XVI
Latin American Symposium
on Chronobiology
October 4 to 7, 2021

ABSTRACTS EBOOK

INDEX

PROGRAM

Monday - October 4

15.00 **Workshops:**
17:00 **Measuring light (Conference Room 1)**
Ángeles Bonmati, Roberto Rodriguez, Graciela Tonello

Circuit-Mapping the Eskinogram (Conference Room 2)
Tristán Bekinschtein, José Duhart, Diego Fernández
18.30 **Inaugural Plenary Lecture: Daniel Cardinali**
19:30 *Chair: Diego Golombek*
Melatonin:The lost silver bullet in COVID-19 pandemic

19:30 Social at the Gathertown bar
21:00

Tuesday - October 5

09:00 **Symposium 1: Clock disruption in pre-clinical models**
11:30 *Chair: Claudia Torres-Farfán*
Ricardo Orozco Solis, Natali Guerrero-Vargas, Beatriz Baño-Otálora, Antonia Tomás-Loba, * Vinícius Tenório Braga Cavalcante Pinto
11:30 Meet the Professors at the Gathertown bar
12:30

13:00 **Plenary Lecture: Gisele Oda**
14:00 *Chair: Horacio de la Iglesia*
Telling the seasons underground: the circadian clock and ambient temperature shape light exposure and photoperiodism in a subterranean rodent
14:30 Poster presentations: ODD NUMBERS
17:00

17:30 **Symposium 2: New Perspectives on Clocks and Sleep**
20:00 *Chair: Adrián Ocampo*
Nara Muraro, Felipe Beijamini, Luciana Benedetto, Leandro Casiraghi, * Ignacio Estevan

20:00 Social at the Gathertown bar
21:30

Wednesday - October 6

09:00 **Symposium 3: Plasticity in rhythmic outputs**
11:30 *Chair: Ana Silva*
Lorena Franco, Esteban Beckwith, Daniel Alves Rosa, Laura Quintana, *Juan Ignacio Ispizua

11:30 Business Meeting in Conference Room 2
12:30

Plenary Lecture: John Hogenesch
13:00 *Chair: Fernanda Ceriani*
14:00 **Building circadian medicine in a pediatric hospital**

14:30 **Poster presentations: EVEN NUMBERS**
17:00

17:30 · **Symposium 4: A Fresh Perspective on Molecular Clocks**
20:00 *Chair: Luis Larrondo*
Gustavo Bueno da Silva Rivas, Andres Romanowski, Filipa Rijo-Ferreira, Nicolás Diaz, *Melisa Malcolm

20:00 Social at the Gathertown bar
21:30

Thursday - October 7

09:00 **Symposium 5:** Circadian rhythms in human subjects
11:30 *Chair: Claudia Moreno*
Juliana Leone, Mario Miguel, Elaine Marqueze, Victoria Garfield

11:30 Social at the Gathertown bar
12:30

13:00 **Workshops:**
15:00 **3.- Social aspects of chronobiology (Conference Room 1)**
Luiz Menna Barreto, Ennio Vivaldi

4.-Translational chronobiology (Conference Room 2)
Luis Larrondo, Malena Mul Fedele, Luis Filipe Rossi

17:30 **Symposium 6: Metabolism and non-photic inputs to the circadian clock**
20:00 Chair: Carolina Escobar
Ivana Bussi, Lorena Aguilar Arnal, Cibele Crispim, Victoria Acosta Rodríguez, * Eva Carolina Soto-Tinoco

20:00 Social at the Gathertown bar
21:30

ORGANIZING COMMITTEE

M. Fernanda Ceriani
Laboratorio de Genética del Comportamiento
Fundación Instituto Leloir

Horacio de la Iglesia
University of Washington

Diego Golombek
UNQ/CONICET

PLENARY LECTURES

Melatonin:The lost silver bullet in COVID-19 pandemic.

