness, we have little understanding of its genetic and cellular basis. One factor that varies both seasonally and by latitude is photoperiod (day length). Many organisms, including the cosmopolitan fly Drosophila melanogaster, display circadian plasticity, adjusting to fluctuating photoperiod by varying the timing of their activity to coincide with changing dawn/dusk intervals. However, a closely related sister species, D. sechellia, is an equatorial island endemic experiencing little photoperiod variation. We found that D. sechellia displays minimal circadian plasticity, and, through a screen of circadian mutants in D. melanogaster-D. sechellia hybrids, identified a role of the neuropeptide Pigment dispersing factor (Pdf) in this phenotype.

We describe species-specific temporal properties of Pdf RNA and protein expression, and collect evidence that differences are due, in part, to cis-regulatory divergence. Finally, we provide evidence that plasticity confers a selective advantage for D. melanogaster at higher latitudes, while D. sechellia likely suffers fitness costs outside its range. Our results highlight this highly conserved gene as a hotspot for circadian plasticity evolution and suggest gene expression evolution as a mechanism for acquiring behavioral plasticity.

Symposia

S 08-03

Punctual parasites: why are rhythms advantageous?

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Life in a rhythmic world dictates the timing of activities for many organisms. This includes parasites, for whom the environments within their hosts and vectors change dramatically over 24 hours. The myriad daily rhythms of hosts and vectors offer opportunities for parasites to exploit and presents dangers to evade. Yet, how parasites have evolved to cope with these environmental rhythms is a fundamental unanswered question. Our research considers parasites in their ecological and evolutionary contexts to ask: "what makes a successful parasite" and "what are their evolutionary limits"? This has enabled us to uncover the sophisticated strategies that malaria (Plasmodium) parasites possess, such as optimizing the balance between transmission and replication, and strategic investment in each sex of transmission stages. Our recent work asks why parasites schedule blood stage replication according to the host's time of day. Malaria parasites - whose rhythms famously cause periodic fevers - are a great model system to explain how parasites evolve to cope with the challenges of environmental rhythms and exploit the opportunities these rhythms offer.

So far, we've established that host feeding rhythms are import to parasites replicating in the blood, determining in-host survival, vulnerability to antimalarial drugs, and the infectiousness of transmission forms, and that rhythms of mosquitoes affect many aspects of transmission. It's about time to integrate the roles of rhythms in all parties across the parasite's lifecycle to understand why timing matters and make translational gains by harnessing parasite rhythms.

Symposia

S 08-04

Circadian and ultradian membrane potential rhythms in hawkmoth olfactory receptor neurons depend on Orco, indicative of an endogenous multiscale membrane clockwork

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Olfactory receptor neurons (ORNs) in the antennae of the nocturnal hawkmoth Manduca sexta are peripheral circadian clocks that express clock genes in a transcriptional-translational feedback loop (TTFL) clockwork. However, it remains unclear how ORNs regulate the circadian mating rhythms. ORNs in the male's pheromone-sensitive long trichoid sensilla exhibit daily rhythms in pheromone sensitivity with maximal temporal resolution during the activity phase at night. This is optimally tuned to support the male's upwind mate search flight that tracks the female's pheromone pulses at frequencies below 10 Hz.

We hypothesize that the endogenous membrane potential oscillations in ORNs are generated via an adaptive posttranslational feedback loop (PTFL) plasma membrane clock that tunes ORN sensitivity and kinetics to resonate with the female pheromone pulses. As a mechanism for active sensing, such a PTFL membrane clock could temporally tune spike threshold, and quickly adjust to the insect's physiological needs. To challenge our hypothesis, we performed long-term in vivo tip-recordings of the spontaneous activity of pheromone-sensitive trichoid sensilla. Spontaneous spiking activity was modulated by both ultradian and circadian rhythms with maximum activity at night. Blocking the evolutionarily conserved olfactory receptor coreceptor (Orco) canceled the circadian but not the ultradian frequency components of the spontaneous activity. In addition, Orco was not under the control of the circadian TTFL. We could replicate our experimental data in a computational model to further explore the role of Orco on the circadian spiking rhythm as a function of 2nd messenger levels.

Currently, we are examining which 2nd messenger-dependent ion channels besides Orco are part of a PTFL clock in ORN membrane. These channels should control circadian changes in spike threshold as a mechanism of active sensing to tune into regular environmental signals via resonance.

Symposia

S 08-05

Towards a molecular understanding of moon-controlled timers: Linking the light valence detector L-Cry to the circalunar clock in the marine annelid Platynereis dumerilii

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Endogenous oscillators are intrinsic timing mechanisms essential for synchronizing metabolism, physiology and behavior to environmental cycles. While the circadian clock, entrained by the daily light-dark cycle, is well studied, molecular mechanisms underlying monthly (circalunar) rhythms remain largely elusive. Marine breeders, such as the bristle worm Platynereis dumerilii, are well-described to possess such a timing machinery. Set by moonlight, it allows the precise timing of reproduction, making it a powerful model to study the ecologically highly relevant impact of natural light conditions on marine species, particularly the phenomenon of monthly clocks.

The light-receptive cryptochrome L-Cry serves as the first functional link to the circalunar oscillator. Its role as a moonlight interpreter enables the distinction between sun- and moonlight under naturalistic conditions, positioning L-Cry in the entrainment pathway.

However, how L-Cry transmits this information to the monthly clock remains unknown.

G3BP1/2 emerged as an attractive candidate to study this link, based both on biochemical/ cellular validation of its interaction with L-Cry, as well as previous findings showing its circalunar regulation at the protein level. Further analysis revealed that G3BP1/2 protein levels are under control of l-cry in a moon-phase dependent manner. Evaluation of G3BP1/2 under lunar free-running conditions implicates it as a mediator between L-Cry and the circalunar oscillator, potentially serving as a gatekeeper for fine-tuning clock entrainment. Functional studies using a pharmacological inhibitor of G3BP1/2, further support its role in circalunar clock entrainment. Whereas control animals gradually shifted their spawning rhythm following an out-of-phase artificial moonlight stimulus, - treated animals responded almost immediately to the new entrainment regime. This faster adjustment closely resembles the re-entrainment behavior of l-cry mutants under artificial light, supporting a model in which G3BP1/2 mediates L-Cry-dependent signaling to the circalunar oscillator.

Symposia

S 08-06

Cryptochrome and magnetic fields in Drosophila

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One of the great mysteries in biology is how animals use the Earth's weak magnetic field to navigate great distances during annual migrations. The circadian clock molecule CRYPTOCHROME (CRY) has emerged as the leading candidate to mediate these remarkable phenotypes. CRYs come in two flavours, light-sensitive as in Drosophila dCRY1 or migratory robin ErCRY4, or non-light sensitive as in mammalian CRYs, the major negative regulators of the circadian clock. CRY is a blue-light flavoprotein which when exposed to light generates radical pairs via a quantum mechanism. These radical pairs oscillate between singlet and triplet states and are magnetically sensitive. A magnetic field can alter the singlet/triplet ratio with effects on CRY conformation, with, presumably, downstream effects.

The classic model developed though in vitro spectroscopy, has a radical pair generated by light via electron skipping between a terminal Trp residue (one of 3 or 4 Trp) and the bound FAD. However, circadian behavioural experiments with Drosophila have revealed that only the 52 residue C-Terminal of dCRY1, without either the Trp tetrad or the FAD binding pocket, is sufficient for generating a circadian magnetic response.[1,2] Drosophila larval neurons that ectopically express the dCRY C-terminal, also show changes in electrophysiological responses when exposed to magnetic fields.[2]
These remarkable results at both whole organism and single cell level suggest an alternative model for how CRY may mediate magnetic field phenotypes. We show how sequences within the CRY-C terminal encode protein binding sites that may target CRY to effector neurons where FAD can regulate ion channel activity and alter neuronal firing. Our results suggest that CRY may be the transducer/amplifier of the magnetic signal and that FAD is the real magnetosensor.

Symposia

S 09-01

Circadian anti-tumor immune responses

Christoph Scheiermann
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The immune system acts in a time-of-day-dependent manner. This talk will focus on circadian anti-tumor immune responses and how these underlying oscillations can be targeted by chrono-immunotherapy. It will further provide mechanistic insights into these therapies.

Symposia

S 09-02

Who, when, where: tissue-specificity in the plant circadian system

Maria A. Nohales
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Circadian clocks are proposed to have evolved as adaptive mechanisms that help organisms anticipate and align their internal biological processes with the external, predictable changes that occur in their surrounding environment over the daily 24-hour cycle. To achieve optimal organismal synchronization with the environment, the circadian network must integrate a wide range of external cues and coordinate a diverse array of endogenous rhythms accordingly. Although traditionally viewed as a cell-autonomous and uniform oscillator, recent research has revealed that the plant circadian network is spatially organized and that tissue-specific circadian oscillators may function to fine-tune plant development. These local clocks may perceive and integrate environmental inputs locally to regulate specific rhythms at the cell-type level, and would then be coordinated across the plant through both intercellular coupling and long-distance communication. However, the mechanisms that drive tissue-specific regulation in the plant circadian system remain poorly understood.

To inform research on such mechanisms, we have performed time-lapse single-nucleus RNA sequencing (snRNA-seq) on Arabidopsis seedlings grown under free-running conditions and have compared gene expression patterns across the different cell types. We have discovered that, while genes encoding core circadian clock regulators robustly oscillate in multiple cell types, around 3000 genes exhibit cell-type specific rhythmic expression patterns. This suggests a high degree of tissue-specific regulation within the plant circadian system. The functional relevance of such tissue-specific clock-output connections for the regulation of plant development in resonance with the environment is exemplified by our recent discovery of a molecular mechanism through which the circadian clock modulates the plant's response to changes in light quality specifically in the epidermis.

Symposia

S 09-03

Asthma… it's about time!

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2 Manchester University NHS Foundation Trust

Asthma is a common and highly rhythmic inflammatory disease of the airways. In this lecture I will present recent data from my research group, using a model of allergic airways disease, showing that the molecular clock in the Club cell of the airway epithelium underpins time of day immune cell trafficking, epithelial barrier permeability and changes in airway mechanics. I will discuss our recently published clinical trial data showing that time of day is highly relevant to the management of patients with asthma.

I will present data showing how time of day impacts the accurate diagnosis of asthma in the clinic and also the treatment of asthma with inhaled corticosteroids (chronotherapy). Finally, I will discuss the public health implications of epidemiological data showing that female night shift workers are particularly at increased risk of asthma.

Symposia

S 09-04

What drives daily rhythms in physiology?

Andrew Beale[1], Andrei Mihut[1], Jo Menzies[1], Tanya Wilson[1], Emily Watson[1], Nathan James[1], Aymen al-Rawi[1], Sew Yeu Peak-Chew[1], Alessandra Stangherlin[2], Aiwei Zeng[3], Rachel Edgar[3], Ed Hayter[4], David Bechtold[4], Antonio di Soccio[5], Claudia Lodovichi[5], Gimmi Ratto[5], John S. O'Neill[1]*

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Abstract: The genetic circuits that facilitate circadian transcriptional regulation have been successfully delineated over the last three decades, however, the mechanisms that actually drive the daily regulation of mammalian biology are quite poorly understood. This talk will consider how circadian timing and the daily regulation of mammalian cell physiology is achieved.

Symposia

S 10-1

Sleep & Rhythm in Neurodegeneration

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Neurodegeneration is one of the most severe burden of disease. However, patho-physiology and -genesis are far from being fully understood. Therapeutic approaches are more or less symptomatic only. Disease modifying treatments are absent.
The introduction of Sleep-Wake Disorders as a distinct chapter in ICD-11 may have refocussed attention. Over the last decade insights have emerged that revive long-known side aspects in neurodegeneration: the impact of sleep and the circadian timing system. The importance of the cholinergic system both for cognition and REM-sleep has been known for over six decades. REM-sleep is substantially modulated by the circadian system. Not surprising specific changes in REM-sleep architecture have recently been accepted as early marker of both: 1. tauopathy (Alzheimer`s) and 2. alpha-synucleinopathy (Parkinson`s and Dementia with Lewy bodies).
In my presentation I will review known disturbances of circadian rhythmicity and specific changes in sleep architecture being involved in the early pathogenesis of neurodegenerative disorders with special emphasis on Isolated REM-Sleep Behavior Disorder (iRBD).

iRBD is characterized by the loss of muscle atonia during REM-sleep leading to outacting of dreams during sleep. Today iRBD is recognised as the most prominent prodromal state to overt alpha-synucleinopathy. Even though a specific circadian rhythm disturbance in the pathophysiology of the disturbance has yet not been established, the effects of a chronobiotic use of melatonin in patients with iRBD suggests a disease modifying effect.

Symposia

S 10-2

Effects of photoperiod in development and adulthood on sleep

Tom Deboer
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Because the earth is tilted relative to its rotation plane around the sun, we experience seasonal changes in daylength in higher latitudes. These changes are affecting behavior and physiology in several different mammalian species, including humans. Recently we have worked on two lines of research investigating these effects in mice. One where we manipulated photoperiod at birth to investigate the effects of perinatal photoperiod (8 hours or 16 hours of light per 24 hours) on sleep in adulthood. The other where we manipulated photoperiod in adulthood and investigated the direct effect on sleep and the response to diazepam, a medicine belonging to the benzodiazepines, which induces sleepiness, increase sleep, and changes the sleep electroencephalogram.

We find that perinatal photoperiod influences sleep in adulthood. Particularly sleep in the light period (the main sleep period of mice) showed a 7.2% difference between animals born in the long and short photoperiod, which is a difference of 1-h over the entire 12 hour light period. Photoperiod in adulthood showed a large effect on the amount of sleep and waking in the dark period with high levels of waking in the long photoperiod, and a significant difference in the effect of diazepam where the animals in the short photoperiod showed a clear increase in REM sleep, which was lacking in the animals in the long photoperiod.
The results will be discussed in the context of previous EEG confirmed sleep data obtained in mammals under different photoperiods, and in the context to humans who sleep as if they are continuously in a long photoperiod.

Symposia

S 10-3

Sleep is for brain cleaning – mechanisms and function of the glymphatic system

Natalie L. Hauglund[1,2,3], Mie Andersen[1], Klaudia Tokarska[1], Tessa Radovanovic[1], Celia Kjaerby[1], Frederikke L. Sørensen[1], Zuzanna Bojarowska[1], Verena Untiet[1], Sheyla B. Ballestero[1], Mie G. Kolmos[1], Pia Weikop[1], Hajime Hirase[1], and Maiken Nedergaard[1,4,5,]*

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As the brain transitions from wakefulness to sleep, processing of external information diminishes while restorative processes, such as glymphatic removal of waste products, are activated. Yet, until recently it is not known what drives brain clearance during sleep. We employed an array of technologies and identified tightly synchronized oscillations in norepinephrine, cerebral blood volume, and cerebrospinal fluid (CSF) as the strongest predictors of glymphatic clearance during NREM sleep. Using optogenetic stimulation, we discovered that the locus coeruleus induced anti-correlated changes in vasomotion and CSF signal in the brain. Furthermore, stimulation of arterial oscillations enhanced CSF inflow, demonstrating that vasomotion acts as a pump driving CSF into the brain. On the contrary, the sleep aid zolpidem suppressed norepinephrine oscillations and glymphatic flow, highlighting the critical role of norepinephrine-driven vascular dynamics in brain clearance. Thus, the micro-architectural organization of NREM sleep, driven by norepinephrine fluctuations and vascular dynamics, is a key determinant for glymphatic clearance.

Symposia

S 10-4

Stimulating waves – Sleep and memory consolidation

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Abstract: For decades, the debate over whether brain rhythms are merely epiphenomena has persisted. A seemingly straightforward way to explore their causal role is not only to perturb them but also to simulate them. This review presents functional relationships between brain rhythms and sleep-associated memory consolidation, as revealed through neuromodulatory exogenous stimulation approaches.

Additionally, inter-individual differences play a crucial role in determining the effectiveness of these stimulation techniques. Investigating the relationship between cognitive ability and electrophysiological activity may help predict susceptibility to exogenous stimulation. Specifically, integrating non-invasive brain stimulation methods supports the idea that individual variations in cortical excitability partially account for the differential effects of closed-loop auditory stimulation on memory retention.

Symposia

S 10-5

Impacts of Night Work and Seasonal Variation on Wearable-Derived Sleep Architecture in Arctic Industrial Workers

Fred Haugen[*1], Fillip Sæther[1,2], Andreas N. Holme[1,3], Dagfinn Matre[1], Knut Inge Fostervold[2] & Line Victoria Moen[1]*

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Background: Arctic shift workers experience unique challenges to circadian regulation due to extreme seasonal variations in daylight and demanding work schedules. This study aimed to investigate the effects of shift type and seasonal photoperiod on wearable-derived sleep architecture in industrial workers located in the Arctic at 71 °N.

Methods: Proxy sleep architecture was assessed using the Oura Ring, a consumer wearable that combines accelerometry, photoplethysmography, and skin temperature to estimate sleep stages. While not equivalent to polysomnography, the Oura Ring provides categorization of sleep into deep, light, and REM stages. Data was collected from 110 rotating 3-shift workers during both light (midnight sun) and dark (polar night) seasons. Linear Mixed Models were used to analyze the influence of shift type (morning vs. night) and season (light vs. dark) on sleep metrics with initial analyses following the intention-to-treat principle and assuming that participants complied with the planned shift schedule.

Results: Shift type significantly affected wearable-derived sleep architecture. Sleep following night shifts was associated with increased deep sleep and reduced light sleep compared to morning shifts. The main effect of shift type on deep sleep was significant for both the proportion ($B = 2.65$, $p < .001$) and the absolute duration ($B = 10.76$, $p < .001$). No significant differences were found in the REM sleep metric. Seasonal photoperiod had no significant effect on sleep architecture. However, large inter-individual variability in sleep related responses was observed.

Conclusion: Initial findings indicate that shift type, rather than seasonal daylight variation, was the primary driver of changes in wearable-derived sleep architecture among Arctic industrial workers. Wearable sleep tracking may provide useful insights into real-world sleep dynamics in extreme working environments. Further per-protocol analyses using payroll data to account for deviations from planned shift schedule are planned.

Symposia

S 11-1

Is there an optimal time of the day to exercise?

Anna Krook[1], *Harriet Wallberg-Henriksson*[1], *Juleen R Zierath*[1]
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Lifestyle interventions such as exercise and dietary regulation are essential in managing and preventing metabolic diseases, including type 2 diabetes. However, the optimal timing of these interventions remains unclear. Emerging evidence suggests that aligning exercise and eating behaviours with the circadian system may enhance glycaemic control. Circadian rhythms play a critical role in glucose homeostasis, and time-of-day-specific responses to exercise have been observed.