Daniel Cardinali
Pontificia Universidad Católica Argentina, Faculty of Medical Sciences

The therapeutic potential of melatonin as a chronobiotic cytoprotective agent to counteract the consequences of COVID-19 infections has been advocated. Because of its wide-ranging effects as an antioxidant, anti-inflammatory, and immunomodulatory compound, melatonin could be unique in impairing the consequences of SARS-CoV-2 infection. Moreover, indirect evidence points out to a possible antiviral action of melatonin by interfering with SARS-CoV-2/angiotensin-converting enzyme 2 association. Melatonin is also an effective chronobiotic agent to reverse the circadian disruption of social isolation and to control delirium in severely affected patients. As a cytoprotector, melatonin serves to combat several comorbidities such as diabetes, metabolic syndrome, and ischemic and non-ischemic cardiovascular diseases, which aggravate COVID-19 disease. In view of evidence on the occurrence of neurological sequels in COVID-19-infected patients, another putative application of melatonin emerges based on its neuroprotective properties. Since melatonin is an effective means to control cognitive decay in minimal cognitive impairment, its therapeutic significance for the neurological sequels of SARS-CoV-2 infection should be considered. Finally, yet importantly, exogenous melatonin can be an adjuvant capable of augmenting the efficacy of anti-SARS-CoV-2 vaccines.

Building circadian medicine in a pediatric hospital

John Hogenesch
Cincinnati Children's Hospital Medical Center

The past two decades have seen critical progress in understanding clock input, output, and the core clock mechanism in several models, including mammals. Despite this progress, relatively little has changed in clinical practice. We're working to change this. First, I will discuss our efforts to harmonize hospital lighting and feeding with the circadian system. Next, I will discuss how drugs are administered in the intensive care and floor units in a circadian fashion and that dosing time does matter for dozens of drugs across many different therapeutic areas. Finally, I will discuss our efforts to understand sleep timing in a healthy pediatric population, as well as, in patients with neurological disorders. I will end the talk with thoughts on how to implement this 'bedside to bench' science and its prospects for informing clinical care.

Telling the seasons underground: the circadian clock and ambient temperature shape light exposure and photoperiodism in a subterranean rodent

Gisele Oda
Laboratório de Cronobiologia Binacional Argentina-Brasil, Universidade de São Paulo, Brazil

The ability to track photoperiod, the proportion of light and darkness hours within a day, is used by organisms to anticipate the seasons and time their seasonal physiology. We investigated if and how this is achieved in a subterranean rodent, the South American tuco-tuco (Ctenomys aff. knighti) combining field work, laboratory experiments and mathematical modeling. Photoperiod measurement has been traditionally studied in lab experiments, under artificially imposed light/dark cycles. In contrast, tuco-tucos´ exposure to light is sporadic and results from their own behavior of emergence to the surface. We investigated the endogenous and exogenous regulation of this behavior and its consequences to photoperiod measurement. In the field, animals carrying biologgers displayed seasonal patterns of daily surface emergence, exogenously modulated by temperature. In the laboratory, constant lighting experiments revealed the endogenous regulation of seasonal activity and the multi-oscillatory structure of the circadian clock. Finally, mathematical modeling corroborated that tuco-tuco's light exposure across the seasons is sufficient for photoperiod encoding. Together, our results elucidate the interrelationship between the circadian clock and temperature in shaping seasonal light exposure patterns that convey photoperiod information in an extreme photic environment. This work is the result of a 13 year collaboration with Dr. Veronica Valentinuzzi (CRILAR, Argentina).

SYMPOSIA

Symposium 1: Clock disruption in pre-clinical models

Beatriz Baño-Otálora
Division Neuroscience and Experimental Psychology | Faculty of Biology, Medicine and Health, University of Manchester, UK

Impact of dim daytime light exposure on the circadian clock in a diurnal mammal

Circadian rhythms evolved under conditions in which there was a huge difference in light intensity between day and night. We now live in a society in which, on the one hand, we experience artificial light at night, and on the other, we deprive ourselves from exposure to bright light during the day by spending most of the waking hours in relatively dimly lit indoor environments. In recent years, there has been a growing understanding of the potential negative effects that exposure to light-at-night can have on human health. However, our knowledge of the impact of exposure to low light intensity during the day is much more limited. Here, we asked whether decreasing daytime irradiance alters circadian rhythms in animal's physiology and behaviour and clock function, using a diurnal rodent, Rhabdomys pumilio. To this end, we measured behavioural and physiological rhythms and master clock electrophysiological activity following stable entrainment to 12:12 light:dark cycles at different daytime intensities (ranging from 1,900 to 18 lx melanopic equivalent daylight illuminance). We found that exposure to dim daytime light reduces not just the reproducibility and robustness of behavioural and physiological rhythms, but also circadian amplitude in spontaneous activity rhythms within the central circadian pacemaker in the mammalian brain. These findings reveal an impact of light on circadian amplitude and highlight the potential importance of daytime light exposure for circadian health.