Notably, we have recently reported that afternoon high-intensity interval training (HIIT) over two weeks improves blood glucose control and insulin sensitivity in men with type 2 diabetes, whereas morning sessions may offer limited benefits. To provide more mechanistic insight into this effect we have studied the effects of a single exercise bout, performed either in the morning or the afternoon in both men and women with or without type 2 diabetes. Current studies are also aimed at understanding how time of day impact not only on glycaemic management, but also on exercise performance in non-diabetic people

Symposia

S 11-2

Enhanced glucocorticoid rhythms drive the outcomes of caloric restriction

*Konstantinos Makris 1, Vlera Fonda 1,2, Fania Feby Ramadhani 1,2, Lina Fadel 1,2, Morgane Davezac 1,2, Teresa Horn 1, Fabiana Quagliarini 1, N. Henriette Uhlenhaut 1,2**

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Caloric restriction extends lifespan and healthspan across species, with feeding times synchronized to circadian rhythms further maximizing its benefits. However, the mechanisms linking diet, diurnal rhythms, and lifespan are not fully understood. In mice, the time point most strongly tied to dietary effects on lifespan coincides with the peak of glucocorticoid secretion (ZT12, lights-off). Caloric restriction raises circulating glucocorticoid hormone levels, but their functional relevance remains untested. Here we show that the glucocorticoid receptor (GR) is critical for the effects of caloric restriction.

Hepatocyte-specific GR mutant mice fail to respond to caloric restriction, indicating that increased glucocorticoid amplitudes support its benefits. Using multiomics techniques in murine liver, we find that nutrient deprivation elicits a nuclear switch of transcriptional activity downstream of JAK/STAT and insulin signaling, enabling the GR to activate a unique diet-specific gene expression program. The results being presented suggest that glucocorticoid rhythms are crucial for caloric restriction-induced metabolic reprogramming.

Symposia

S 11-3

Restoring 24-hour substrate rhythmicity to improve glycemic control by timing of lifestyle factors

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Nowadays, many people spend a large part of the day in suboptimal and artificial lightning conditions and are physically active and ingest food and drinks at times when the intrinsic circadian system may not be optimally prepared for these activities. Inappropriate timing of these environmental triggers (light exposure, feeding, activity) – or lack thereof – can lead to detrimental biological adaptations thereby worsening metabolic health, and eventually contribute to disease development.

We previously found that inducing circadian misalignment by a 12h rapid shift in healthy, lean men, reduced skeletal muscle insulin sensitivity. We also showed a complete lack of rhythmicity in whole-body energy substrate oxidation, muscle mitochondrial function, plasma FFA levels, and the expression of major molecular clock genes in skeletal muscle from humans predisposed to diabetes, as opposed to healthy, lean volunteers. Particularly, individuals at risk for developing type 2 diabetes displayed a higher nocturnal respiratory exchange ratio (RER) indicating that they do not switch to fat oxidation during the night, as compared to young, lean individuals. In subsequent intervention studies in people with or at risk for type 2 diabetes, we could show that changes in the timing of behavioral interventions, such as food intake, exercise (training) and natural light exposure, impact on the 24h rhythmicity of metabolism and/or glucose homeostasis. Optimizing the timing of lifestyle strategies to enhance alignment with the circadian system may reinforce these strategies in their ability to improve metabolic health and glucose homeostasis.

Symposia

S 11-4

Cellular Clocks on Steroids: How Glucocorticoids Signal Time of Day to Circadian Clocks

Anna Edmondson[1], Ivan Schlamovitz[1], Andrew Zeller[1] Andrei Mihut[1], Andrew Beale[1], Nathan James[1], John O'Neill[1]*

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Glucocorticoid (GC) steroid hormones are vital for signalling time of day throughout the body. Yet the mechanism by which GCs synchronize cellular clocks is not completely understood. A plausible model has been proposed, following the classical genomic signalling pathway of its cognate nuclear hormone receptor, the glucocorticoid receptor (GR). The GR activates transcription of the Period genes that encode PER proteins, leading to an increase in PER abundance, and changes in PER activity elicit circadian phase shifts. However, much of the evidence for this model is correlatory; uncertainty surrounds which PER paralogs are important; and non-genomic GR signalling pathways, recently demonstrated in a range of other biological contexts, cannot be excluded.

Using transient-transcriptome sequencing (TT-seq) to interrogate genes nascently transcribed or repressed by the GR, coupled with mRNA-seq and whole-cell (phospho)proteomics, we demonstrate that, contrary to the proposed model, Per1 is regulated by GCs post-transcriptionally. Moreover, chemi-genetic knockdown of endogenous PER2 reveals that it does not function as a primary GC effector. By generating a CRISPR GR KO rescue system, we find that interactions with the GR's AF-2 region are essential for clock synchronization, whilst cytoplasmic to nuclear translocation of the GR is dispensable. Refining the canonical model, we propose a two-tiered mechanism in which GR activation results in both a rapid translational and slower transcriptional induction of Per1. These mechanistic insights may open up new avenues for targeted chronotherapies in individuals with disrupted circadian rhythms.

Symposia

S 11-5

Night work affects diurnal rhythms of cortisol, melatonin, and testosterone in permanent night workers

Anne Helene Garde[1,2], Kirsten Nabe-Nielsen[1,2], Åse Marie Hansen[1,2], Anastasi Kosmadopoulos[3], Anders Aagaard[1], Heidi Lammers-van der Holst[4], Rasmus H. Reeh[1,5], Ann Dyreborg Larsen[1]*

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Night work induced circadian disruption is associated with increased the risk of adverse health effects. Yet, the underlying mechanisms remain poorly understood. It has been suggested that permanent night work experience better adaption and less circadian disruption of endogenous rhythms compared with rotating shift work. The aim of the present study is to investigate the acute effects of night work on diurnal rhythms of cortisol, melatonin and testosterone among permanent night workers.

Twenty-three men with permanent night work participated in the study. They collected saliva every 4 hours while awake over a 24-hour period on three separate days: the second and fourth consecutive night shifts, and a day off. For each 24-hour period, the mesor, amplitude and acrophase of cortisol, melatonin and testosterone were assessed. Regression analyses with repeated measures adjusted for age were used to evaluate differences in rhythms during 24-hour periods with night shifts compared to days off (ref).

For cortisol, mesor was higher during 24-hour periods with night shifts (+0.63 pmol/L, SD=0.31 pmol/L; p=0.048). For melatonin, mesor (+6.3 pmol/L, SD=2.8 pmol/L; p=0.040) and amplitude (+9.5 pmol/L, SD=4.0 pmol/L; p=0.030) were higher during 24-hour periods with night shifts. For testosterone, the acrophase was delayed in 24-hour periods with night shift (from 13:08 h to 10:22 h; p=0.049). The acrophases of cortisol (13:56 h) and melatonin (13:05 h) on days off did not differ from that on 24-hour periods with night shift.

Acute changes in levels of cortisol and melatonin on night shifts compared with days off - with lowest levels during the days off - were observed among permanent night workers. In addition, the timing of testosterone, but not cortisol and melatonin, changed between night shifts and days off, indicating that the circadian rhythms of the hormones become internally desynchronized in relation to night shifts even among permanent night workers.

Symposia

S 11-6

Diurnal Variation in Human Plasma Metabolites and Circadian Hormones Across Seasons

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Physiological and pathological states influence human 24-hour metabolite profiles. Seasonal variation in diseases with altered metabolism suggests circulating metabolites may exhibit seasonal patterns. This study aimed to investigate seasonal and diurnal variation in plasma metabolites and circadian hormones (melatonin and cortisol) under controlled laboratory conditions.

Eight healthy young adults (3 females; 26 ± 5.4 years; BMI 22.1 ± 1.9 kg/m^2; females on oral contraceptives) participated in two 72-hour in-laboratory sessions, one each in mid-summer and mid-winter. The protocol included 24 hours under dim light (<8 lux, semi-recumbent posture) followed by a seasonally-adjusted light/dark cycle for 48 hours, with standardised meals, and nocturnal sleep scheduled across three nights. Blood was sampled every 2 hours for targeted UPLC-MS/MS metabolomics and hourly for hormone quantification.

Melatonin and cortisol exhibited robust 24-hour rhythms across seasons. Overall, melatonin area under the curve (AUC) did not differ significantly between seasons; however, males demonstrated a ~1-hour advance in dim-light melatonin onset (DLMO) and melatonin acrophase during summer. Targeted metabolomic analysis identified most metabolites as rhythmic in at least one of the 4 groups (males-summer, males-winter, females-summer, females-winter) (93%, (127/137)), with 55% (71/137) having consistent 24-hour rhythms across all 4 groups. Summer-winter concentration differences were minimal; t4-hydroxyproline and sphingolipids (SM C24:1, SM C26:0) were elevated in winter. Principal component analysis indicated stronger time-of-day effects compared to seasonal influences.

Plasma metabolite rhythms remained largely resistant to seasonal photoperiod changes, though some modest phase shifts occurred (<4 h in most cases). In both sexes, most metabolites (87%, (76/87)) were phase advanced in summer, paralleling the earlier DLMO. Serotonin showed the largest phase advance (~7 hours) in summer, potentially relevant to seasonal affective disorder (SAD).

These findings highlight the importance of considering diurnal and seasonal timing in metabolic studies and support the need for larger, longitudinal investigations.

Symposia

S 12-1

Emerging links between the sleep and circadian system and cardiometabolic disease

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In the UK more than 4.3 million people live with Type-2 Diabetes (T2D), and 2.4 million more are at increased risk of developing the disease. T2D, obesity, hypertension, and fatty liver disease frequently cluster together in the same individuals, termed cardiometabolic multimorbidity. The risk factors for cardiometabolic disease (CMD) are multiple but sleep and circadian rhythm disruption (SCRD) is emerging as an important driver of disease incidence and severity. But importantly, SCRD is also promoted by CMD; thereby creating a vicious cycle perpetuating poor health and wellbeing.
Here, we employ a genetic co-localisation approach in the UK Biobank to find loci that associate with both a SCRD, and a CMD phenotype, or trait. This approach was designed to identify the shared genetic architecture between the two interrelated systems: CMD, and SCRD. This identified a number of loci with varying confidence intervals and estimated statistical significance. One locus attracted attention due to its high statistical significance and tightly defined genetic interval around a single genetic variant (rs12140153).

The putative causal SNP (rs12140153) is a missense variant within PATJ, a gene encoding a membrane-bound protein associated with tight junctions. PATJ has been identified in previous GWAS studies of body mass index (BMI) and with C-reactive protein (CRP; a marker of systemic inflammation). PATJ is widely expressed in human tissues (with protein levels highest in brain (cerebellum), GI tract, muscle and immune related tissues). We further analysed a different, cardiometabolic-focussed biobank in Oxford, UK and identified individuals with rare, deleterious coding region variants.These individuals had significant changes in serum lipid concentrations, especially affecting sphingolipids.Sphingolipids are implicated in cardiometabolic disease, and cellular sphingolipid metabolism is associated with changes in circadian function.
In summary, large human cohort analysis has the potential to identify new genetic drivers of SCRD and CMD.

Symposia

S 12-2

The Potential of Circadian Medicine to Prevent Delirium in the ICU and Post-Intensive Care Syndrome

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Delirium is a severe acute complication in critically ill patients. It is characterized by fluctuating symptoms that follow circadian patterns. Delirium is associated with prolonged hospital stays, higher mortality, and long-term impairments after intensive care unit (ICU) stay. Especially the cognitive component of delirium plays a central role in Post-Intensive Care Syndrome (PICS). PICS is a major burden for ICU survivors, family members, and healthcare systems. [1–3]
Circadian rhythm and sleep disruption in the artificial ICU environment could be contributing factors to delirium development. ICU patients are typically exposed to constant light, noise, and sedation, with little exposure to natural daylight rhythms. We have established dynamic lighting screens in our ICU [4]. The modified ICU rooms were designed to create an environment that supports the natural circadian rhythms of ICU patients through a multi-component approach , potentially reducing stress [5–7]. The modified room concept includes visual content elements to stimulate cognition, reduce anxiety, and adjust the intensity and color temperature throughout the day [5–7]. In a first pilot trial, we observed, that treatment in modified ICU rooms is associated with reduced delirium development [4]. However, the impact of circadian interventions on acute and chronic ICU outcomes is largely unexplored.
In this talk, we provide an overview of delirium and PICS in the context of circadian disruption. We discuss how dysregulated circadian physiology can be detected in the ICU and highlight circadian interventions, such as dynamic lighting systems, that have the potential to improve acute and long-term outcomes.

Symposia

S 12-3

Minding the Implementation Gap in Circadian Medicine: Lessons from ADHD.

Andrew N. Coogan

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The application of chronobiological principles in medicine and public health holds much promise to improve patient and population outcomes through the development of new pharmaceutical and behavioural approaches and refining the utility of existing therapies. However, in many regards, translation of circadian science into routine clinical practice has been frustratingly slow. In this presentation I will explore some reasons for this "implementation gap" in circadian medicine, with a particular emphasis on behavioural paradigms.

To illustrate these challenges I will use the example of circadian rhythm research in adult attention deficit hyperactivity disorder (ADHD), a topic that I have worked on for twenty years. I will discuss a number of specific barriers to translation: 1) lack of fundamental aetiological understanding of diagnosis-specific circadian changes; 2) lack of knowledge about the levels of inter-individual variance in circadian rhythm manifestation within diagnostic groups; 3) the general transdiagnostic implementation gap that limits the translation and consolidation of psychological and behavioural interventions; and 4) the education gap for practitioners regarding the consideration of chronobiological processes as foundational for health. I will conclude by making recommendations for overcoming these barriers to better leverage chronobiology for human health.

Symposia

S 12-4

Impact of early vs. late time-restricted eating on glucose and lipid profiles and internal circadian time in overweight or obese women

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Background: Time-restricted eating (TRE) is a promising strategy to improve metabolic outcomes but there is a lack of carefully controlled trials.

Aims: We investigated whether timing of the eating window during the isocaloric TRE affects diurnal rhythms of circadian clock and improves glycemic control and lipid profile.
Methods: A randomized cross-over trial was completed by 31 overweight or obese non-diabetic women (age 58.4y; BMI 30.5kg/m^2). They followed a two-week early TRE (eTRE, eating 8a.m.-4p.m.) and two-week late TRE (lTRE, eating 1p.m.-9p.m.) being asked to consume their usual food quality and quantity. Glycemic control was assessed by continuous glucose monitoring (CGM). Lipid profiling was conducted by high throughput shotgun plasma lipidomics. Internal circadian time was assessed by the BodyTime assay in blood monocytes.

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Results: Participants showed a high timely adherence, unchanged dietary composition, minor daily calorie deficit (eTRE:-167kcal) and weight reduction (eTRE:-1.1kg, lTRE:-0.4kg). Circadian phase in blood monocytes was advanced by 40 min after the lTRE compared to the eTRE. The 24-hour glucose profiles were also shifted accordingly to the eating timing. The mean 24h glucose levels, mean amplitude of glycemic excursion (MAGE), and glucotypes were unchanged by both TRE interventions. Mean of daily difference (MODD) index slightly decreased during both eTRE (p=0.037) and lTRE (p=0.003).

The eTRE intervention affected 103 lipid species, reducing ceramide (P = 0.043) and phosphatidylcholine (P = 0.043) classes, and altered the activity indices of desaturases D5D, D6D, and D9D, as well as elongase ELOVL6. The lTRE altered D5D index but caused no substantial changes in lipid species and classes.
Conclusions: A nearly isocaloric TRE induced changes of circadian systems but does not clinically relevantly alter glycemic control in overweight or obese women. However, eTRE has more pronounced effects on the plasma lipid profile compared to lTRE, suggesting that TRE effects depend on the eating timing.

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S 12-5

Clocking the clot: A novel use for a circadian clock compound to restore clot breakdown pathways inhibited by inflammation

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Stroke, the second leading cause of death globally, which normally has a rate of 60 events/hour, surges to 190 events/hour between 8:00 and 9:00am. Stroke is characterised by aberrant blood clotting, followed by recruitment and activation of immune cells, particularly macrophages. Plasma concentration of a key clot breakdown inhibitor plasminogen activator inhibitor-1 (PAI-1) also peaks during this morning increased stroke risk. Given such timed features of stroke incidence and the role of macrophages, the aim of this project was to target specifically the circadian clock in macrophages with a clock modulatory compound SR9009 in effort to limit unwanted clotting events.

Mouse peritoneal and bone marrow-derived macrophages were treated with SR9009 and bacterial component lipopolysaccharide (LPS) to mimic an immunothrombotic event. Following treatment, RNAseq, qPCR and ELISA were performed. Clot breakdown potential was assessed by cell-based clot lysis and plasmin generation assays.

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We show for the first time that untreated macrophage secretion of clot breakdown inhibitors PAI-1 and PAI-2 displayed circadian rhythmicity ex vivo. Consequently, macrophages isolated during the active phase generated half the amount of plasmin than macrophages isolated during the resting phase, leading to inhibited clot breakdown. SR9009 treatment suppressed macrophage expression of genes involved in leukocyte migration, inflammation and fibrinolysis, with the most significantly suppressed gene being Serpinb2 (PAI-2). SR9009 halved PAI-1 and PAI-2 production compared to control, and in functional assays SR9009 treatment doubled plasmin generation and restored clot breakdown to levels comparable to untreated cells.

These data show that the clock modulatory compound SR9009 selectively restores clot breakdown via inhibition of inflammation-induced anti-fibrinolytic protein generation. We have established the contribution of the circadian phase of macrophages to clotting dynamics and demonstrated that SR9009 may have a potential application in restoring normal clot lysis in individuals with dysregulated fibrinolytic activity in the morning hours.

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S 12-6

Targeting BMAL1 to develop various circadian medicines

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Circadian clock at molecular level function through the complex transcription-translation feedback loops (TTFLs) with BMAL1 works as the core component to ensure 24-hour periodicity. BMAL1 has been proven to be the only clock gene in mice whose sole absence would abolish circadian rhythms. Targeting circadian clock offers therapeutic opportunities to treat diverse clock-related disorders, including cancer, autoimmune disease, sleep disorder and metabolic conditions. We recently reported a breakthrough on drug development of BMAL1, providing an inverse agonist of BMAL1 that enable dose-dependent modulation of clock-controlled genes and down-regulation of inflammatory pathways in macrophages.

Here, We compare the newly developed BMAL1 compound CCM (Core Circadian Modulator) with other clock modulators to elucidate the merit of CCM as a tool in cell-based circadian studies, and focus on the insights we obtained during drug development to emphasize the therapeutic potential of BMAL1 compounds to treat various clock-related disorders.

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The circadian clock in the deep sea

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The deep-sea mussel Bathymodiolus azoricus lives at hydrothermal vents along the Mid-Atlantic Ridge, at depths ranging from 840 to 3350 meters. In this environment, sunlight is absent, and pressures range from 84 to 335 bar (sea level: 1 bar). These vents feature extreme conditions when viewed from a human standpoint, with temperatures varying from 4°C in ambient seawater to >350°C in black smoker fluids. Mussels prefer cooler vent waters (4–9°C) and experience alternating exposure to anoxic, acidic, metal-rich vent fluids (e.g., O_2 = 0 mM, pH 3–3.4, H_2S = 5-10 mM) and oxygenated seawater (O_2 ~ 0.2 mM, pH ~ 7.8, H_2S = 0 mM) due to tidal cycles (12.4h) that create a chemically and physically dynamic habitat. Our previous work showed that B. azoricus exhibits biological rhythms, including rhythmic valve movements and gene expression patterns at 1700 m depth. These rhythms are primarily driven by tidal cycles, with a detectable ~24-hour rhythm. Interestingly, in laboratory conditions with a 12:12 light-dark cycle, the dominance of these rhythms reverses.

To investigate whether B. azoricus possesses endogenous clocks and functional circadian clock genes, we developed molecular tools and maintained primary cell cultures from foot tissue for over two years. Temporal experiments on these cultures revealed circadian rhythms under constant conditions. Additionally, an endogenous B. azoricus enhancer was identified, and its functionality was confirmed in S2 cell assays with the mussel's core circadian clock genes. While several of these genes function similarly to their terrestrial counterparts, this research shows that even at the edge of life, deep-sea animals utilize circadian mechanisms to orchestrate their physiology.

Symposia

S 13-2

Effects of environmental factors on rhythms in mosquito-host interactions

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Mosquitoes transmit numerous human pathogens, such as the dengue virus and Plasmodium falciparum, and are responsible for nearly one million deaths yearly. In addition to rising insecticide resistance, it has become increasingly evident that current strategies to control mosquito populations are being confounded by the resilience conferred by mosquitoes' high levels of behavioral and physiological plasticity. For example, some nocturnal mosquitoes have shifted their biting activity in response to the use of insecticide-treated bednets and are now active early in the morning. Thus, biological rhythms are crucial to disease transmission as they allow mosquitoes to be active and responsive to host cues at times of the day when hosts are available. However, despite clear epidemiological relevance, we know very little about the mechanisms underlying the interaction between the chronobiology and the olfactory behavior of mosquitoes.

Combining analytical chemistry, quantitative analysis of behavior, electrophysiology, and transcriptomic analyses, we found that daily rhythms in genes coding for sensory proteins underlie the host-seeking patterns of the yellow fever mosquito Aedes aegypti. Additionally, we observed that mosquito activity patterns are plastic and can be influenced by the light and temperature conditions experienced during larval development. Results will be discussed in the context of the evolution of host preference and the potential for vector control strategies.

Symposia

S 13-3

Sleep Cycle Development: Ultradian Activity Rhythms During Sleep in the First Year of Life

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During the first year of life, human sleep undergoes remarkable alterations; sleep bouts consolidate, and nighttime sleep duration increases. The ratio of time spent in the two fundamental sleep states, REM and non-REM sleep, evolves, as well as their alternation in so-called NREM-REM cycles. In infants, these cycles are 50-60 min long, while by school-age, they already reach an adult-like period length of ~90 min, calling for studies of the functional aspects of ultradian sleep cycle dynamics.

In adults, we have previously shown that ultradian sleep cycle dynamics are revealed by non-linear conversion of locomotor activity into Locomotor Inactivity During Sleep (LIDS). However, it remains unknown if such methodology would reveal sleep cycle dynamics already in infancy.

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Through the lens of LIDS, we examined the ultradian dynamics in longitudinal actigraphy recordings of 152 healthy full-term infants across 10 nights at 3, 6 and 12 months after birth, as well as concomitant recordings of their parents. As expected for sleep cycles, our study shows robust rhythmic activity patterns during infant sleep with a predominant period of 50-60 min, while parents also display rhythmic activity, but with significantly longer ultradian periods (80-90 min). Consistent with the evolution of sleep cycles already at an early age, we also find that LIDS rhythms lengthen between 3 and 12 months of life. Interestingly, we also observe differences related to breast-feeding habits and sleep environment.

This study shows that locomotor activity can be used to measure ultradian sleep cycle dynamics in infants; from its development to its biological and contextual determinant. This is particularly relevant since studies suggest an active involvement of sleep in brain maturation. Overall, by opening opportunities for longitudinal studies in real life, LIDS analyses will contribute to improving our understanding of the significance of ultradian sleep cycle dynamics for health and development.

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S 13-4

Light pollution alters immune rhythmicity and function in nocturnal and diurnal wild rodents

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Artificial Light at Night (ALAN) is an increasing environmental stressor that disrupts natural light-dark cycles and has the potential to affect immune function, yet its impact on adaptive immunity and immune rhythms remains poorly understood, particularly in diurnal species. Here we investigated how ALAN influences rhythms in the immune system,secondary antibody responses, and survival in two wild rodent species with contrasting activity patterns: the nocturnal Acomys dimidiatus and the diurnal Acomys russatus. Immunization assays revealed that under natural conditions, the secondary response was time-dependent, with increased titers when immunization occurred during the rest phase. However, this temporal variation was absent under ALAN exposure, suggesting a disruption of adaptive immune dynamics.

Additionally, daily lymphocyte rhythms in peripheral blood followed a clear daily pattern in control animals, but these oscillations were altered by ALAN exposure in the nocturnal species and abolished in the diurnal species. Finally, Kaplan-Meier survival analysis indicated increased mortality in ALAN-exposed animals, though the extent to which immune disruption contributes to this outcome remains unclear. These findings suggest that ALAN systematically alters immune regulation, potentially affecting host defense and physiological stability in both diurnal and nocturnal species.

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S 13-5

Weak coupling between energetic status and timing of seasonal reproduction in an Arctic ungulate.

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In mammals, seasonal breeding is a widely observed trait, reflecting the annual environmental cycle of bioenergetic favourability. Since the length and severity of the winter season varies between years, it is of interest to explore how photoperiodic and metabolic information interact to yield plastic seasonal reproductive timing. Here, based on historical data, we demonstrate that neither body mass nor adiposity are strong proximate predictors of date of conception in wild reindeer (Rangifer tarandus).

Weak coupling between energetic status and the photoperiodic synchronization of reproduction accounts for the increasing discrepancy between the phenology of forage (energy supply) and the phenology of reproduction (energy demand) observed across the last 4 decades in two populations of Rangifer. The ultimate and mechanistic causes of the comparative lack of phenological plasticity in cervids as opposed to boreal rodent species are discussed.

Symposia

S 13-6

Entrainment in natural conditions

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The art of keeping in time with the environment for any organism involves careful choreography of cellular processes, and small adjustments of movement and their timing relative to each other, at different scales from the co-localisation of proteins inside a cell,to keeping the whole organism rotating following the direction of the sun. The circadian clock is a central choreographer for filtering in and out the environmental signals that are necessary or superfluous. In sampling the whole transcriptome of the plant Arabidopsis halleri in its natural habitat, we discovered rhythms in known circadian clock associated transcripts that are entrained to light conditions (irradiance) and others that are entrained to temperature conditions. We created local temporal phase response curves that reveal the nature of entrainment to temperature can be either parametric or non-parametric.

We further studied the competing zeitgebers of light and temperature for each transcript, and found that sometimes temperature can transiently accelerate the clock and sometimes decelerate it, independent of irradiance, and sometimes irradiance is the prevailing zeitgeber independent of temperature. Thus, the clock under field conditions is influenced by small adjustments in tempo and movement, to keep the organism dancing in time. We are exploring this further with mathematical models that explain in more detail how the plant keeps in time with the environment.

Symposia

S 14-1

Mouse Circadian Proteome Atlas and Post-translational Regulations in the Circadian Clock

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The circadian clock drives daily rhythms of gene expression and physiologies. Advances in next-generation DNA sequencing have provided extensive insights into RNA expression, but more functional information at the protein level with sufficient depth has been limited by technical challenges. In this study, we generated a comprehensive mouse circadian proteome atlas by analyzing 32 tissues including the suprachiasmatic nucleus (SCN) using the next-generation mass spectrometer Orbitrap Astral. Data-independent acquisition of 500 samples including developmental samples revealed the spatiotemporal profiles of 18,751 proteins, accounting for 73% of all proteins registered in UniProt. Proteome and phospho-proteome analyses of whole-cell and nuclear proteins in the liver revealed circadian changes in protein quantity and quality and also global changes in hPER2-S662G mutant mice, a genetic model of human familial advanced sleep phase (FASP). This multi-tissue circadian proteome atlas provides a fundamental resource for understanding when, where, and which proteins are expressed and function.

This atlas not only provides a valuable resource for studying circadian regulation at the protein level but also contributes to understanding the roles of posttranslational modifications and protein dynamics beyond transcriptional regulation. Circadian rhythms 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 test tubes, i.e., in vitro. KaiC protein is not conserved in eukaryotes, but some circadian events maintain their rhythmicity even in the absence of transcriptional rhythms. These facts raise the possibility that protein dynamics, such as posttranslational regulation, protein complex formation and conformation changes, can drive circadian oscillations also in eukaryotes. In the last part of this talk, I will introduce our recent findings showing the presence of a circadian protein oscillator without transcriptional rhythms in eukaryotes.

Symposia

S 14-2

Towards Human Systems Biology of Sleep/Wake Cycles: The Role of Calcium and Phosphorylation in Sleep

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Around 2012, we reached to a new hypothesis: sleep homeostasis may not rely on "sleep substances" but could be better explained by mechanisms that track "wakefulness substances," such as calcium if there is its integration/memory mechanism. This led us to focus on calcium in regulating sleep. Building on Dr. Setsuro Ebashi's discovery of calcium as a signalling molecule, we hypothesized that calcium might both stimulate neurons and promote sleep. To test this, we conceived next-generation mammalian genetics concept and developed the "Triple-CRISPR method" in 2016, achieving knockout mice with over 95% efficiency. Using this method, we created 25 knockout mouse lines targeting calcium channels and pumps, showing that calcium promotes sleep by acting as a brake on neuronal excitability.
We also developed CUBIC, a tissue-clearing technique, which demonstrated that calcium suppresses neuronal excitability. Further research identified calcium-dependent kinases, such as CaMKIIα/β, which integrate calcium history to promote sleep. We also found three critical phosphorylation sites in CaMKII responsible for sleep induction, maintenance, and cancellation. Additionally, we identified PKA as a sleep-suppressing kinase, and PP1 and Calcineurin as sleep-promoting phosphatases.
Our recent studies have highlighted ion channel mechanisms contributing to slow-wave oscillations (SWA), a key indicator of sleep need. We systematically examined sleep phenotypes in knockout mice lacking specific voltage-dependent potassium channels and identified that slowly-activating potassium channels Kcnq1/Kcne1 modulate the frequency of SWA, while the M-current channel Kcnq2 regulates SWA amplitude. Furthermore, knockout studies of M-current upstream kinase PKC isoforms suggest that a PKC member plays a role in regulating SWA amplitude. Additionally, hyperpolarization-activated cyclic nucleotide-gated (HCN) channels were found to impact SWA characteristics and mediate the effects of the sedative dexmedetomidine through a noradrenaline-dependent pathway. These findings underscore the importance of potassium and HCN channels their upstream Gq/Gi pathways in sleep-homeostasis- and drug-dependent SWA regulations.

Symposia

S 14-3

Beyond Timekeeping: Roles of Post-Translational Modifications of Clock Proteins

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The circadian clock is an intrinsic timekeeping system that drives daily rhythms in physiology and behavior. At the molecular level, it is governed by transcriptional-translational feedback loops involving core clock proteins, such as CLOCK, BMAL1, PER, and CRY. Post-translational modifications (PTMs), including phosphorylation and ubiquitination, play critical roles in regulating the stability, activity, and localization of these proteins, thereby fine-tuning the period, amplitude, and robustness of circadian rhythms. Several mutations affecting the PTMs of clock proteins were previously identified in pedigrees exhibiting familial advanced sleep phase, an extreme morning preference trait (Toh et al., Science, 2001; Xu et al., Nature, 2005; Hirano et al., eLife, 2016), indicating their crucial roles in the regulation of human sleep-wake behaviors.

Beyond maintaining clock function, emerging evidence suggests that PTMs of clock proteins influence broader cellular processes, linking circadian regulation to metabolism, neural activity, and disease states. FBXL3 is a crucial component of the ubiquitin-proteasome system that regulates the stability of CRY proteins, key elements of the circadian clock (Siepka et al., Cell, 2007; Godinho et al., Science, 2007). In this study, we investigated the neurological consequences of FBXL3 deficiency using Fbxl3 knockout mice. Our findings reveal that the loss of FBXL3 leads to an epileptic phenotype characterized by abnormal EEG patterns, particularly those associated with absence seizures. These results suggest that PTMs of clock proteins have diversified roles in neural excitability and brain function through clock-dependent or independent mechanisms.

Symposia

S 14-4

Digital twins for circadian-sleep health?

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Bright light soon after waking or blue-blocking glasses in the evening are standard therapies for sleep timing problems. These approaches are grounded in well-known circadian principles, encapsulated in phase response curves, which have established that light after the circadian minimum has the effect of speeding up the biological clock, and light before the circadian minimum has the effect of slowing down the biological clock. Challenges for application of these circadian principles are that the time of the circadian minimum is not usually known, there are substantial differences between individuals in their phase-shifting response to light and poor adherence. Hierarchical coupled oscillator mathematical models have been developed that can take as input the full 24-h light profile and predict the impact on circadian and sleep timing at an individual level. We have shown we can construct personalized mathematical models which take individual light data and successfully fit to individual sleep timing and duration data from healthy unemployed controls, people living with schizophrenia and healthy older adults.

We have used these models to highlight possible environmental (light) versus physiological factors underpinning sleep timing phenotypes. This quantitative approach highlights that sleep timing problems may be a consequence of aberrant self-selected light exposure patterns but also that individual differences in physiology matter. We will present a digital twinning approach in which light and sleep timing data are combined with mathematical models in close to real time, personalized to take account of individual differences in physiology. Using the predictive capabilities of the mathematical modelling framework, this approach could provide quantitative advice in the form of behavioural nudges on how much, when and for how long to seek light to better align sleep and circadian rhythms to lifestyles.

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S 14-5

Perinatal photoperiod has lasting effects on the sleep-wake cycle and the circadian system

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Seasonal changes in daylength affect circadian rhythm of behaviour, sleep pattern and SCN neuronal signalling. Exposure to different photoperiods during development was shown to determine physiological and behavioural traits in adulthood, but little is known about the effect on the circadian system. We therefore tested the long-term influence of perinatal photoperiod on sleep, locomotor activity and SCN signalling.
We exposed male and female mice to a short (8h light:16h dark; LD 8:16), intermediate (LD 12:12), or long photoperiod (LD 16:8) during the perinatal period (approximately E1 to P28). Subsequently, all animals were transferred to LD 12:12, and remained there for at least 3 weeks. We recorded wheel running behaviour (n=26-40 per photoperiod/sex group), EEG (n=5-8) to determine changes in the pattern of activity and sleep-wake cycle. We also quantified the neuronal signalling response of SCN cells to GABA application using calcium imaging in hypothalamic brain slices (n=13-17).

Long photoperiod-developed animals had a shorter behavioural activity duration under LD 12:12 compared to short photoperiod-developed animals ($p<0.001$). Additionally, the long photoperiod animals showed more sleep and less waking in the light (inactive) phase than the short photoperiod group (waking: $p=0.023$; NREM sleep: $p=0.032$). Lastly, the effect on GABA polarity of SCN neurons was strikingly different from results of earlier studies in adult mice exposed acutely to different photoperiods and animals from a long photoperiod had relatively fewer SCN cells responding excitatory to GABA ($p=0.014$).
We conclude that perinatal photoperiod has a long-term influence on sleep and locomotor activity. While exposure to different photoperiods during adulthood has been observed to lead to a short-term alteration, lasting no more than several days after transition to LD 12:12 or DD, the changes in physiology and behaviour after perinatal photoperiod exposure that we observed, were present a much longer time after exposure.

Symposia

S 14-6

Influence of Circadian Phenotypes on Excessive Sleepiness, Sleep Disturbance and Performance in Patients with Shift-Work Disorder

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Introduction: Vulnerability to nighttime sleepiness and disturbed daytime sleep are considered primary pathophysiological mechanisms underlying shift work disorder (SWD). Here we evaluated how different circadian phenotypes influence clinical symptoms and performance. Methods: After three consecutive night shifts, 204 patients with SWD were assessed for circadian phase of dim light melatonin onset (DLMO) or offset (DLMOff) from hourly saliva samples (2030-0830h). Patients were categorized as advanced (~10%), normally entrained (~48%), delayed (~15%), or low nocturnal melatonin (~18%) circadian phenotypes as compared to published data from day workers. Nocturnal melatonin levels were not determined for ~10% of patients. Multiple sleep latency test (MSLT) and Karolinska Sleepiness Scale (KSS) sleepiness, as well as Psychomotor Vigilance Test (PVT) performance (2330-0800h), and daytime polysomnography (1000-1800h) were assessed.

Results: Patients with SWD showed excessive sleepiness (MSLT=2.0±0.1 min) and disturbed sleep (total sleep time=355.2±4.1 min; sleep efficiency=74.0±0.8%). Advanced circadian phenotypes showed shorter MSLT latencies early during the nightshift compared to other circadian phenotypes (all $p<0.05$), but showed less KSS sleepiness compared to other circadian phenotypes (all $p<0.05$), and fewer PVT lapses compared to normally entrained and low nocturnal melatonin circadian phenotypes ($p<0.05$) both near the end of the nightshift.

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Furthermore, advanced circadian phenotypes showed shorter daytime sleep duration and lower sleep efficiency compared to delayed circadian phenotypes ($p<0.05$). Delayed circadian phenotypes showed longer MSLT latencies and lower KSS in the first half of the nightshift compared to normally entrained phenotypes ($p<0.05$).

Conclusion: Advanced circadian phenotypes start nightshifts well into their biological night and end nightshifts and sleep during their biological daytime, whereas delayed circadian phenotypes start nightshifts during the biological daytime and end nightshifts and sleep during the biological nighttime. Although all patients with SWD show clinically meaningful nighttime excessive sleepiness and daytime sleep disturbance, circadian phenotype impacts clinical outcomes as would be expected by circadian theory.

Symposia

S 15-1

Cortical Interneurons Convey Infraslow Oscillations During Sleep

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Sleep is characterized by a specific regulation of cortical circuits that enables plasticity underlying memory consolidation. Vasoactive Intestinal Polypeptide (VIP) interneurons exert a superior control over cortical circuit activity by inhibiting both parvalbumin and somatostatin-positive interneurons, thereby eventually disinhibiting pyramidal cells. We used in-vivo two-photon Ca^{2+} imaging to quantify activity of cortical layer 2/3 VIP interneurons in naturally sleeping male mice during wake, slow wave sleep (SWS), and rapid eye-movement (REM) sleep, as well as during spindle and slow oscillation (SO) events. VIP cell activity was lower during SWS than REM sleep epochs, and acutely upregulated during both spindles and SO events, presumably to facilitate memory processing in cortical circuits. Notably, in all sleep and wake stages, VIP activity showed a profound ~0.02 Hz infraslow rhythm, which during SWS epochs was closely but inversely phase-coupled to the well-known infraslow rhythm of spindle activity and possibly also originates from noradrenergic brainstem networks.