Natali Guerrero-Vargas
Departamento de Anatomía, Facultad de Medicina, Universidad Nacional Autónoma de México

Scheduled feeding prevents depressive and anxiety-like behavior in rats due to circadian disruption

Individuals who regularly shift their sleep timing, like night and/or shift-workers suffer from circadian desynchrony. Shift-work is suggested to be a risk factor for the development of mood disorders such as the burn out syndrome, anxiety, and depression. Food intake restricted to the normal activity phase is a potent synchronizer for the circadian system and can prevent the detrimental health effects associated with circadian disruption. We explored whether adult male Wistar rats exposed to an experimental model of shift-work (W-AL) developed depressive and/or anxiety-like behaviors and whether this was associated with neuroinflammation in brain areas involved with mood regulation. We also tested whether time-restricted feeding (TRF) to the active phase could ameliorate circadian disruption and therefore would prevent depressive and anxiety-like behaviors as well as neuroinflammation.

Here we showed that TRF prevented behavioral changes and decreased neuroinflammation markers in the brain of rats exposed to shift-work. Present results add up evidence about the importance that TRF in synchrony with the light-dark cycle can prevent neuroinflammation leading to healthy mood states despite circadian disruptive conditions.

This study was supported by grants PAPIIT-IA 206620 and División de Investigación, Facultad de Medicina UNAM.

Ricardo Orozco Solis
Laboratorio de Cronobiología y Metabolismo, Instituto Nacional de Medicina Genómica, INMEGEN, México

The circadian clock in the astrocytic control of energy balance

Hypothalamic circuits compute systemic information to control metabolism. Astrocytes residing within the hypothalamus directly sense nutrients and hormones, hereby modulating neuronal outputs. Consequently, these glial cells play an important role in metabolic control, which is not fully understood. Besides, growing research indicates that the circadian clock in the hypothalamic nuclei contributes to the regulation of peripheral metabolism. We sought to explore the role of the astrocytic circadian clock in the control of energy balance. To tackle this question, we used genetically modified mice in combination with a number of metabolic, behavioral and physiological approaches. Our current data demonstrates that under high caloric diet, young

adult mice bearing an astrocyte-specific disruption of the clock show time-specific higher energy expenditure, improved systemic response to glucose, and favor carbohydrates as fuel source over fat when compared with their control littermates. Moreover, these mice present higher preference for high palatable caloric food during the dark period. These results suggest that the astrocytic circadian clock could play a role on circadian regulation of energy balance by controlling both metabolic and non-metabolic responses.

Antonia Tomás-Loba

Circadian Rhythm and Cancer Unit, Chronobiology Laboratory, Department of Physiology, Murcia University, Spain

Circadian rhythm and cancer: an approximation with diurnal animal models

As a way to anticipate changes, the circadian system (CS) influences body physiology keeping it ready and healthy to respond to rhythmic fluctuations. Thus, shift work, chronic jet lag, sleep disturbances, or any other chronodisruption (CD), directly impact health status. Besides, several epidemiological studies have linked CD to an increased incidence of specific cancers. In support of that, CD was classified as a probable carcinogen by the World Health Organization in 2007. Liver physiology is subjected to a tight circadian regulation mostly influenced by the feeding time. Hepatocellular carcinoma is the most abundant type of liver cancer (LC) and the 3rd leading cause of cancer-death worldwide. LC is estimated to increase more than 61.9% in 2040, this dramatic increase is unequivocally related to today's modern lifestyle. Of note, it is the correlation between the effort invested in cancer research and clinical trials that come to completion: only 3.4% of clinical trials in cancer treatment get the approval of the FDA. One important argument for this low success rate could be due to the current use of nocturnal experimental animals. Thus, it is of utmost importance to understand the relevance of the CS in physiology and liver cancer using diurnal animal models. In our lab, we are starting to open a new avenue in cancer research using Arvicanthis niloticus, a diurnal rodent that can develop type 2 diabetes and liver cancer spontaneously.