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S 15-2

Sleep deprivation enhances decision making in larval zebrafish

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Sleep deprivation is known to drastically affect cognitive function including decision-making and attention across many different species. In this study, we leveraged the small size and conserved brain structure of larval zebrafish to investigate the consequences of sleep disruption in the context of two well-described behaviors, a visual and an olfactory-based decision-making task. We find that in both paradigms, sleep disruption leads to an improvement in performance. Specifically, we show that sleep disruption increases reaction time and improves performance in a visual motion discrimination task, an effect that we attribute to longer integration periods in disturbed animals. With the use of a drift diffusion model, we predict specific circuit changes underlying these effects. In olfactory decision making we find that sleep disruption leads to increased odor sensitivity, which we show is likely mediated by cortisol. Our findings set the groundwork for further investigation of the underlying circuit changes in the brain that occur as a result of sleep disturbance across different species.

Symposia

S 15-3

Network synchrony creates neural filters promoting quiescence in Drosophila

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Animals require undisturbed periods of rest in which they undergo recuperative processes. However, it is currently unclear how brain states arise that are able to dissociate an animal from its external world, allowing for quiescent behaviors, while retaining vigilance to salient sensory cues. Here, we describe a neural mechanism in Drosophila that creates neural filters that engender a brain state allowing for quiescent behavior by generating coherent slow-wave activity (SWA) between sleep-need- (R5) and locomotion-promoting neural networks.

The coherence of SWA is subject to circadian and homeostatic control and can be modulated by sensory experience. Optogenetic mimicry of coherent SWA reveals that temporally fine-tuned R5 oscillations reduce responsiveness to visual stimuli by rhythmically associating neural activity of locomotion-promoting cells, effectively overruling their output. These networks can regulate behavioral responsiveness by providing antagonistic inputs to downstream head direction cells. Thus, coherent oscillations provide the mechanistic basis for a neural filter by temporally associating opposing signals resulting in reduced functional connectivity between locomotion-gating and navigational networks. We propose that the temporal pattern of SWA provides the structure to create a 'breakable' filter, permitting the animal to enter a quiescent state, while providing the architecture for strong or salient stimuli to 'break' the neural interaction, consequently allowing the animal to react.

Symposia

S 15-4

Sleep: A Worm's Eye View

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One of the simplest, yet molecularly accessible, sleep-capable model organisms is C. elegans. Our lab uses a combination of genetics, functional imaging, optogenetics, and physiological analysis to explore sleep in C. elegans. We have shown that C. elegans relies on a single sleep-active neuron called RIS to induce sleep. RIS is active during sleep bouts; its ablation abolishes sleep, and its optogenetic activation induces sleep. Thus, RIS is highly similar to sleep-active neurons found in mammals.
RIS is regulated by upstream circuits that translate wakefulness into sleep pressure. Cellular stress and antimicrobial peptides—produced during a wounding response—activate RIS via EGFR signaling and through the stress-sensing ALA neuron. This increases sleep following stress, injury, or infection. Sleep deprivation reduces survival during starvation and after wounding, and increases aging phenotypes.

C. elegans enables genetic screening for sleep-related genes and led to the discovery of aptf-1, an AP2 transcription factor crucial for RIS function. Translational studies in mouse models showed that AP2 transcription factors are also essential for sleep in mammals, highlighting a conserved role across species. The genes and mechanisms controlling sleep in C. elegans therefore likely reflect conserved principles of sleep regulation.

Symposia

S 15-5

Genetic basis of the natural polymorphisms of sleep-wake rhythms in Drosophila

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Life on Earth is shaped by regular environmental changes like the day-night cycle, seasons, and the lunar cycle. Biological clocks allow adaptation to these changes. Genetic polymorphisms play a key role in shaping development, physiology, and behavior. My thesis aims to identify natural genetic variants that influence circadian behavior and to study known variants to uncover the molecular basis of behavioral variability, starting from screens of the Drosophila Genetic Reference Panel (DGRP, Mackay et al., 2012), a collection of 200 wild-derived lines originated from flies inbred for 20 generations and fully sequenced. The project focuses on a line with a period length of 32 hours, and a defect in temperature compensation (period length increases with increasing temperatures). We found at least two mutations responsible for the phenotypes, which we mapped to the circadian gene timeless (specifically to a region implicated in temperature compensation) and in the Dop1R2 gene. The tim mutation by itself results in accumulation of TIM and decrease in PERIOD (PER) protein levels, resulting in a circadian period of about 28 hours. Adding the Dop1R2* mutation lengthens the period to about 31 hours but has no effect in a tim^+ background. Downregulation of Dop1R2 in the LNvs reproduced the long-period phenotype in the tim* genetic background only, suggesting that dopamine signaling in PDF cells interacts with the clock machinery. Modifying dopamine levels in neural tissues similarly affects circadian behavior. We noticed that affecting other neurotransmitters systems or signaling pathways in the tim* mutation background also resulted in a period lengthening similar to the original wildtype line's phenotype. Our hypothesis implies that tim has a central role in clock regulation depending on the physiological state of the fly, e.g., acting as a buffer during physiological stress. To confirm this hypothesis, behavioral tests in different physiological conditions are ongoing.*

Symposia

S 15-6

VIP and its receptor (VPAC2R) in circadian timing of the sleep-wake cycles

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Abstract: 286 words

In mammals, the circadian rhythm of cells and tissues are controlled by a molecular machinery within the suprachiasmatic nucleus (SCN), regulating downstream functions such as locomotor activity, endocrine functions, and sleep/wake cycles. The neuropeptide VIP and its receptor, the VPAC2 receptor (VPAC2R), represent a key component in maintaining a stable clock function within the SCN. In the absence of either component, circadian behavior is compromised due to desynchronization among SCN neurons. Orexin (hypocretin) neurons in the lateral hypothalamus are crucial in maintaining wakefulness. Although the SCN is known for playing a key role in regulating sleep/wakefulness, the specific efferent neuronal pathways mediating its circadian control over sleep/wakefulness remain poorly defined.

The aim of this study was to map VPAC2R expression in the mouse SCN and lateral hypothalamus to elucidate whether VPAC2R is expressed in orexin neurons, indicating a direct output pathway from the SCN. Immunohistochemical staining using VIP, VPAC2R, AVP, PK2 and orexin antibodies were performed on brain sections from both wildtype and VPAC2R deficient mice. Confocal microscopy combined with 3D reconstruction software was used to examine the localization of VPAC2R and neuropeptides at high magnification. In wild type mice, the VPAC2R was expressed throughout the SCN with the highest expression corresponding to the dorsomedial shell region (no staining in VPAC2 KO mice). Moreover, VPAC2R was co-expressed in VIP neurons (few) and AVP/PK2 neurons. VIP nerve fibers from the ventral SCN were innervating VPAC2R expressing neurons in the SCN shell. VIP nerve fibers, likely originating from the SCN, formed a dense network around and innervated orexin neurons in the lateral hypothalamus that were expressing VPAC2R.

These neuroanatomical findings suggest a direct influence from the SCN on one of the main centers involved in sleep-wake cycle regulation.

Symposia

S 16-1

Neuronal circadian circuit controlling metabolism during sleep revealed by an engineered transcriptional switch

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Disruption of intrinsic circadian molecular rhythms promotes obesity and metabolic syndrome, but how circadian neurocircuits regulate energetics across the sleep-wake cycle – and the induction of torpor in small mammals – is not well established. Here we developed a reversible genetic switch in mice that enables bidirectional virally targeted control of the molecular clock within circuits, enabling us to delineate how the genetically defined neuronal clock subpopulations drive rhythmic regulation of feeding and energetics. By using a newly developed genetic model for intersectional targeting of AVP cells, we combined stereotaxic targeting, chemogenetics, photometry and circuit-dependent Tetanus toxin-mediated chronic neuronal silencing, to identify a circadian neurocircuit projecting from arginine vasopressin (AVP) cells within the suprachiasmatic nucleus (SCN), with a key role in satiation, energetics, hormonal rhythms, as well as body temperature and induction of torpor.

We further established that this SCN-AVP output was dependent upon a functional molecular clock, by using CRISPR-Cas9 as well as a new genetic mouse model with interwoven Cre-FRT sites targeted to the core clock activator gene Bmal1. The latter model enabled us to reversibly abrogate Bmal1 transcription specifically within the identified SCN-AVP neurocircuit. This demonstrated that restoration of Bmal1 transcription targeted to the SCN-AVP circuit rescued the metabolic abnormalities that were disrupted following SCN-targeted Bmal1 disruption, including cold-induced torpor. Our results exploit the requirement for the clock transcriptional feedback loops within pacemaker neurons to establish pathways for the rhythmic integration of behavior and metabolism.

Symposia

S 16-2

Defining the islet cell-specific binding landscape of core-clock protein Bmal1

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The circadian clock system has evolved by most organisms as an anticipatory mechanism driving rhythmic oscillations of physiology with approximately 24-hour cycles (circa diem, from Latin “about a day”). A key function of the circadian timing system is to orchestrate metabolism in different organs according to the needs imposed by rest-activity cycles. Light exposure during the night, late meals, and reduced sleep hours associated with modern lifestyle desynchronize intrinsic clocks from environmental cycles. Type 2 diabetes mellitus (T2D), a major public health challenge today, is one of the consequences of circadian misalignment.

Our works demonstrated that circadian oscillators operative in mouse and human α- and β-cells ensure temporal orchestration of transcriptional and lipid landscape impacting on the islet function, which is perturbed in context of type 2 diabetes (T2D). Furthermore, we unravelled the critical role of the core-clock protein Bmal1 in regulating β-cell compensatory regeneration. Employing temporal multi-omics studies in mouse and human α- and β-cells across 24 hours, including CUT@RUN sequencing, we have now identified islet cell-specific binding landscape of Bmal1 protein. At present, we dissect the molecular pathways linking the islet cellular clocks and insulin and glucagon secretion via thus identified Bmal1 functional targets, and their disruption in T2D context.

Symposia

S 16-3

The circadian control of energy metabolism: implications for people with obesity, type 2 diabetes, and shift workers.

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The circadian timing system consists of a central brain clock in the suprachiasmatic nuclei and peripheral clocks in tissues including metabolic organs such as the liver, the pancreas and adipose tissue. Circadian disruption, that is the disturbed synchrony between the central brain clock, peripheral clocks, the light/dark cycle and behavioral rhythms, leads to reduced sleep quality, and probably contributes to the current pandemic obesity and type 2 diabetes. Shift workers are an extreme example, but most people in our 24-7 may suffer from circadian disruption. The Stenvers group performs rodent studies, state-of-the art human brain imaging, and clinical intervention trials, to study: 1) metabolic adaptation to circadian phase shifts 2) the central and peripheral clock rhythms in people with obesity and type 2 diabetes, and 3) improvement of circadian synchrony and sleep in daily life.

Symposia

S 16-4

Effects of chronodisruption on gut circadian clock rhythmicity in human intestinal organoids

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Introduction

Biological clocks align physiological processes with the astronomical cycle through external cues. In the intestine, circadian clocks are regulated by the light-dark cycle via the master clock and locally by feeding cues. Chronic disruption of circadian rhythms, as occurs in rotating shift work and obesity, promotes the development of metabolic and intestinal disorders. Studying diurnal rhythms in the human gut is challenging. This study investigated whether rhythmicity of clock genes differs between 3D intestinal organoids (enteroids) from patients with obesity and normal-weight organ donors who stayed at the intensive care unit, two conditions known to disrupt circadian rhythms.

Methods

Jejunal enteroids from normal-weight organ donors (n=8) and patients with obesity (n=8) were synchronized, and mRNA was collected every 4 hours over 28 hours. Rhythms in core clock gene expression were analysed using RNA-sequencing and confirmed by RT-qPCR.

Symposia

S 16-4

Results

RNA-seq analysis revealed that the number of clock genes exhibiting rhythmic expression was higher in enteroids from patients with obesity than in those from normal-weight organ donors. Notably, the overall amplitude of 16 core clock genes was 14% higher (P<0.001) in obese organoids. RT-qPCR analysis confirmed that BMAL1 (peak: ZT 13.25 hours) and NR1D1 (peak: ZT 18.91 hours) mRNA expression was rhythmic (P<0.05) in obese enteroids but not in normal-weight enteroids. However, PER2 expression was rhythmic in both groups (P<0.05), peaking at ZT 5.78 hours (normal-weight enteroids) and ZT 3.04 hours (obese enteroids).

Conclusion

Disruption of light/dark cycle (eyes closed) and feeding cues (fasting) in organ donors probably reduced the amplitude of the rhythm in core clock gene expression. In contrast, rhythms in patients with obesity are still entrained by the light/dark cycle but also impacted by dysregulated feeding cues. The type of chronodisruption is important and should be considered when implementing chronotherapeutic strategies.

Funded by EU Horizon Shift2Health (No 101080788).

Symposia

S 16-5

The impact of misalignment with the external light cycle on diabetic retinopathy

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Rationale: Circadian disruption can be experienced when one lives in a misalignment of their internal clocks with the environmental light cycles. In this study, we investigated whether a form of circadian disruption experienced by extreme chronotypes impacts the progression of diabetic retinopathy.

Methods: Ins2 Akita, hyperglycemic, male mice and healthy controls, at two months of age, were housed for 4 months in a forced desynchrony conditions within the limits of entrainment with light cycles (T22.5 and T27 cycles) that resemble circadian disruption of late and early extreme chronotypes correspondingly. Eye disease endpoints were assessed with in vivo retinal imaging (fundus imaging, OCT), ex-vivo immunohistological approaches for acellular capillaries and vascular morphology, and retina tissue was used for mRNA sequencing. Two Way ANOVA (diabetes, chronotype) was used to identify statistically significance ($p<0.05$)

Results: Retinal thickness was significantly reduced in diabetes by 7% compared to controls and was further reduced by another 7% in both forced desynchrony conditions. The number of acellular capillaries in diabetes was increased by 48% in the diabetic mice undergoing forced desynchrony with effects on the intermediate and deeper vascular layers, where significantly reduced vessel area, vessel length and increased E-lacunarity. The mRNA sequence of the retina confirmed that forced misalignment in diabetes impacted the retina vasculature more than in control. Endothelins were among the top genes mostly affected by forced misalignment in diabetes, while a more inflammatory pathway activation was also observed.

Conclusions: Overall, a misaligned light schedule to the internal clock that simulates the social jet lag experienced by extreme chronotypes leads to an acceleration of the retinal structure and microvascular dysfunction observed in diabetes. Circadian disruption could therefore be a modifiable risk factor for prevention of diabetic retinopathy.

Symposia

S 16-6

Tert deletion impairs circadian regulation of blood pressure in spontaneously hypertensive rats

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Aging is associated with both impaired clock function and an increased incidence of cardiovascular disease. Deletion of the Tert gene leads to telomere shortening, which is associated with aging and age-related cardiovascular diseases, but its effects on the circadian regulation of blood pressure (BP) have not yet been studied. To address this gap, we developed a rat model with genetic deletion of the Tert gene on a spontaneously hypertensive rat background (SHR-Tert-/-) in which telomeres were shortened in the F3 generation. We analyzed the effects on locomotor activity, oxidative stress, telemetrically measured parameters of cardiovascular system (CVS) function, and clock gene expression in various tissues. SHR-Tert-/- showed reduced physical fitness, which was reflected in more fragmented nocturnal activity with longer breaks and a poorer correlation between spontaneous activity and telemetrically measured parameters of CVS function. Day/night BP amplitudes were reduced and the circadian rhythm of systolic BP was completely abolished in constant darkness. In the rostral ventromedial medulla of the brainstem, the number of tyrosine hydroxylase (TH)-immunopositive cells was reduced, which could explain the lower average BP values. In the heart (and other tissues), the day/night expression of clock genes was significantly impaired, suggesting an impairment of the synchrony of the clocks. In addition, the level of protein oxidation was increased in heart tissue. Our results suggest that deletion of Tert impairs circadian regulation of BP via a mechanism downstream of the central clock, through a reduced rhythm of sympathetic activity innervating the heart and other tissues, leading to impaired circadian regulation of local peripheral clocks. These findings provide a possible link between age-related telomere shortening and impaired rhythmicity of cardiovascular function.

The project no. LX22NPO5104 is funded by the European Union Next Generation EU and the Research Project RVO: 67985823.

Symposia

S 17-01

Astrocytic control of circadian rhythms

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Circadian rhythms depend on ~24h self-sustained intracellular oscillations of clock gene expression present throughout the body. In mammals, these cellular oscillations are coordinated at the organismal level by the suprachiasmatic nucleus (SCN) of the hypothalamus, which synchronises coherent ~24h patterns of physiology and behaviour and aligns the body as a whole to the light-dark cycle. We have shown that the SCN uniquely operates as an astrocyte-neuronal 24h pacemaker: neurons and astrocytes of the SCN are active at opposite times of the day (day-time, and night-time, respectively), and they are both capable of cell-autonomously rescuing patterns circadian rest-activity behaviour when their clock function is restored in mouse models of genetic clock ablation (Brancaccio et al., 2019, 2017; Hastings et al., 2018). In this talk, I will focus on the nature of information encoded by astrocytes and the mechanisms by which they contribute to circadian rhythmicity. In particular, the role of GABA selectively produced in astrocytes by polyamine degradation as an internal circadian synchroniser of circadian rhythms in the SCN ("astrozeit") will be discussed (Hoekstra et al., 2024; Ness et al., 2025).

Symposia

S 17-2

Dissecting the Neural Network of Mammalian Circadian Clock

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Circadian clock is a biological timing system evolved to align animal behaviors and physiology with daily changing environment. Although almost every cell possesses a molecular circadian oscillator in mammals, suprachiasmatic nucleus (SCN) is considered as the master pacemaker of circadian clock orchestrating the circadian rhythms across the body through a vast neural network in the brain. So far, the structure and function of this neural network of circadian clock remain still unclear. Our previous study (Wen et al., 2020) has identified comprehensive neuron subtypes of SCN, providing a unique opportunity to investigate the functional roles of these subtypes in circadian regulation (Xie et al., 2023). In this talk, I will report our latest studies combining single-cell RNAseq, cross-species comparison, calcium imaging, targeted manipulation, electrophysiology and behavioral analysis to dissect the central neural circuit of SCN in mammalian circadian clock.

Symposia

S 17-3

The role of oligodendroglia in sleep and circadian rhythms

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Neuron-centric mechanisms have dominated sleep research, with the discovery of hypocretins/orexins revolutionizing sleep science. Despite this, nearly 70 million people suffer from sleep disorders in the United States, with 40-90% of individuals with neurogenerative disorders displaying sleep dysfunction. I posit that one putative reason for this is that sleep science has neglected the role of glia in sleep. One component underexplored in our understanding of glia in sleep regulation is the interaction between circadian control of glia and homeostatic regulation of sleep. The role of the circadian system in glial cell modulation has been implicated in models of brain disorders known to include sleep abnormalities, including Alzheimer's disease (AD) and the demyelinating disorder multiple sclerosis (MS). Numerous lines of evidence support that altered circadian biology—through shift work or genetic polymorphisms of clock genes—are significantly associated with AD and MS prevalence. Sleep deprivation in mice decreases myelin thickness, a microstructural change that dysregulates circuit function. These data suggest an interplay between circadian-mediated sleep states and myelin processes, but the direction of causality and how this relates to neurodegeneration remains unknown. Given that Bmal1 is the only non-redundant circadian gene, we developed a conditional clock gene knockout ($Bmal1^{fl/fl}$) and cell type-specific Cre driver mousemodel (NG2::Cre) to knockout Bmal1 from OPCs in embryonic development. Our data strongly suggest a significant deficit in OPC proliferation, density, morphology, oligodendrogenesis, and myelination in OPC-Bmal1-KO mice lacking a functional molecular clock in OPCs compared to OPC-Bmal1-WT mice. We find significant sleep fragmentation, driven by fragmented NREM sleep, in our dysmyelinated mice following OPC-specific Bmal1 loss. These findings establish for the first time a role for myelin-forming cells or myelin in sleep regulation.

Symposia

S 17-4

GABA transmission in AVP neurons of the SCN is essential for regular estrous cycling in mice

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The central circadian clock of mammals is located in the suprachiasmatic nucleus (SCN) of the hypothalamus. The SCN regulates circadian rhythms in various behavioral and physiological functions. In rodents, the luteinizing hormone (LH) surge that induces ovulation is time-dependent and occurs in the evening of proestrus. Moreover, SCN-lesioned rats and hamsters fail to exhibit the LH surge. While the projection pathway from the SCN to gonadotropin-releasing hormone (GnRH) neurons—responsible for triggering the LH surge—has been studied, the functional neural circuitry that conveys this timing information remains unclear.

In the present study, we first employed a method termed "isolation of the SCN (iSCN)," which severs afferent and efferent neural connections of the SCN except for retinal inputs. In wild-type mice, iSCN disrupted the estrous cycle, which normally recurs every 4–5 days, as assessed by vaginal smears and wheel-running activity. Then, we focused on arginine vasopressin (AVP) and vasoactive intestinal peptide (VIP), neuropeptides abundantly expressed in the SCN. Using Avp-Cre and Vip-Cre mice, we investigated the roles of AVP and VIP neurons in female reproductive function. Since most SCN neurons are GABAergic, we generated mice (Avp-Cre or Vip-Cre; Vgat flox/flox) in which the vesicular GABA transporter (VGAT) was deleted in AVP or VIP neurons, and examined whether the estrous cycle persisted. Irregular estr*ous cycles were observed only in Avp-Cre; Vgat flox/flox mice. When an adeno-associated virus (AAV) designed to restore Vgat expression (AAV-DIO-Vgat-mCherry) was injected into the SCN, estrous cycling was rescued.*

These findings highlight a critical role for GABAergic transmission from AVP-expressing SCN neurons in regulating female reproductive physiology, particularly in the timing mechanism underlying the preovulatory LH surge.

Symposia

S 17-5

Integrity of the circadian clock determines regularity of high frequency and diurnal LFP rhythms within and between brain areas

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It is well established that numerous brain regions generate autonomous molecular circadian rhythms, and electrical activity of neurons exhibits 24-hour oscillations. However, the extent to which oscillations of electrical activity in the living animal is driven by endogenous rhythms, environmental time cues, and their interplay is not fully understood. Additionally, the influence of circadian rhythms on general electrophysiological dynamics of neuronal populations remains to be elucidated. Therefore, we proceeded to measure local field potential (LFP) time series in wild-type and Cryptochrome 1 and 2 deficient (Cry1/2$^{-/-}$) mice in the suprachiasmatic nucleus (SCN) and the nucleus accumbens (NAc) under regular light conditions (LD) and constant darkness (DD). We employed an innovative telemetry approach to record freely behaving animals across several weeks. We then used refined analyses to systematically profile LFP time series activity. Our analyses demonstrate that endogenous and environmental rhythms, and particularly their interplay, strongly determine circadian rhythmicity of LFP signals and their frequency components. Additionally, they also shape neuronal patterns on much smaller non-circadian time scales (i.e., hours, minutes, seconds, and milliseconds). For instance, on the millisecond scale, the typical fluctuating electrophysiological dynamics of neuronal activity become highly synchronized and homogeneous within and between brain regions in Cry1/2$^{-/-}$ mice, especially under DD. Furthermore, the simultaneous recording of the SCN and the NAc revealed that their electrical signals can predict each other in wild-type mice under LD. However, when rhythms are disrupted, the NAc signal can no longer predict the SCN signal. These findings demonstrate the indispensable role of functional circadian rhythms in orchestrating both circadian and non-circadian neuronal synchronization. Our findings indicate that environmental time cues exert a comparable influence to that of endogenous clocks, and that their interaction represents the optimal state. This shows that chronotherapies based on the stabilization of environmental rhythms have very concrete effects on brain functionality.

Symposia

S 17-6

Role of Ca^{2+} signaling for Oscillation of Circadian Transcriptional Circuits

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Our group is focusing on roles of Ca^{2+} signaling in circadian clock system. In mammals, circadian Ca^{2+} signaling is transduced by CaMKII that phosphorylates CLOCK to promote E-box-dependent gene expression (Kon et al., Genes and Development, 2014). Recently, we found that Na^{+}/Ca^{2+} exchanger (NCX) mediates cold Ca^{2+} signaling for temperature compensation, and the role of NCX is conserved among mammals, insects, plants and cyanobacteria (Kon et al., Science Advances, 2021). The study suggests that Ca^{2+} signaling was involved in ancestral clock system.

In order to investigate a hierarchical structure in the clock system, we developed mammalian cellular and fruit fly models that lost transcriptional rhythms without disrupting genes encoding the clock-related transcriptional factors. In the mammalian cells, expression of known clock genes was not sufficient to generate autonomous transcriptional rhythms of Per2 and Bmal1. Importantly, their anti-phasic expression rhythms were sustained by once-daily activation of Ca^{2+} signaling. The forced oscillation of transcriptional circuits was also observed in Drosophila mutant of Ca^{2+} signaling. Importantly, we found that circadian Ca^{2+} rhythms were present in mammalian cells lacking Bmal1. These results lead a novel clock model that the circadian transcriptional circuits are sustained by Ca^{2+} oscillation.

Symposia

S 18-1

Hair test reveals plasticity of human chronotype

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Abstract: Circadian clocks govern daily physiological and behavioral processes and are crucial for health, yet disruptions can lead to various diseases. Chronotype, the state of circadian timing, varies between individuals and is reflected in behaviors such as sleep-wake patterns, cognitive performance, and physical activity. This interindividual variability is influenced by both genetic factors and environmental cues, but the relative contributions of each remain unclear, particularly in terms of plasticity - how much chronotype can shift in response to lifestyle and environmental factors. The gold standard for chronotype assessment, dim-light melatonin onset (DLMO), is invasive and impractical for large-scale studies, while blood-based molecular biomarker tests, which estimate internal time, show promise but are limited by practicality. Here, we introduce HairTime, a novel assay that estimates chronotype from a single hair sample collected at one point during the day. HairTime was developed and evaluated in two studies: a training study and a validation study, where it demonstrated a strong ability to predict chronotype, with DLMO as the comparison. This non-invasive method is suitable for large-scale, longitudinal studies and clinical practice. We assessed HairTime using over 4,000 samples, observing a normal distribution of chronotype across the population, with its estimation associating with age, sex, and notably, work schedules. The association with work schedules reveals the plasticity of chronotype, with circadian timing being earlier on workdays, highlighting that societal factors can influence and modify an individual's internal rhythm. Additionally, we explore the concept of circadian amplitude, finding that lower amplitude rhythms in hair follicle cells are linked to reduced chronotype prediction accuracy. Our results highlight that both intrinsic circadian mechanisms and external factors, such as lifestyle and work schedules, shape chronotype. HairTime offers an innovative tool for understanding circadian rhythms, facilitating personalized chronotherapy to improve health outcomes by aligning treatments with an individual's biological rhythms.

Symposia

S 18-2

PDF-mediated plasticity of the evening locomotor activity in Drosophila melanogaster

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At higher latitudes, day lengths can change drastically throughout the year. As a result, most animals need to reliably track the seasonal changes in order to adapt their behavior. Likewise, Drosophila melanogaster adjusts its bimodal activity to changing environmental conditions. Pioneering studies in Drosophila have mapped its two major activity bouts to three distinct populations of neurons within the clock network. The Pigment Dispersing Factor (PDF)-positive s-LN_v contribute to the morning activity, while the LN_d, together with the ITP-positive LN contribute to the evening activity. A third population of neurons, the DN_{1p}, appears to contribute to both, the morning and evening activity. PDF, as one of the major clock neuropeptides, has been shown to play a role in both the synchronization and phasing of the molecular clock as well as the neuronal activity in the clock neuron populations.

Here, we show that PDF signaling from both the s-LN_v and l-LN_v is required for the proper timing of the evening activity. Recording the flies' locomotor activity under temperature cycles in constant light shows that the evening activity is composed of two distinct activity peaks. While there is no phase difference between the two evening activity components under short days the phase angle increases with increasing day length. Using PDF-receptor rescue experiments and ablation of either s-LN_v or l-LN_v, we characterize the contributions of each neuron group and map the activity peaks to distinct neuron populations of the evening neurons.

We further use an activation-dependent sensor for the PDF-receptor to investigate unique and shared targets of s-LN_v and l-LN_v. Together with synaptic connectivity data, our analyses provide insights into the pathways involved in the seasonal plasticity of evening activity in Drosophila.

Symposia

S 18-3

Systemwide transcriptome and behavioral plasticity as an adaptation strategy to changing energy demand

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Prior studies have revealed that the phase of the central circadian clock (SCN) displays an internal representation of the external light-dark cycle and is therefore similar in nocturnal and diurnal species. How this signal is translated into differential temporal organization of physiology and behavior remains a long-lasting question. In order to understand what biological principles drive the daily timing of activity, we used a model wherein we can manipulate energy balance, and switch mice to be nocturnal or diurnal by regulating how much wheel-running activity will be rewarded with a food pellet (work-for-food). Insufficient energy intake to meet energy expenditure demands of physical activity can result in systemic neuroendocrine and metabolic abnormalities in activity-dependent anorexia and relative energy deficiency in sport (REDs), affecting >40% of athletes. To assess the molecular changes in response to energy deficiency, we implemented the same paradigm and replicated several aspects of REDs in mice with high physical activity and gradually reduced food intake, which results in weight loss, compromised bone health, organ-specific mass changes, and altered rest-activity patterns. We subsequently wanted to assess the changes in brain and peripheral sites that are involved in regulating sleep-arousal, feeding-fasting, and locomotor activity. To this end, we performed rhythmic transcriptome analysis of 38 tissues, including 18 brain regions, from nocturnal and diurnal male and female mice. Rhythmic gene expression across tissues comprised a different set of rhythmic genes with minimal overlap between nocturnal and diurnal mice. Rhythmic gene expression was shifted in phase and reduced in amplitude in diurnal mice. Overall, we found distinct gene expression signatures including clock genes in different hypothalamic, thalamic and cortical structures of nocturnal and diurnal mice. Our findings contribute in defining the mechanisms and brain networks that enable nocturnal-diurnal switches, and may provide important implications for human sleep, shift-work and metabolic health.

Symposia

S 18-4

The balancing act of the SCN

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The suprachiasmatic nucleus (SCN) regulates circadian variations in physiological setpoints by targeting various brain areas within and outside the hypothalamus. The SCN prepares us every morning to become active, and therefore, it needs to prepare the physiology of our body in advance for this vital behavioral change. Consequently, the SCN alters hormonal rhythms and Autonomic Nervous System activity, transmitting a precise message to select organs and adjusting their sensitivity to changes in hormones, metabolites, and other essential factors associated with behavioral changes.
All these parameters vary within very narrow boundaries at a specific time, whereby day-to-day variation is less than 5% at any particular hour. However, the circadian peak values, for example, Corticosterone, can be at least ten times higher than the circadian trough values, indicating the need for an elaborate feedback system to inform the SCN and other participating nuclei about the actual levels reached.

Challenging the body with infections, food shortages or excess, and low or high temperatures will alter the physiological setpoints of the body. Under fasting conditions, setpoints for body temperature and glucose levels are lower at the beginning of the inactive phase. However, starting the active phase, the expected increase in glucose and temperature levels occurs to support activities associated with food acquisition. Thus, the SCN adjusts physiological setpoints in accordance with the time of day and in response to the challenges faced by the body. The SCN is enabled to do this by receiving information about the body's condition. Therefore, when the body gets stimuli contradicting normally expected physiology, such as eating or activity during the inactive period, this information reaches the SCN, allowing it to adapt its output and correct this imbalance. Consequently, frequent violations of the SCN message, such as shift work or night eating, can lead to disease development.

Symposia

S 18-5

A systems biology approach to analyze intercellular coupling of peripheral circadian clocks

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A large number of molecular, cellular, physiological and behavioral processes such as gene expression, metabolism, immunity, food intake and sleep, oscillate according to a 24-hour rhythm synchronized with the day/night cycle. These oscillations are controlled by the circadian clock, an endogenous mechanism whose disruption has known adverse consequences on health. The molecular oscillator that governs the circadian clock operates autonomously and is present in almost all cells. In addition to being synchronized by light, hypothalamic central clock neurons are coupled to each other via neurotransmitters, a mechanism necessary to maintain coherent, robust, and high-amplitude oscillations at the tissue level. Despite the lack of evidence for a similar mechanism in peripheral organs, recent work has shown that hepatocytes maintain coherent circadian oscillations in vivo in the absence of extrahepatic synchronization. The TGFb signaling pathway has also been implicated in the phase adjustment of peripheral cells. Along the same lines, the TGFb signaling pathway has been proposed to mediate the phase adjustment of peripheral cellular clocks but this role in vivo is not yet known. To explore the hypothesis of intercellular coupling in the liver, we developed a multidisciplinary approach combining mathematical modeling, 3D-cell culture, real-time luminescence and signal processing methods. Using an ODE based reduced model of a network of coupled oscillators, we predict that cells with different period converge towards a common period upon diffusive coupling.Experiments with spheroids mixing hepatocytes with different periods fully validate this prediction, demonstrating that cells communicate to adjust their periods. Although the underlying mechanism remains to be understood, the experimental results also suggest that direct contact between cells is important for observing this intercellular period locking phenomenon. Studying synchronization at the tissue level will help understand and treat the negative effects of circadian misalignment and disruption.

Symposia

S 19-1

Models of food anticipatory circadian timekeeping

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Among the most important functions of circadian clocks is to coordinate behaviour and physiology with daily cycles of food access and intake. To achieve this, circadian clocks must be entrainable by stimuli that report the phase of feeding cycles. Daily light-dark (LD) cycles may be one such stimulus, and may be sufficient for organisms that rely on energy sources perfectly correlated with light exposure (e.g., photoautotrophs). For many organisms, access to food may be limited to specific times within the day or night, which may change over time. In these cases, circadian control of food seeking behaviour would be optimized by the evolution of mechanisms that permit flexible timing of behavioural rhythms relative to the solar day.

This could involve adjustments in the phase of a light-entrainable clocks by converging food input pathways, or entrainment of separate clocks by parallel food and light input pathways. A third possibility is that mealtimes are encoded in the phase of light-entrainable clocks, with no requirement for food-entrainable clocks. I will review archival and new evidence by which to evaluate models of food anticipatory circadian timekeeping, and identify knowledge gaps and opportunities.

Symposia

S 19-2

A dopamine regulated circadian resonator underlying sensorimotor integration

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The daily temporal pattern of animal behaviour is determined not only by the central circadian pacemaker located in the suprachiasmatic nucleus (SCN). In rhythmic behaviours such as food anticipation, the methamphetamine-sensitive circadian oscillator (MASCO), and other forms of time memory, the mesolimbic pathway is strongly implicated. We present a mathematical model of our hypothesis that a dopamine regulated circadian resonator (DARCR) underlies sensorimotor integration and time memory. In particular, sensorimotor integration involves dopamine dependent glutamate/GABA feedback circuitry. The mesolimbic pathway acts as a resonator in that it is rhythmic in response to input but is not intrinsically oscillatory. We study this resonance with amphetamine input and photic input, and how it is regulated by the central circadian pacemaker. The model includes a phenomenological description of the SCN, as well as a neuronal population of dopaminergic neurons, a neuronal population of striatal neurons, and extracellular dopamine in the striatum. These neuronal populations are described by their average firing rates and mean cell body voltages. We include the effects of neurotransmitters glutamate, GABA, and dopamine; release, diffusion, and reuptake of extracellular dopamine; and amphetamine as reducing synaptic connection strengths between the SCN and the DARCR as well as dopamine transporter activity. Both the SCN and DARCR drive locomotor activity output. Simulation, parameter sensitivity analysis, and period bifurcation analysis explains wheel running activity patterns under chronic amphetamine including a disengagement of the SCN and DARCR, relative coordination between the two, and bicircadian rhythms. The model provides an integrated framework to explain the overt expression of rhythmic controls of behaviour. The model reproduces complex patterns of rhythmic behaviour that are produced in mice in response to a variety of photic stimuli and chronic amphetamine. We hope that the model provides insight into the basis of sensorimotor integration and time memory.

Symposia

S 19-3

The post-translational nature of mammalian circadian timekeeping

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In mammalian cells, circadian timekeeping has long been thought to be driven by a network of transcriptional-translational feedback loops resulting in, and reliant upon, the rhythmic expression of 'clock genes'. Recently however, several independent lines of evidence have questioned whether this simple model is adequate to explain, not only how cells time ~24h, but also how circadian regulation of biological function is achieved [1, 2, 3, 4]. Through comparative chronobiology we have explored an alternative hypothesis: that circadian timing and regulatory mechanisms are primarily post-translational in nature.

We identify an evolutionarily conserved, circadian phosphorylation motif and characterise its cellular functions. Chemical and genetic manipulation of phosphomotif effectors in cells results in striking circadian phenotypes, supporting their function as novel post-translational components of the cellular clockwork. We conclude that daily oscillations in 'clock gene' expression may play an accessory, rather than causal, role in circadian timekeeping and physiology.