*** Vinícius Tenório Braga Cavalcante Pinto**

Centro de Medicina Circadiana - Faculdade de Medicina, Universidade Federal de Alagoas, Maceió – Brasil.

RNAseq analysis of the prefrontal cortex of mice exposed to a daylength transition model
(Short talk selected from posters)

Several epidemiological studies have described a seasonal variation of suicide rates and mania symptoms with peaks in spring and early summer. We previously demonstrated that mice exposed to a photoperiodic transition protocol present a mania-like behavior that is prevented by lithium administration. In this work, we evaluated transcriptome alterations in the prefrontal cortex (PFC) of this model. We used thirty mice (C57BL/6) divided into: Equatorial photoperiod (12:12LD) group (n=15); DTM (Daylength Transition Model) group (n=15), initially subjected to 12:12LD regime before stabilization at short photoperiod (8:16LD) for 8 days, then an increasing photoperiodic condition (1h/day) until reach 16:8LD where the samples were collected (ZT06). PFC were pooled in three replicates (n=5 for replicate) for each group for RNAseq. Sequences were annotated using Kallisto and the differentially expressed genes (DEGs) were determined using Deseq2 (Fdr<0.05). We identified 102 DEGs (49 up-regulated in DTM). Some of these DEGs were previously associated with neuropsychiatric disorders, including Schizophrenia, Autism, Parkinson and Alzhheimer. Serpinh1, a repressed gene in DTM was previously identified to be downregulated in the brain of suicidal patients. These results indicate that important neurological changes occur at the molecular level in the prefrontal cortex of a potential model for bipolar disorder and suicidal behavior induced by photoperiodic transitions.

Symposium 2: New Perspectives on Clocks and Sleep

Felipe Beijamini

Federal University of Fronteira Sul, Brazil

Later school start time and adolescents sleep, sleepiness and emotion

Adolescen's insuficiente sleep is a public health concern. In a multifactorial condition, adolescents are prone to go to bed later, as well as wake up later than children or adults. However, school start times are historically set for early times, (7AM to 745AM, in Brazil), resulting in situation of chronic sleep deprivation for adolescents. Developing a more inclusive educational system is needed, and school start time is an important factor that must be addressed. Consequently, we need to evaluate the effect of delaying school start time in adolescent's

sleep, sleepiness, mood, cognition and several other health aspects in order to be able to advocate for a significant change in school start time, specially during adolescence.

Luciana Benedetto

Departamento de Fisiología, Facultad de Medicina, Universidad de la República, Uruguay

Does sleep influence lactation in the postpartum rat?

Since the decade of the eighties, it has been claimed that milk ejection (ME) in the mother rat must be preceded by NREM in order to occur. Also, sleep deprivation (SD) using gentle handling (GH) in the female rat has been associated to an impossibility to eject milk. However, this is not a quality shared by all mammals, including the human female. Although SD is a stressful situation, most common methodologies used in laboratory environments, usually involve aversive stimulus/situations, with additional unwanted effects. Electrical stimulation of the brainstem reticular formation to prevent sleep might generate better behavioral outcomes. The aim of our study is: 1) determine if there is any correlation between sleep and ME. 2) compare ME occurrence and the amount of milk ejected during SD period using two different techniques: GH and deep brain electrical stimulation (DBES) in postpartum rats. The main findings show a positive linear correlation between the number of ME and most sleep stages, including NREM sleep ($r = 0.4$, $p = 0.01$). Also, the frequency of ME was significantly reduced during GH (1.0 ± 0.4) compared to control values (3.6 ± 0.4; $p = 0.02$) and DBES (2.8 ± 0.4; $p = 0.03$), but no differences between control and DBES were found. We conclude that the mother rat is able to eject milk when she is sleep deprived, but DBES seems to be a better methodology to prevent sleep in the postpartum rat, as she ejects milk similarly to control values.