Symposia

S 19-4

Epigenomic and transcriptomic oscillations are interlinked with linear trajectories in the developing cell

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To explain the coordination of molecular events in the developing cell, we propose that linear changes in epigenetic regulation, gene transcription, and other molecular processes essential for cell differentiation are mediated — and potentially regulated — by their oscillatory dynamics. Supporting this model, we demonstrate an association between oscillatory and linear dynamics in cytosine modifications in mouse intestinal organoids, as well as in the transcriptomes of C. elegans. Furthermore, we show that transcriptomes of single cells exhibited developmental chrono-heterogeneity, enabling reconstruction of oscillatory cycles which correlate with linear changes. Oscillation-mediated linear dynamics may represent an evolutionary invention for encoding molecular time and orchestrating developmental processes.

Symposia

S 19-5

A functional analysis of circadian pacemakers in mPers-deficient mice

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The Period (Per) gene was first identified in Drosophila as a key regulator of circadian rhythms, with mutations causing altered or loss of behavioral rhythmicity. In mammals, three homologs, Per1, Per2, and Per3, have been discovered. This study compares the circadian properties of mice deficient in each Per gene, both singly and in combination, under constant darkness (DD). It analyzes their free-running periods (τ) and phase response curves (PRCs) to 6-hour light pulses (6h LP). Each Per-deficient mouse line showed unique circadian characteristics. Per1-deficient mice (including double mutants lacking Per1 and Per3) displayed high-amplitude PRCs with large phase shifts near CT18, consistent with Type 0 PRCs. Per2-deficient mice exhibited shorter τ and Type 1 PRCs, with crossover points around CT17. Notably, some Per2-deficient mice lost circadian rhythmicity after a single 6h LP around the crossover points but regained rhythm after a second pulse 12 days later. Per3-mice, lacking Per1 and Per2, did not maintain stable circadian rhythms in DD but could transiently reestablish a rhythm (~19.5 h period) in response to a 6h LP. Those behavior suggests that rhythm loss was not due to the cessation of circadian oscillation but rather the uncoupling of the multiple oscillators that constitute the circadian pacemaker. Overall, the study demonstrates that each Per gene contributes distinctively to the regulation of τ and light responsiveness in the mammalian circadian system.

Symposia

S 20-1

Light, circadian rhythms, and psychiatry

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Light has profound effects on physical and mental wellbeing. In addition to the synchronisation of the circadian clock, light exposure acutely improves mood and alertness. Recently, it was shown that there are greater than 50-fold differences between people in the sensitivity of the circadian system to light. Either being highly sensitive, or having very low light sensitivity could make an individual vulnerable to sleep and circadian disruption. Additionally, medications including antidepressants and mood stabilisers can alter the sensitivity of the circadian system to light, either increasing or decreasing the response. These effects may lead to either positive or detrimental clinical outcomes for patients with mood disorders depending on a number of factors. This talk will explore the implications of inter-individual differences in sensitivity to light for the regulation of sleep, circadian rhythms, and mood in the context of mood disorders. Additionally, the available evidence for impacts of psychiatric medications on circadian light sensitivity in humans will be reviewed, and the clinical implications of this work and future avenues requiring investigation will be summarized.

Symposia

S 20-2

Sampling in the real world: What can ambulatory biosampling tell us about our rhythms?

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Assessment of hormone rhythmicity outside of laboratory settings is challenging. One particular challenge is that hormonal rhythms of the hypothalamic-pituitary-adrenal axis have complex dynamic properties that can only be crudely estimated when sampled infrequently. In this talk I will show how an ambulatory biosampling technique based on microdialysis can be used to assess the structure and properties of adrenal hormone rhythms in real world settings, using endocrine diseases as examples, and discussing how this approach provides information that could lead to improvements in the type and timing of hormone replacements. I will also show examples of how the ambulatory technology can be used to assess other rhythmic systems, including melatonin secretion, and an experimental method for tissue quantification of catecholamines and their metabolites. Finally, I will reflect on the potential offered by the combination of biological and wearable sensing information collected in detailed 'laboratory-at-home' like protocols.

Symposia

S 20-3

Circadian Medicine in the Intensive Care Unit

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Circadian rhythms coordinate physiological processes across all organ systems, yet in the intensive care unit (ICU) – where patients are most vulnerable – these rhythms are often severely disrupted. The ICU environment, characterized by irregular light-dark signals, continuous nutrition, and round-the-clock interventions, contributes to this disruption and is increasingly recognized as a contributor to adverse health outcomes in critically ill patients. Therefore, integrating circadian principles in critical care represents a promising strategy to improve patient outcomes in the ICU. In this talk, I will discuss our recent research in this field, highlighting our work on the use of data from electronic health records (EHR) that are collected as part of routine clinical care to study 24-hour rhythms in physiological processes in patients in the ICU as well as our results from a recently-completed clinical trial in which we investigated the impact of cyclic daytime enteral nutrition compared to continuous enteral nutrition on circadian rhythms and clinical outcomes in the ICU. Furthermore, I will discuss the challenges and recommendations related to integrating circadian medicine in critical care that were defined in a workshop that brought together international experts with backgrounds in circadian biology, critical care, and implementation science. Altogether, this talk will explore the potential of circadian medicine in the ICU, using our data to bridge the gap from basic circadian biology to real-world clinical applications.

Symposia

S 20-4

Can translational studies help to determine the optimal time point for cancer therapy in humans?

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Hepatocellular carcinoma (HCC) occupies the fourth cause of cancer death worldwide with mortality rate of 8.2% (782,000 deaths) and 841,080 new cases in 2018 and therapeutic options still remain limited. We therefore investigated in a translational approach with mice whether a chronotherapeutic treatment of hepatocellular carcinoma (HCC) may improve treatment efficacy and mitigate side effects on non-tumoral liver (NTL). HCC was induced in Per2::luc mice by single injection of diethylnitrosamine (DEN) and chronic treatment of phenobarbital in drinking water. Control groups received chronic phenobarbital treatment only or were left untreated. Tumor growth was controlled in vivo by magnetic resonance imaging. Tumor-bearing mice and controls were irradiated at four time points of the day. Proliferation and DNA-double strand breaks were analyzed in irradiated and nonirradiated animals by detection of Ki67 and γ-H2AX. Prior to whole animal experiments, organotypic slice cultures were investigated to determine the dosage to be used in whole animal experiments. Irradiation was most effective at the proliferation peaks in HCC at ZT02 (early inactivity phase) and ZT20 (late activity phase). Irradiation effects on NTL were minimal at ZT20. As compared with NTL, nonirradiated HCC revealed disruption in daily variation and downregulation of all investigated clock genes except Per1. Irradiation affected rhythmic clock gene expression in NTL and HCC at all ZTs except at ZT20 (late activity phase). Irradiation at ZT20 had no effect on total leukocyte numbers. Our results indicate ZT20 as the optimal time point for irradiation of HCC in mice at which the ratio between efficacy of tumor treatment and toxic side effects was maximal.

An important question is whether the data from nocturnal mice can help to determine the optimal time point for radiotherapy of HCC in diurnal humans. Time points which are determined in nocturnal species (mouse) might be easily transferred to diurnal species (e.g., human) by using the rest-activity cycle as parameter which obviously shows the same phase relation with chronotherapeutic strategies and antimitotic therapies in mice and humans. Rest activity cycles can be recorded by means of non-invasive techniques in mice by means of infrared detectors and in humans by wrist-worn motion detectors and wearables. We therefore propose to introduce a new term: the time after activity onset (TaAO) and use this for translational studies.

Symposia

S 20-5

Effect of cyclic daytime versus continuous enteral nutrition on circadian rhythms in patients in the Intensive Care Unit: a randomized controlled trial

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INTRODUCTION

Circadian rhythms are severely disrupted in patients in the intensive care unit (ICU), which is associated with poor clinical outcomes. One potential contributor is the 24-hour continuous administration of enteral nutrition. As rhythmic feeding-fasting cycles are potent synchronizers of the circadian system, optimizing these cycles might help restore rhythms in the ICU.

OBJECTIVES

The CIRCLES study aims to investigate the impact of cyclic daytime versus continuous enteral nutrition on 24-hour rhythms in ICU patients.

METHODS

In this randomized controlled trial, patients with an expected stay >48 hours and intention to receive enteral nutrition were randomized to continuous (standard care) or cyclic feeding (08:00–20:00). Outcomes were 24-h rhythm amplitudes of core body temperature, mean arterial pressure, heart rate (variability) – assessed by cosinor analysis - and melatonin on study day 3–4, as well as clinical outcomes (glucose levels, insulin requirements, caloric intake, and gastric residual volumes) Outcomes were compared using t-tests or Mann-Whitney U-tests.

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RESULTS

Eighty-five patients were enrolled; 62 completed follow-up until study day 3-4. The 24-hour rhythm amplitudes in vital signs were consistently, although not significantly, higher in the cyclic group compared to the continuous group. Regarding clinical outcomes, no significant differences were observed in daily caloric intake and insulin requirement, although glucose levels and gastric residual volume were significantly higher in the cyclic daytime feeding group. Melatonin analyses are ongoing and results will follow shortly.

CONCLUSIONS

Our results show that cyclic feeding during daytime hours is safe and feasible in critically ill patients, highlighting its potential use as a circadian intervention in the ICU. Although amplitudes of 24-hour rhythms in vital signs were consistently higher in patients receiving cyclic daytime feeding, these differences were not statistically significant. Therefore, further studies are needed to confirm cyclic feeding as intervention to strengthen circadian rhythms and improve ICU outcomes.

Symposia

S 20-6

Preliminary Results of a Scoping Review of use of Wearables and Internet of Things Technologies in Circadian Medicine

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Introduction

Wearable and Internet of Things (IoT) technology have increasingly been used to monitor daily rhythms and promote circadian health. These devices offer a non-invasive method to track various physiological parameters that are indicative of circadian health, yet the evidence is scattered across disciplines.

Objective

To map and characterize wearable and IoT technologies used and reported in the literature to measure or modulate daily rhythms, identifying gaps and translational opportunities to support future research at the intersection of these emerging technologies and circadian medicine.

Symposia

S 20-6

Methods

A scoping review is being conducted following the Joanna Briggs Institute Manual and drafted using PRISMA ScR. MEDLINE, Embase, Web of Science, Scopus, CINAHL, IEEE Xplore, ClinicalTrials.gov, and WHO ICTRP databases were searches from 2010 to April 30, 2025, without language restrictions. Two reviewers are independently screening records and charting data. The protocol will be registered on the International Registered Report Identifier.

Results

Our preliminary search yielded 4602 records; remaining 3918 after de-duplications and 242 reports, representing 189 unique studies. Research spanned 34 countries. We have identified until now 57 distinct wearables (34 smartwatches/rings, 12 skin-patches, 7 neuro-headbands, 4 ingestibles) and 22 ambient IoT systems (eg. Smart-lighting, meal-timing apps). Most studies are observational (58%). Primary circadian endpoints were activity-derived rhythmicity indices (68%) light-exposure metrics (42%), skin/core-temperature phase (27%) and glucose rhythmicity (11%). Formal validation against a gold standard was not represented in the majority of the studies (>70%).

Conclusions

The conducted preliminary search implies that there are enough heterogeneous references to complete the scoping review. Wearable and IoT technologies show significant potential in circadian medicine, particularly for monitoring and treating circadian rhythm disruptions in neurological diseases and mood disorders. These devices offer a scalable and non-invasive method to track circadian health, though further research is needed to standardize methodologies and validate their clinical use.

Symposia

S 21-1

Interplay of Circadian Clocks, DNA Damage, and Sleep Patterns in Cnidarians with Divergent Chronotypes

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Circadian clocks govern daily rhythms in physiology and behavior across metazoans, yet their evolutionary origins and cellular functions in early-diverging lineages remain unresolved. Cnidarians, with their diffuse nerve nets and divergent chronotypes, offer a unique opportunity to investigate how endogenous timekeeping systems emerged and what roles they play in maintaining organismal homeostasis. In this study, we examined two distantly related cnidarians—the upside-down jellyfish Cassiopea andromeda and the starlet sea anemone Nematostella vectensis—to determine how circadian clocks shape behavior and contribute to neuronal genome stability.

We show that diel behavioral rhythms in both species are modulated by a combination of light input and internal clocks. C. andromeda exhibits a primarily light-driven quiescence pattern with nocturnal inactivity and midday napping, while N. vectensis demonstrates circadian-regulated rest that peaks around dawn. Disruption of the core circadian gene Clock in N. vectensis ($NvClk^{\Delta/\Delta}$) altered the timing and structure of daily behavioral quiescence, confirming a central role for the molecular clock in modulating activity cycles.
Remarkably, we found that alignment between behavioral rhythms and circadian phase is crucial for neuronal health. Exposure to UV and experimental sleep deprivation elevated levels of neuronal DNA damage, particularly in animals with disrupted circadian timing. Conversely, behaviors aligned with circadian phase were associated with enhanced genome stability, suggesting that one of the ancestral functions of circadian clocks and sleep may have been to optimize cellular repair and reduce oxidative and genotoxic stress.
Together, our findings highlight the evolutionary conservation of clock-driven behavioral regulation and point to a mechanistic link between circadian timing and neuronal genome maintenance in basal metazoans. These results suggest that circadian clocks may have evolved, in part, to coordinate protective cellular processes with environmental light–dark cycles, even in animals with simple nervous systems.

Symposia

S 21-2

The sexy clock of the bee: The influence of sex and caste on circadian rhythms

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Mating success depends on many factors, but first of all, a male and a female need to meet at the same place and time. We characterized circadian rhythms in adult emergence and locomotor activity under different illumination regimes for males, gynes (unmated queens), and mated queens. We developed a method to monitor adult emergence from the pupal cocoon and found no circadian rhythms in this behavior for either males or gynes. These results are not consistent with the hypothesis that the circadian clock regulates emergence from the pupa. Consistent with this premise, we found that both gynes and males do not show circadian rhythms in locomotor activity during the first three days after pupal emergence, but shortly after develop robust circadian rhythms that are readily shifted by a phase delay in illumination regime. We conclude that they do not need strong rhythms in adult emergence and during their early adult life in their protected and regulated nest environment, but do need strong activity rhythms for timing flights and mating-related behaviors. Next, we tested the hypothesis that the locomotor activity of males and gynes have a similar phase, which may improve mating success. We found that both males and gynes have strong endogenous circadian rhythms that are entrained by the illumination regime, but males show rhythms at an earlier age, their rhythms are stronger, and their phase is slightly advanced relative to that of gynes.

An earlier phase may be advantageous to males competing to mate a receptive gyne. By contrast to virgin gynes, mated queens are active around the clock with attenuated or no rhythms in the presence of brood, but switch to activity with circadian rhythms when the brood is removed. Our results are consistent with the hypothesis that sex-related variations in circadian rhythms is shaped by sexual selection and the need for efficient brood care.

Symposia

S 21-3

Clocks in the dark: Adaptations of circadian clocks in cave animals

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The Circadian Clock and DNA damage repair represent key regulatory systems which operate at the interface between cells and their environment and perform highly conserved functions. However, evolution in extreme environments such as under perpetual darkness can have profound effects on these mechanisms and provides novel insight into their function and regulation by environmental factors. We have focused on exploring the circadian clock as well as DNA repair in a species of cavefish, the blind Somalian cavefish, Phreatichthys andruzzii. This is an extreme example of a cave-dwelling fish species that has been completely isolated from surface water within phreatic layers beneath the Somalian desert for several millions of years and consequently displays extreme troglomorphic phenotypes including complete loss of the eyes, scales and body pigmentation. We have revealed that this species retains a circadian clock which is not entrained by light but is strongly regulated by the timing of feeding. This striking phenotype is associated with a series of loss-of-function mutations which affect a subset of opsin photoreceptor genes as well as transcription control mechanisms that target light-inducible clock genes via the D-box enhancer element. Furthermore, in P. andruzzii we have documented the loss of the highly conserved, light-dependent photoreactivation function whereby photolyase proteins enzymatically repair UV-induced DNA damage. Interestingly, consistent with the absence of sunlight exposure, 6-4 and DASH photolyase have accumulated multiple loss-of-function mutations in this cavefish, however the conservation of normal CPD photolyase function reveals a light-independent role for this protein in the management of oxidative stress-induced DNA damage.

Symposia

S 21-4

Low-Temperature Dynamics in Single Cyanobacterial Cells Highlight Critical Features of Circadian Clock Response

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We studied how the circadian clock in individual cyanobacteria responds to temperatures below 25°C. Using time-lapse fluorescence microscopy, we separated the clock's behavior from general cold-shock responses by identifying specific signs of cell death. By focusing on surviving cells, we found that the clock's response to temperature changes aligns with the Stuart-Landau oscillator. This model captures the dynamics of oscillatory systems near critical points where rhythmic activity begins or ends, providing a framework to understand how external changes influence oscillatory behavior.

We then applied this same Stuart-Landau analysis to previously published KaiC protein phosphorylation data after temperature drops. In both living cells (in vivo) and isolated protein systems (in vitro), we observed similar behavior: their oscillations disappeared at a critical temperature, consistent with a supercritical Hopf bifurcation—an event where rhythmic activity switches on or off.

To explain how temperature affects the clock's properties, we propose two simplified models:1) Temperature-sensitive positive feedback, where temperature changes alter the coupling constant and phase dynamics of the system. 2) Temperature-compensated delayed negative feedback, a stabilizing process that operates with a time delay and remains consistent across temperatures.

Our results provide strong constraints for future models by revealing a specific time scale for transitory regimes in the cyanobacterial circadian system and its temperature dependence.

Symposia

S 21-5

How beetles tick- Insights into the circadian system of the model species Tribolium castaneum

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While the principal mechanism and some components of circadian clocks are conserved across animals, comparisons among Bilateria revealed important differences in the molecular make-up of those pacemakers. Although beetles represent the most species-rich animal taxon, their circadian systems remain poorly understood. Here, we present a comprehensive analysis of circadian activity patterns and provide first insights into their molecular underpinnings in the model beetle species Tribolium castaneum.

Our behavioral measurements revealed entrainable, temperature-compensated free-running rhythms in the locomotor activity of the red flour beetle. However, unlike most species typically studied in chronobiology, this beetle exhibits considerable inter-individual variation in diel activity patterns. While some individuals show clear behavioral rhythms, a noteworthy proportion display arrhythmic activity. To further investigate the underlying molecular clock, we used RNA interference to target the negative limb of the T. castaneum circadian clock, whose composition varies among insects. Knockdowns of period and cryptochrome 2, but not of timeless, compromised behavioral rhythms. This suggests a clock that most closely resembles the one described for heteropterans, such as the firebug.

Our study provides comprehensive new insights into beetle circadian behavior and its molecular foundations, beginning to fill a remarkable gap in the broader map of insect chronobiology. Furthermore, our results highlight the extent of individual variation in clock-driven behavior in T. castaneum. The range of genetic tools available for this beetle makes it a promising model to study the origin of individual variation in circadian rhythms in the future.