Leandro Casiraghi

Department of Biology, University of Washington

Human sleep, then and now: from moonlight to the light bulb

The evolution of human sleep, in particular since the industrial revolution and the widespread use of electric light, is still a matter of intense study. In this sense, working with communities that live in pre-industrial, isolated conditions provides a unique window into the sleep in more primitive human groups. In this presentation I will summarize the work done by the de la Iglesia lab in the last decade with different Toba/Qom communities in the north of Argentina, and what we have learnt from it regarding the effect of electric light and natural environmental variables on sleep. First I will talk about how sleep is delayed in Toba/Qom communities with full access to electricity and how this effect is particularly strong in the winter season. Then I will describe how we tested whether and how these effects of electric light and seasons reflected changes in the master circadian clock. Lastly, I will discuss our recent work describing the modulation of human sleep across the moon cycle both in isolated Toba/Qom communities as well as in industrialized populations.

Nara Muraro

Instituto de Investigación en Biomedicina de Buenos Aires (IBioBA)-CONICET

Physiological bases of Drosophila circadian and sleep neurons

Circadian rhythms have been extensively studied in *Drosophila*, however, still little is known about how the electrical properties of clock neurons are specified. Our work focuses on understanding how neurotransmitter inputs and voltage-gated ion channels allow neurons to achieve particular action potential firing patterns, which in turn translate into complex circuital mechanisms that regulate behavioral outputs. The lateral ventral neurons (LNvs) of *Drosophila* come in two flavors, the small type (sLNvs) with a recognized role in circadian synchronization and the large type (lLNvs) that function as arousal neurons, inhibiting sleep. Although these overt behavioral effects have been studied, information is lacking about the physiological bases of these neuronal clusters and their interaction. We have performed a behavioral genetic screen through the downregulation of candidate ion channels in the LNvs finding that the hyperpolarization-activated cation current Ih is important for the behaviors that the LNvs command. Using whole-cell patch clamp electrophysiology we demonstrate that Ih is necessary to achieve a high frequency bursting firing pattern of both types of LNvs. Moreover, using a technically challenging simultaneous recording of a sLNv and a lLNv we have discovered that firing among these neuronal types is synchronized. We will use this novel preparation to explore the LNv microcircuit.

* Ignacio Estevan

Instituto de Fundamentos y Métodos, Facultad de Psicología, Universidad de la República

Bidirectional association between light exposure and sleep in adolescents

(Short talk selected from posters)

During adolescence, changes in both circadian and homeostatic factors are related to the delayed sleep timing observed in adolescence, which is related to restrictions in their sleep duration. In addition, the circadian factor allows light exposure and sleep pattern to be related. We recorded 15 high-school students for 11 vacation days and 12 school term days using GENEactiv accelerometers. Using a repeated-measures analysis, we explored the day-to-day bidirectional association between light exposure and sleep behavior across a period with extreme variability in social pressures. When the previous day light was 10 times more intense the sleep onset was more than 30 min earlier and the sleep duration was almost 20 min longer. In addition, when sleep ended 1 h later the light intensity had a reduction of more than 20%. Sleep onset and offset were both later during vacation than on school days (almost 2 h and 4 h, respectively), and free days (almost 1 h), while sleep duration was almost 2 h longer on vacation and free days than on school days. On the other hand, light exposure intensity was two times higher during vacation days when adjusted by sleep timing. Although we found that light exposure was associated with longer sleep duration, the influence of school start times was greater and ended up prevailing, which explained the short sleep durations observed on school days.

Symposium 3: Plasticity in rhythmic outputs

Daniel Alves Rosa

Federal University of Goias

Hidden cardiometabolic dysfunctions associated with internal forced desynchronization