Symposia

S 21-6

The membrane hand of the circadian clock in flies and mice: Experimental and modelling insights

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Circadian rhythms are a universal feature of life, from single-celled organisms to humans. They operate at molecular, cellular and electrophysiological levels to align physiology and behaviour with the 24-hour light-dark cycle and seasonal changes. At the core lies the molecular clock - comprising rhythmically expressed clock genes that regulate the circadian transcription of downstream genes, including ion channels. In both mammalian and Drosophila clock neurons, this transcriptional feedback loop underlies daily changes in electrical activity essential for time-of-day signalling. Strikingly, silencing electrical activity disrupts molecular oscillations and abolishes behavioural rhythms, suggesting two interdependent circadian oscillators: a nuclear molecular and a membrane electrical – the two hands of the circadian clock. This project aims to elucidate the components and mechanisms of the membrane hand using flies, mice and computational modelling.

Across both species, we observe daily rhythms in membrane properties such as resting potential, firing rate, input resistance, capacitance and excitability, with most parameters peaking in phase. However, some features differ: for example, action potential shape and size vary across the day in opposite directions - mouse neurons fire larger, narrower spikes at night, when fly neurons show broader, smaller spikes. Voltage-clamp recordings reveal only small day/night differences in ionic currents, suggesting that subtle changes in conductance or morphology are sufficient to control excitability. Direct comparisons are complicated by differences in neuronal structure and recording location. In Drosophila, spikes are recorded far from the initiation zone, leading to attenuation and filtering. To overcome this, we exploit the unique morphology of large ventrolateral neurons (LNvs), which have two spike initiation zones - each producing distinct spike shapes. Using anatomical reconstructions and measured biophysical properties, we developed a compartmental LNv model that will allow us to reconstruct original spike shapes and test whether conserved ion channel sets underlie circadian electrical rhythms across species.

Symposia

S 22-1

Mathematical modeling and controlling of spatiotemporal dynamics in Arabidopsis circadian clock

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Individual plant cells possess a circadian clock that times internal processes to the environmental day-night cycle. Mathematical models of the cellular clock network have driven a mechanistic understanding of the plant circadian system. However, these models are typically either 'whole plant' models that ignore tissue or cell-type-specific behavior, or 'phase only' models that do not include clock gene components explicitly. To reveal the design principles of the plant circadian system, mathematical models must address spatial differences observed in tissues and cells in plants, including period and phase differences between cells and spatial waves of gene expression between organs. Here, we present a single-cell mathematical model of a genetic network and implement its ensemble on a spatial template of the plant. Several strategies are introduced to simplify the single-cell model. First, clock genes peaking at similar phases are grouped into the same species. Second, processes of protein productions are represented by distributed-delay expressions of the corresponding mRNA. Third, the model parameters are optimised so that the peak phases of the gene expressions match those of the experiments. Then, we couple the single-cell models locally via the levels of core clock mRNA.

In our network model, sensitivities of the individual cells to light inputs vary across the plant. We found that differences in sensitivities to environmental input can explain the experimentally observed differences in clock periods in different organs, and we show that a plausible coupling mechanism can generate the experimentally observed waves in clock gene expression across the plant. Local sensitivity to environmental inputs combined with cell-to-cell coupling allows for flexible yet robust circadian timing under noisy environments. A strategy for controlling the spatiotemporal dynamics of the plant circadian system based on the phase sensitivity of the individual cells is further discussed.

Symposia

S 22-2

Unlocking the rhythm: Deciphering the role of rhythmic stability for circadian dynamics through single-cell analysis and mathematical modeling

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Many mammalian proteins display circadian oscillations in their abundance, including core clock proteins like CRYPTOCHROME1/2 and PERIOD1/2. These oscillations arise from either genes being turned on and off rhythmically or from the proteins being broken down at different rates throughout the day. Whereas rhythmic production is well studied, little is known about how and whether protein degradation changes at different times of the day. In this study, we analyzed how stable these clock proteins are at different points in their cycle across tens of thousands of individual cells expressing either the circadian repressors CRY1, CRY2, or PER1 as fluorescent fusion proteins. Through continuous measurements of endogenous clock protein levels, we found that the stability of the repressors is changing diurnally: these core clock proteins tend to be more stable when they are building up compared to when their levels are decreasing. To understand how phase-dependent stability changes can impact the repressors' rhythms, we developed a simple mathematical model that showed that the stability changes —being higher during the buildup— can actually amplify protein amplitude. Taken together, our results suggest that the body's internal clock might be compensated for or might adjust for different protein turnover rates that could otherwise induce period alterations, thus ensuring that the rhythm stays on track.

Symposia

S 22-3

Fundamental limits to (Deep) Learning circadian clock phase from single-cell snapshot data

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Recent advances in single-sample circadian time inference provides a potentially powerful alternative to traditional time-series based approaches. Single-sample techniques typically leverage population level RNA rhythms, but the feasibility of single-cell phase detection still remains an open question. Answering this question has proved difficult due to technical drop-outs in current single-cell sequencing technologies, and it has been suggested that overcoming these technical issues may allow for single-cell phase decoding. We have recently argued using low technical noise, multiplexed smFISH measurements that phase inference from single cells might be more fundamentally limited by transcriptional noise, and that some level of 'pseudo-bulking' is likely essential [1]. However, with the ever-increasing sophistication of computational algorithms, in particular Deep-Neural Net architectures, it remains unclear what the limits are to single-cell circadian phase inference.

A powerful approach to estimating circadian phase from high dimensional RNA measurements is to identify a circular latent space in 2D, where the angle represents the clock phase. Generative models such as Variational Auto-Encoders (VAEs) are particularly well suited for this approach, due to the possibility of enforcing biologically relevant priors on the latent space. Here I will describe our recent results on performing Deep Embedding on circular latent spaces using hierarchical priors, within a VAE framework. I will explain how a Negative Binomial distribution, which best represents measured clock RNA distributions, can also be incorporated into this framework. Using this mathematical formulation, I will present results of phase inference from smFISH and scRNA-seq datasets across cell-lines and mouse tissue samples. Our results suggest that while lesser number of cells and genes can be used to identify circadian phase compared to currently available techniques, the fundamental limitation remains and phase inference may not be achievable at single-cell resolution.

Symposia

S 22-4

Modelling Entrainment in Arabidopsis

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Natural light is defined by multiple factors including photoperiod length, intensity, and wavelength, all of which regulate the dynamics of Arabidopsis circadian oscillators. Mechanistically, light regulation of the circadian oscillator occurs through multiple synergistic and/or independent molecular signalling pathways. Phenotypically, it manifests as entrainment under periodic environments and as modulation of free-running rhythms under constant conditions. Mathematical modelling provides a systematic approach to studying highly interlocked dynamical systems such as circadian oscillators and the entrainment pathways that regulate them. The structure of Arabidopsis circadian oscillators is well captured by current models but the mechanisms by which entrainment occur are poorly described, often with light input treated as binary on or off.

We have developed models capable of performing reliable simulations and predictions of circadian dynamics under various light conditions (photoperiods, intensities, and wavelengths), and use these models to study the mechanisms of light entrainment and modulation. Systemic analyses of model simulations reveal the roles of individual light signalling pathways in circadian entrainment. Parameter sensitivity analyses identify key regulatory edges that mediate dynamic plasticity in response to changes in light intensity. These results contribute to a deeper understanding of how light inputs structure circadian timekeeping in Arabidopsis.

Symposia

S 22-5

WANTED: Nonlinearities & Long Delays

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Circadian clocks represent autonomous, self-sustaining oscillations, also known as "limit cycles." Oscillator theory provides a framework for understanding these limit cycles, along with synchronization and entrainment. It has been demonstrated that self-sustained rhythms require nonlinearities, such as sigmoidal relationships or molecular switches, often referred to as "bistability." We discuss various mechanisms that contribute to significant nonlinearities, including cooperativity, sequestration, and positive feedback. Notably, these features are prevalent in both ultradian and circadian clocks.

Another essential condition for establishing self-sustained circadian rhythms is a long delay of at least 6 hours. Mathematical theory correlates the duration of this delay in negative feedback loops with the period of oscillations. Many transcriptional-translational feedback loops (TTFLs) exhibit delays of approximately 1 hour, resulting in oscillation periods of around 2 to 4 hours. Examples of such systems include somite clocks in mammals, NFkB oscillations, and p53 pulses.

To achieve rhythms lasting about a day, particularly long delays are necessary. Potential mechanisms include slow phosphorylation, complex formation, and epigenetic processes. Different species utilize distinct mechanisms: for instance, nuclear import in Drosophila, slow phosphorylation in Neurospora, dynamics of protein complexes, and histone (de)acetylation mediated by p300/CBP, HDAC3, or Sirt1 in mammals.

We emphasize that oscillator theory can provide valuable insights into quantitative molecular chronobiology by highlighting the critical roles of nonlinearities and long delays in rhythm generation.

Symposia

S 22-6

PyCycleBio: modelling non-sinusoidal-oscillator systems in circadian biology

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Abstract: 300 words maximum

Protein, mRNA, and metabolite abundances can exhibit rhythmic dynamics, such as during the day/night cycle. Leading bioinformatics platforms for identifying biological rhythms often utilise single-component models of the harmonic oscillator equation, or multi-component models based upon the Cosinor framework. These approaches offer distinct advantages: modelling either temporally-resolved regulatory behaviour via the extended harmonic oscillator equation, or complex rhythmic patterns in the case of Cosinor. Here, we have developed a new platform to combine the advantages of these two approaches. PyCycleBio utilises bounded-multi-component models and modulus operators alongside the harmonic oscillator equation, to model a diverse and interpretable array of rhythmic behaviours, including the regulation of temporal dynamics via amplitude coefficients.

We demonstrate increased sensitivity and functionality of PyCycleBio compared to other analytical frameworks, and uncover new relationships between data modalities or sampling conditions with the qualities of rhythmic behaviours from biological datasets-including transcriptomics, proteomics, and metabolomics. We envision that this new approach for disentangling complicated temporal regulation of biomolecules will advance chronobiology and our understanding of physiology. PyCycleBio is available at: https://github.com/Glycocalex/PyCycleBio, and the Python package is available to install at: https://pypi.org/project/pycyclebio/. PyCycleBio can also be used at https://colab.research.google.com/github/Glycocalex/PyCycleBio/blob/main/PyCycleBio.ipynb with no installations necessary.

Symposia

S 23-1

Host immune responses and circadian rhythms in the context of malaria and leishmaniasis

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Malaria and leishmaniasis are diseases caused by insect vector-borne infections with parasites Plasmodium spp. and Leishmania spp., respectively. Infection with each of these parasites is associated with an inflammatory component that is central to disease progression and outcome. Since various immune functions show circadian rhythms, we wondered whether Plasmodium and Leishmania infections are regulated by host circadian clocks. In Leishmnia major-infected mice, we found that time of infection impacted the inflammatory response and parasite load. These circadian rhythms were lost in mice lacking clock function in immune cells. Similarly, in a mouse model of cerebral malaria (mice infected with Plasmodium berghei ANKA), disease progression varied according to time of infection, along with parasite load in red blood cells (RBCs). Systemic desynchronization of host clocks, using a chronic jet lag protocol, let to decreased parasite growth throughout the course of the infection, and a loss of rhythmicity. This was paralleled by a loss of plasma glucose rhythm. Accordingly, impairing host glucose rhythms led to a loss of blood parasite load rhythms. Interestingly, in both experimental leishmaniasis and cerebral malaria, circadian rhythms can be traced down to the cellular level, with rhythms of infection, signaling pathway activation, and cytokine secretion in cultured macrophages in response to Leishmania promastigotes as well as Plasmodium-infected RBCs. Lastly, since endogenous circadian rhythms were found in other unicellular eukaryotes (including Plasmodium spp.), we aimed to find out whether such circadian rhythms exist in Leishmania. L. major promastigotes were subjected to temperature cycles alike those experienced by the parasite in its insect vector, and the transcriptome investigated under alternating temperatures or constant temperature. A significant number of transcripts were found to follow the temperature rhythms, involved in pathways related to response to reactive oxygen species, metabolism and cytoskeleton. These results many suggest new therapeutic avenues for parasitic diseases.

Symposia

S 23-2

Circadian Control of Asthma: New Molecular Insights

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Background and Aim: Asthma is a chronic inflammatory airway disease characterized by time-of-day variability in symptoms and severity. In this study, we aimed to evaluate whether the molecular circadian clock is dysregulated in peripheral blood leukocytes of asthmatic patients and whether it may serve as a diagnostic biomarker and therapeutic target.

Methods: We monitored clock protein expression by flow cytometry in peripheral blood leukocytes from patients with mild asthma over a 24-hour period and validated our findings in a separate cohort of patients with moderate asthma. To investigate the interaction between inflammation and the molecular circadian clock, isolated leukocytes were stimulated with patient sera, inflammatory mediators, and clock-modulating ligands. The therapeutic potential of these ligands was assessed both in vitro and in a murine model of allergen-induced airway inflammation.

Results: Leukocytes from patients with mild asthma exhibited altered period length and phase shifts in the oscillation of all clock proteins, along with a reduced overall amplitude. In patients with moderate asthma, altered clock protein levels correlated with disease severity and allergy status. In vitro stimulation with inflammatory components induced similar changes. Inhibition of CCR3/ERK and EGFR signaling using an inverse ROR agonist reset the molecular circadian clock in eosinophils and exerted anti-inflammatory effects by reducing eosinophil migration in vitro. Furthermore, the clock-modulating compound SR1001 demonstrated bronchoprotective effects in two in vivo models, confirming its therapeutic potential.

Conclusion: Our findings indicate that clock proteins may represent therapeutic targets in asthma. Pharmacological inhibition of ROR signaling produced significant anti-inflammatory and bronchoprotective effects, supporting its potential as a treatment strategy for asthma and other eosinophilic disorders.

Symposia

S 23-3

Exploring the Role of Circadian Rhythms in Neurodevelopmental Disorders

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CDKL5 deficiency disorder (CDD) is a severe X-linked neurodevelopmental condition with no current cure. Patients suffer from intellectual disability, refractory epilepsy, motor impairments, and a range of comorbidities including sleep disturbances and gastrointestinal dysfunctions, which significantly impact the quality of life and wellbeing of both patients and caregivers. Despite the high prevalence of sleep problems in CDD, their underlying mechanisms remain largely unexplored. In particular, the contribution of circadian disruption to the pathogenesis of the disorder is still unknown.

In this study, we investigated circadian rhythms in a widely used CDD mouse model (CDKL5 knockout, KO). Thermographic recordings across the diurnal cycle revealed that CDKL5 KO mice exhibit increased locomotor activity and elevated body temperature during light-phase transitions (light-dark and vice versa), suggesting abnormal circadian regulation.
To uncover the molecular basis of these behavioral phenotypes, we performed diurnal transcriptome profiling at 6 ZT in the suprachiasmatic nucleus (SCN) and hippocampus. RNA sequencing revealed an increased number of rhythmically expressed genes in KO mice compared to wild-type (WT) littermates in both brain regions. Gene Ontology analysis of oscillating genes uniquely detected in the SCN of KO mice highlighted a significant enrichment in immune- and inflammation-related pathways. These findings suggest that immune responses may be under circadian control in the SCN of KO mice, contrasting with the non-rhythmic expression seen in WT animals. This alteration may reflect a broader immune dysfunction in the brain, potentially driven by disrupted circadian regulation in absence of a functional CDKL5 protein and linked to previous evidence of subclinical immune dysregulation in CDD patients.
For the first time, our study uncovers circadian transcriptome dysregulation in the brain of CDKL5 KO mice. These insights could advance our understanding of CDD pathophysiology and open new avenues for circadian-based therapeutic strategies to alleviate sleep and neurological disturbances in patients.

Symposia

S 23-4

Time-restricted feeding provides limited microglial immunometabolic improvements in diet-induced obese rats

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Time-restricted eating has shown great promise for improving metabolic health in obese humans, but its mechanism is still not completely resolved. In this study, we investigated how time-restricted feeding (TRF) affects microglial immunometabolism using Wistar rats. In high-fat diet (HFD)-fed induced obesity, restricting food intake to the active phase reduced fat mass, reinforced the rhythmicity of the microglial transcriptome, and prevented an increase in hypothalamic microglial cell number. However, TRF failed to reverse HFD-induced microglial immune dysfunction and metabolic disturbances, including suppressed electron transport chain activity, increased lipid metabolism gene expression, and impaired metabolic flexibility. These findings suggest that obesity-driven microglial immunometabolic reprogramming persists despite TRF and may contribute to obesogenic memory and weight regain after dietary interventions-induced weight loss.

Symposia

S 23-5

Uncovering the circadian basis of vascular homeostasis

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A quiescent, resilient layer of blood vessel-lining endothelial cells (ECs) is vital for maintaining organ function and enabling healthy aging. Much has been learnt in recent years about the mechanisms controlling the resilient EC phenotype. In turn, little is known if and how mechanisms of vascular resilience are affected by environmental rhythmic stimuli (extrinsic (e.g., light-dark, feeding-fasting) or intrinsic (e.g., blood pressure, hematopoiesis)). Performing day/night analyses of organotypically differentiated ECs, we identified distinct organ-specific time-of-the-day dependent transcriptomic programs of resting ECs in adult mice.

These translated functionally into temporal vascular adaptations during the day that critically sustain steady-state tissue health and function. Our ongoing work with EC-specific clock gene targeting infers circadian determinants of vascular health in physiology and response to a pathological challenge. Collectively, adding the day/night cycle as a critical, hitherto in the field of vascular research heavily underappreciated research dimension, the present study sheds novel insights into dynamic molecular changes that underlie vascular homeostasis and critically sustain the physiological need for daily oscillations in organ function.

Symposia

S 23-6

Chronic inflammatory arthritis alters the circadian rhythms of liver macrophages

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The circadian clock generates daily physiological rhythmic oscillations with a period length of approximately 24 hours. These are aligned to the external light-dark environment and regulated by the internal physiological and pathological status. Macrophages, as central components of the innate immune system, are considered key orchestrators of circadian, immune, and metabolic functions. Chronic inflammatory arthritis is often associated with altered liver metabolic and immune function, but the casual mechanisms remain not fully understood. Using the collagen-induced arthritis (CIA) mouse model, we are investigating the role of circadian liver-resident macrophages, Kupffer Cells (KCs), in this process. Single nuclear RNA sequencing revealed differentially expressed genes through circadian times in KCs, and that these changed in CIA condition.