In the 7/24's society and mainly in shift workers are observed a growing incidence of obesity, dyslipidemia, diabetes, hypertension, and cardiovascular events. Several evidence shown that parts of these cardiometabolic dysfunction can be attributed to changes in the circadian timing system. We recently shown that rats chronically exposed to a symmetrical light-dark cycle of 22 h (T22) – an animal model of forced internal desynchronization – showed increased cardiometabolic risks. Here, we'll focus on extend our understanding recent about cardiometabolic outcomes by three approaches using T22 as circadian desynchronization model in rats. The first refers results related to assess the effects of the food's restriction at dark phase of LD cycle throughout the T22 protocol, observing whether the cardiometabolic effects previously founds could be associated with an altered daytime of food intake. Second, under T22, rats express two simultaneous components of locomotor activity rhythm, with different periods. Sometimes theses rhythms are in coincidence with activity phase (CAP) or in opposite phase, non-coincidence phase (NCAP). Then our approach refers to assess the rat's cardiac function by Langendorff heart model at distinct activity phase (i.e., CAP and NCAP). Finally, we'll also report here that the rat offspring from mothers exposed to T22 cycles during pregnancy shown a cardiac remodeling in its adult life, suggesting a hidden risk of cardiometabolic diseases.

Esteban Beckwith

Instituto de Fisiología, Biología Molecular y Neurociencias (IFIBYNE-UBA-CONICET), Buenos Aires, Argentina

A fat body-brain axis controls sickness behaviour

Infected animals undergo behavioural changes that are collectively called "sickness behaviours". Some of these changes are infection specific, but most infections cause a common suite of behavioural changes that include anorexia and disruption of sleep/wake activity cycles. These behavioural changes have significant effects on human and animal health and well-being. Despite the importance of sickness behaviours, they are relatively poorly understood. Here, we employ Drosophila to study the genetic and molecular underpinnings of sickness behaviour. This model provides many benefits since several behaviours are well understood and the fly has a relatively simple and well-studied immune response, allowing us to connect infection-induced changes in behaviour to their underlying mechanisms. We have used a new behavioural profiling platform to analyse the interaction between locomotor activity and infection in Drosophila. We find that both pathogenic and non-pathogenic bacterial infections can cause sleep disruption. We then tested various bacterial infections and Drosophila immune mutants, to determine how pathogen recognition and immune pathway activation contribute to the sleep disruption we observe. We find that fat body-derived spätzle, and an active Toll signalling pathway in neurons, are necessary for the marked increase in activity triggered by bacterial recognition. We propose that pathogen sensing activates a fat body-brain axis that directly influences over behaviour.

Lorena Franco
Departamento de Física Médica, Centro Atómico Bariloche, Comisión Nacional de Energia Atómica Bariloche, Rio Negro, Argentina

Modulation of the circadian clock by mating-triggered signals in Drosophila melanogaster

After mating the female physiology undergoes several important changes, which are reflected in their rest-activity cycles. To explore the hypothesis that the mating modifies the normal function of circadian clock and the temporal organization of the behavior, we performed a high resolution analysis of locomotor activity using a video tracking method. By comparing the rest-activity cycles we observed that mated females lose their ability to anticipate the night-day transition, when compared to males and virgins. Our results show that this postmating response is mediated by the action of the sex peptide (SP) principally on pickpocket (PPK) neurons, since the decreased expression of the SP receptor (SPR) in these neurons restores the ability to anticipate the light/dark transition in mated females. We show the existence of synaptic input provided by the PPK neurons onto the pigment-dispersing factor (PDF)-positive ventral lateral neurons (sLNv). Since PDF levels has been associated to the generation of morning activity peak, we hypothesized that SP signal is involved on PDF levels modulation. Indeed, herein we demonstrate that mated females have reduced levels of PDF at the dorsal protocerebrum compared with males and virgin females and the downregulation of SPR receptor in PPK neurons mimics the male levels. Our results point to a model whereby mating-triggered signaling impinges the clock network to modulate changes in the temporal organization of circadian behaviors.

Laura Quintana
Instituto de Investigaciones Biológicas Clemente Estable – MEC. Montevideo, Uruguay

Seasonal rhythms in the hormonal modulation of aggression: contributions from a native teleost fish