By deconvoluting the liver bulk RNA-sequencing data through circadian time, we showed that the circadian rhythmicity of around 30% of the oscillatory genes in the KC transcriptome were altered (lost, gained, or changed). Pathway analysis of the genes with altered rhythmicity further showed that the regulation of actin cytoskeleton, cytokine signalling, and oxidative stress pathways were significantly enriched and of biological importance. Based on these findings, we further investigated the circadian regulation of KC activities by BMAL1 (Basic Helix-Loop-Helix ARNT Like 1), a core clock component, at the basal state or in response to inflammatory stimuli. Starting with using bone marrow derived macrophages (BMDMs) as a general model, we found that Bmal1 deletion caused BMDMs to be less adherent, increased their motility, and modified their responsiveness to LPS priming. Similar morphological changes were also observed in KCs. We plan to further explore the role of BMAL1 in KCs in health and disease, and how the crosstalk between KCs and hepatocytes affects liver metabolism in a broader scope.

Symposia

S 24-1

Interactive effects of sleep, circadian timing, and light on mood and cognition in adolescents

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During adolescence, changes in sleep and circadian rhythms have been well documented, including later timing of sleep, restricted sleep duration particularly on weeknights, and irregular sleep-wake patterns. Limited studies have examined the trajectories of these changes over time. Furthermore, role of ambient light exposure in these developmental changes is not well understood. We are undertaking the Circadian Light in Adolescence, Sleep and School (CLASS) Study, which uses a longitudinal design to examine the associations of sleep-wake timing, circadian timing and light exposure with academic performance and sleepiness during a critical stage of development. Participants were aged 12–13 years (school Year 7) on enrolment, and were recruited from the general community in Melbourne, Australia. They were monitored at five 6 monthly time points over 2 years. To date, we have studied 168 adolescents (aged 12.81±0.40 years, 56% female) at the first timepoint (T_0). As expected, we found that sleep onset time (particularly on free days) became later and sleep duration (on school days) decreased over time. We are currently examining the interactions between sleep and circadian changes over time and measures of daytime functioning and mood.

Symposia

S 24-2

Individual variability in light-dark patterns and circadian entrainment

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Light is the primary time cue responsible for setting the central circadian clock. Large inter-individual differences exist in how sensitive the circadian system is to light. Currently it is not well understood how these individual differences interact with the light environment in the real world to determine sleep and circadian timing. Interactions between human circadian physiology (such as circadian light sensitivity) and environmental conditions cannot easily be explored systematically in the real world. Using a modelling approach gives us the ability to test complex interactions, yielding important insights about how we can expect these features to interact, and to identify candidate strategies to improve entrainment in cases at greater risk of disruption by light.

This talk will explore how interactions between light sensitivity and light behaviour can affect circadian entrainment, drawing from simulation studies and data collected in real-world settings. Using a validated computational model of the human circadian clock and its response to light, we explored the nuanced relationships between home evening lighting environments and light sensitivity. This talk will also explore avenues for accounting for differences in circadian physiology to more accurately track individual-level circadian timing in real world settings, and consider the implications for developing personalized interventions. Our results contribute to our understanding of how circadian light sensitivity can be used to tailor individual-level solutions that support optimal sleep and circadian timing.

Symposia

S 24-3

New Perspectives on the Molecular Control of Circadian Timing and Sleep

Circadian Stability Under Pressure: Molecular Brakes on Light, Sleep, and Timing

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Circadian clocks promote synchrony between internal physiology and environmental cycles. This temporal alignment ensures that processes such as sleep, hormone secretion, metabolism, and behaviour remain coordinated with the 24-hour light–dark cycle. While much is known about how light entrains the master circadian pacemaker in the brain, the mechanisms that preserve coherence across the broader circadian network, especially under environmental disruption, remain less well understood.

In this talk, I will present new data that challenge classical models of circadian entrainment. Rather than focusing solely on mechanisms that drive adaptation to shifts in environmental cycles, our findings reveal a parallel regulatory layer: molecular “brakes” that actively constrain the pace of circadian realignment. These systemic constraints help preserve temporal coherence between central and peripheral oscillators. They also buffer the organism against physiological desynchrony during abrupt environmental changes, such as those induced by jetlag or shift work.

Using behavioural monitoring, multi-organ gene expression profiling, and spatial transcriptomics, we show that this regulatory buffering is crucial for preserving sleep quality and metabolic stability during circadian misalignment.

By reframing circadian adaptation as a balance between flexibility and restraint, our work introduces a new conceptual model for understanding resilience to chronobiological stress. I will discuss the broader implications of this framework for basic chronobiology and its translational relevance to mitigating the health impacts of disrupted circadian timing in modern, 24/7 societies.

Symposia

S 24-4

Ultradian cycles within sleep: How are they shaped by circadian and sleep homeostatic processes?

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The timing and structure of sleep depend on circadian and homeostatic processes, meaning they are heavily influenced by both biological time of day and sleep-wake history. An additional layer of chronobiological complexity is often overlooked: ultradian rhythms within sleep. In humans, these are ~90-minute cycles commonly defined by the alternation of non-REM and REM sleep, but widely expressed across physiological systems including movement. Movement patterns can be harnessed to study ultradian cycles in sleep when polysomnographic measures are unavailable or to overcome the non-continuous nature of non-REM-REM cycle classification.

Symposia

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To investigate how circadian, homeostatic and ultradian processes interact during sleep, we analysed datasets from multiple human in-patient forced desynchrony protocols (N_{Total}=66 participants, 20h/28h/42.85h T-cycles with typical 2:1 or sleep-restricted 3.3:1 ratios of wakefulness:sleep) and one insufficient sleep protocol (N=16, 5 days of 5-h vs 9-h sleep, randomised crossover). We used scored polysomnographic records to compute non-REM-REM cycles and wrist-actigraphy recordings to extract ultradian movement patterns during sleep by non-linear transformation of activity to inactivity. Inactivity parameters were quantified using stationary sloped cosine fits and non-stationary singular spectrum analyses. Circadian and homeostatic effects on all signals were analysed via linear mixed effects models.

Our analyses revealed effects of circadian phase on ultradian cycle length: longer cycles during the biological night (relative to melatonin maximum) for both non-REM-REM cycles and inactivity cycles. We also uncovered homeostatic signatures in inactivity patterns within and between participants, with subtle differences between protocols. Overall, higher homeostatic sleep pressure was associated with a lower amplitude of the inactivity rhythm, higher inactivity levels at sleep initiation, higher mean levels of inactivity, and a steeper rate of inactivity decline. These findings suggest that inactivity patterns may be an informative circadian, homeostatic and ultradian marker that could be exploited in large cohorts to dissect links between sleep and health.

Symposia

S 24-5

Misalignment of peripheral circadian rhythms is associated with irregular sleep

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Background: The circadian rhythm produces approximately 24 hour cycles in our behaviour and physiology. It is controlled centrally by the suprachiasmatic nucleus in the brain, but acts in a top-down fashion, regulating circadian oscillators in peripheral systems such as the heart. Disruption to the circadian rhythm is linked to poor health outcomes. Misalignment in the timing of circadian rhythms across tissues, relative to each other and the central oscillator, may be one form of disruption. Here, we relate misalignment between circadian rhythms with objectively measured sleep irregularity.
Methods: We analysed concurrently recorded wearable measures of heart rate (HR), skin temperature (TEMP), activity counts (ACT), sleep (Empatica EmbracePlus) and glucose (GLU) (FreeStyle Libre 2) recordings spanning 7-22 days (mean: 12.3, std: 3.6) from 13 healthy volunteers. For each participant, circadian rhythms were extracted from each measure using Singular spectrum analysis, and the circadian phase series was derived using the Hilbert transform. Alignment was measured using the phase-locking value (PLV) derived between each pair of measures. Sleep regularity was quantified using the sleep regularity index (SRI) from the sleep time series. For each pair of measures, the PLV for was compared against SRI using the Spearman's ρ across all participants.
Results: Using HR, TEMP, ACT and GLU, there were 6 unique PLVs for each participant. Except for HR/ACT (ρ=-0.02) and HR/GLU (ρ=0.10), all pairs were weak-moderately positively correlated (overall mean ρ=0.27) with SRI across participants, the highest being PR/TEMP and TEMP/GLU (both ρ=0.48). However, no correlation was statistically substantial (all $p > 0.05$).
Conclusions: Our results hint at a link between stronger alignment of peripheral circadian oscillators and improved sleep regularity, measured objectively using wearable devices. Future work should further investigate the relationship with other behavioural measures such as meal timing, mood, and exercise, using continuous measures of phase locking.

Symposia

S 24-6

Mapping the conflict between social time and sun time: sleep patterns from a large wearable data donation project confirm spatiotemporal solar influences year-round

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Human behavior follows complex daily, weekly and yearly patterns. To which extent these are based on social time (cultural norms and schedules and resultant zeitgeber cycles) and to which extent on sun time (solar light-dark cycles) remains widely debated. Using 1.7-2.7 years of longitudinal wearable data from over 100,000 participants across Germany, we analyzed the systematic variation in sleep patterns linked with free and workdays, geographic location, urbanization, season and Daylight Saving Time on a fine-grained spatiotemporal resolution.

Our analysis confirms a continuous East-West pattern in sleep timing in line with solar progression from East to West. This pattern was strongest on weekends, in non-metropolitan regions and summer, while blunted on weekdays, in metropolitan regions and in winter, in line with more rigid schedules and lower intensity of solar light-dark cycles. Notably, we also detected a North-South pattern in sleep timing that was opposite for weekdays and weekends, leading to amplified workday-weekend differences (social jetlag) in the North.

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S 24-6

Furthermore, sleep behavior changed with seasons irrespective of location, showing the latest and longest sleep during solar winter. While sleep duration and sleep offset exhibited yearly oscillations, sleep onset oscillated in a half-yearly fashion, peaking both in winter and in summer. Lastly, we found the adaptation of sleep timing to Daylight Saving Time was incomplete in our sample, leading to later average sleep timing (in local clock time) during the months when DST was in effect.

Taken together, our large-scale results from objective measures confirm and extend previous studies suggesting not only social but also systematic solar influences on human sleep behavior. With sleep linked tightly with health, understanding these patterns at population scale is essential for identifying modifiable risk factors and informing public policy on issues such as school and work times, daylight saving time and time zone allocation.

Symposia

S 25-1

Hypothalamic tanycytes as mediators of maternally programmed seasonal plasticity

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Maternal photoperiodic programming (MPP) allows juvenile development to be matched to forthcoming seasonal environmental conditions. In mammals, this phenomenon is driven by in utero effects of maternal melatonin on the production of thyrotropin (TSH) in the fetal pars tuberalis (PT) and consequent TSH receptor-mediated effects on tanycytes lining the 3rd ventricle of the mediobasal hypothalamus (MBH). Using LASER capture microdissection and transcriptomic profiling we show that TSH-dependent MPP controls the characteristics of the ependymal region of the MBH in juvenile animals. In Siberian hamster pups gestated and raised on a long photoperiod (LP) and thereby committed to a fast trajectory for growth and reproductive maturation, the ependymal region is enriched for tanycytes bearing sensory cilia and receptors implicated in metabolic sensing. Contrastingly, in pups gestated and raised on short photoperiod (SP) and therefore following an over-wintering developmental trajectory with delayed sexual maturation, the ependymal region has fewer sensory tanycytes. Post-weaning transfer of SP-gestated pups to an intermediate photoperiod (IP), which accelerates reproductive maturation, results in a pronounced shift towards a ciliated tanycytic profile and formation of tanycytic processes. We suggest that tanycytic plasticity constitutes a mechanism to tailor metabolic development for extended survival in variable overwintering environments.

Symposia

S 25-2

Photoperiod and temperature interaction in voles

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Voles are a group of key species in ecosystems around the world. Global warming is challenging ecosystems, because photoperiodic responses may not match seasonal temperature development, leading to sub-optimal seasonal timing. Here we present a series of studies in different vole species from different geographical locations. The photoperiodic responses in reproduction and growth shows a strong interaction with ambient temperature. It is important to understand such relationship in order to understand the effects of timing mismatch in ecosystem health evaluation.

Symposia

S 25-3

The winter blue-greens: how cyanobacteria anticipate the seasons

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Do bacteria care about the seasons? All throughout the tree of life, organisms have evolved adaptive responses that allow them to deal with the seasonal variations their environment undergoes every year. Usually, organisms rely on photoperiod as an anticipatory cue of future environmental conditions, and particular photoperiods trigger major changes in physiology, behavior and/or metabolism. Migration, flowering, diapause and hibernation are but a few examples of such responses. Despite being widespread among eukaryotes, the phenomenon of photoperiodism has never been seriously considered within the realm of prokaryotes, likely due to the assumption that bacteria generally have such short life-cycles that an elaborate mechanism to predict the comparably slower seasonal changes would be excluded in favor of simple direct responses. In our studies, we tested whether cyanobacteria could use photoperiod as a cue of future temperatures, and whether their survival to cold would be different depending on their photoperiod exposure. We observe that cyanobacteria exposed to short (winter-like) photoperiods survive ice-cold temperatures 2-3x better than those exposed to long (summer-like) photoperiods.

This effect necessitates a functional circadian clock – similarly to many plants and animals – and synergizes with low temperatures to promote survival. Remarkably, we also observed short days induce an increase in the desaturation of the lipid membrane to levels akin to that observed after actual cold exposure. We also quantified gene expression through RNAseq, and observed that exposure to short days alters global programs of gene expression, hinting at potential pathways as well as other possible photoperiodic responses in cyanobacteria.

Symposia

S 25-4

Longitudinal variation in sleep and circadian rhythms across season and photoperiod: Results from the Ecology of Human Sleep (EcoSleep) Cohort Study

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The EcoSleep Study tracked a cohort (N=12) over 12 months to study sleep determinants and how sleep varies within and between participants in real life. Continuous measures included melanopic light exposure, bedroom light/temperature/humidity/air pressure, actimetry, and glucose monitoring. Monthly 3-day sessions assessed at-home sleep EEG, ecological momentary assessment (4×/day), and evening melatonin onset and CAR. Monthly questionnaires included MCTQ, ISI, PSQI, ESS and FSI. Linear mixed models used categorical season or numeric photoperiod as predictors. Twelve participants enrolled (n=2 dropouts, n=1 withdrawal, n=1 reduced data collection). Eight participants (aged 21–33, 27±4 years, 80% female) completed data collection by April 2025. The mobile EEG showed good accuracy (87%) compared to a clinical setup in a subset (n=5). Photoperiod models outperformed seasonal models, which are reported: EEG-derived TST (n=80 nights, N_{ID}= 10) decreased by 5.4 minutes/hour of photoperiod (t(77.954)=-2.746, p=0.00749). This equates to -41.5 min in June vs December, suggesting an inverse relationship of 1:10 with ~6 minutes less sleep/60 minutes longer photoperiod. Latencies to N2/N3/REM decreased by 2.5 (p=0.0086), 3.2 (t(73.156)=-3.086, p=0.0029), and 4 minutes (p=0.0749), respectively, i.e. participants reached N2/N3/REM faster by 19.25, 23.87, and 30.8 minutes in June. The remaining sleep variables showed expected numerical trends (n.s.). Chronotype (MSFsc) was earlier by 24.5 min (β=-0.05 min; t(45.53622)=-2.295, p=0.0264) in June. MSF and MSW showed a numerical trend to advance by 15 and 10 minutes. ISI, PSQI, ESS, and FSI showed no significant effects in photoperiod models but numerical trends in seasonal models. Body weight was significantly reduced by ~1.4 kg in June (β=-0.17, t(143.02081)=-5.708, p<0.001). Preliminary results support the feasibility of long-term sleep and circadian data collection. Time-of-year effects were found for TST, latency to N2/N3/REM, chronotype, and weight. Most variance is explained by within-participant variation, highlighting the importance of individual approaches to characterising sleep and circadian rhythms.

Symposia

S 25-5

"How daily and seasonal timing aids polar adaptation in Antarctic krill (Euphausia superba)"

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Biological clocks are conserved widespread molecular mechanisms that help organisms anticipate daily and seasonal environmental changes and allows them to adjust their physiology and behavior accordingly. Antarctic krill (Euphausia superba) is a key species endemic to the Southern Ocean and one of the biomass richest species on Earth. It sustains the Southern Ocean ecosystem by serving as prey for a variety of predators including whales, penguins, and seabirds, and contributes to carbon storage through daily vertical migrations (DVM). Krill's success in this extreme environment relies on precise adaptations to daily and seasonal environmental cycles, the mechanistic basis of which remains largely elusive.

Here, we present experimental evidence that krill behavior and physiology are underpinned by circadian and circannual clocks.

During field campaigns in the Southern Ocean, we screened the locomotor activity of individual krill in different seasons under light-dark cycles and constant darkness conditions. Our data show how the krill circadian clock, in combination with light, drives a distinct bimodal activity pattern that could facilitate behavioral rhythms, such as DVM. Rapid damping and flexible synchronization of swimming activity suggest that the krill clock is adapted to a life at high latitudes.

Additionally, in a long-term experiment spanning over almost two years, krill were kept under simulated Southern Ocean photoperiods and constant darkness while physiological parameters were monitored monthly. The results indicate that sexual maturity is regulated by photoperiod, whereas lipid accumulation is under circannual control, which suggests varying needs for flexibility between the processes of reproduction and energy storage.

These findings provide critical insight into the mechanisms behind daily and seasonal environmental adaptations in krill, a prerequisite for predicting their resilience to ongoing environmental changes.

Symposia

S 25-6

Seasonal signaling between the pars tuberalis and tanycytes: a comparative study between a mammal and bird

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Mammals and birds rely on photoperiod to synchronize their seasonal physiology and behavior with annual changes in the environment. The pars tuberalis (PT) of the pituitary gland decodes photoperiodic information and regulates seasonal timing. It achieves this through thyroid-stimulating hormone (TSH) signaling to tanycytes, specialized glial cells that line the third ventricle of the mediobasal hypothalamus, which drive changes in seasonal status. Although TSH signaling between the PT and tanycytes play a crucial role in seasonal processes, additional signaling interactions are believed to contribute to seasonal responses. To explore novel PT-tanycyte communication pathways, we combined laser capture microdissection and transcriptomic analyses of the PT and tanycyte areas from both summer and winter states to investigate the ligand – receptor dynamics between the two tissues. We further compared data between the Golden hamster and the Svalbard ptarmigan, two highly seasonal animals, to identify novel conserved seasonal signaling pathways between the PT and tanycytes.

We focused on G protein-coupled receptors (GPCRs) and their ligands present in both species, with an emphasis on the bidirectional signaling between the tissues and the differential expression between short photoperiod (SP) and long photoperiod (LP). Our preliminary findings reveal seasonal differences in somatostatin and neuromedin B signaling from tanycytes to the PT. In both species, somatostatin expression was elevated during LP; however, neuromedin B showed contrasting patterns: in the hamster, its expression was induced under SP, whereas in ptarmigan, it was significantly upregulated under LP. These results suggest that somatostatin signaling is conserved across the two species, while neuromedin B exhibits species-specific adaptations, highlighting diverse strategies for coping with seasonal changes. A better understanding of the signaling interactions between the PT and tanycytes will improve our knowledge of how seasonal processes are regulated in vertebrates.
rhythms.

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