Organisms that inhabit the temperate zone exhibit various seasonal adaptive behaviors, including reproduction and aggression. Both of these behaviors are usually associated with gonadal androgens and estrogens during the breeding season. Gymnotus omarorum, a teleost electric fish which is native of Uruguay, presents a clear seasonal variation in gonadal development. Nevertheless it displays robust territorial aggression all year long in both males and females. We hypothesize that this persistent aggression is driven by seasonal hormonal mechanisms, which are mainly gonadal in the breeding season and brain-derived during the non-breeding season. Acute blocking of aromatase, the enzyme which converts androgens to estrogens, significantly decreases aggression intensity in the non-breeding, but not breeding, season. This reveals a seasonal role of fast-acting estrogens upon territorial behavior. The quantification of plasmatic and brain steroids shows that the brain is the sole producer of estrogens in both males and females during the non-breeding season. In addition, preliminary evidence shows there is a seasonal variation in the expression of genes directly linked to the androgenic-estrogenic systems in the preoptic area, a key node of the social brain network. In all, this teleost model reveals the seasonal plasticity in the role of neuroestrogens in the control of aggression, a strategy that has been shown to be common across distant vertebrate species.

***Juan Ignacio Ispizua**
Laboratorio de Genética del Comportamiento, IIBBA-FIL

The impact of glial signals on neuronal structural plasticity
(Short talk selected from posters)

Recently, we described that a functional glial clock is necessary for circadian plasticity in the small lateral ventral neurons (sLNvs), a group of key pacemaker neurons of D. melanogaster. Circadian structural plasticity involves rhythmic changes in the degree of arborization and fasciculation of their dorsal termini. The sLNvs express PDF, a neuropeptide relevant in the synchronization of the clock network that oscillates in phase with this remodelling process. We have previously demonstrated that circadian plasticity modifies the way the pacemaker circuitry is wired regularly, but its impact on behaviour and the molecular basis that control this process are yet to be defined. Building upon our previous results, we examine in depth the impact of neuronal-glial connectivity. Using GFP reconstitution analysis (GRASP), we found that sLNv termini contact directly with two different glial subtypes (astrocyte-like and ensheathing glia) and that these contacts are time-of-the-day dependent. Interestingly, blocking adult glio-transmission has different effects on PDF levels and plasticity depending on the type of glia recruited and the length of the treatment (12 or 24 hours). Preliminary experiments show that preventing clock oscillations in different glial subtypes affect circadian plasticity distinctively. Taken together, our results suggest a complex glial implication in the modulation of adult structural plasticity with distinct roles for different glial subtypes.

Symposium 4: A Fresh Perspective on Molecular Clocks

Gustavo Bueno da Silva Rivas

Postdoctoral Research Associate, Department of Biology, Texas A&M University

CWO promotes CLK-CYC activation via the conserved repressor CIPC

The Drosophila circadian clock is driven by a transcriptional feedback loop in which the bHLH transcription factor CLOCK-CYCLE (CLK-CYC) binds E-boxes to transcribe genes encoding the PERIOD-TIMELESS (PER-TIM) repressor, which releases CLK-CYC from E-boxes to inhibit transcription. The bHLH-Orange transcription factor CLOCKWORK ORANGE (CWO) reinforces PER-TIM repression by binding E-boxes to maintain PER-TIM bound CLK-CYC off DNA, but also acts through an unknown mechanism to promote CLK-CYC transcription. To determine how CWO activates CLK-CYC transcription, we identified CWO DNA binding targets that are upregulated in the absence of CWO repression, conserved in mammals and preferentially expressed in brain pacemaker neurons. Among the genes identified was the Drosophila ortholog of mammalian Clock Interacting Protein Circadian (Cipc) that acts to repress CLOCK-BMAL1 transcription. Reducing or eliminating Drosophila Cipc expression shortens circadian period while overexpressing Cipc lengthens circadian period in flies, consistent with previous analysis showing that Drosophila Cipc represses CLK-CYC transcription in S2 cell culture. Long period rhythms of cwo mutant flies are largely rescued when Cipc expression is reduced or eliminated, indicating that increased Cipc expression mediates period lengthening of cwo mutants. These results suggest a mechanism for CWO-dependent CLK-CYC activation: CWO inhibition of CIPC repression promotes CLK-CYC transcription.

Nicolás Diaz

University of Washington-Department of Ophthalmology

Wounding Induces Facultative Opn5-Dependent Circadian Photoreception in the Murine Cornea

Autonomous molecular circadian clocks are present in the majority of mammalian tissues. These clocks are synchronized to phases appropriate for their physiologic role by internal systemic cues, external environmental cues, or both. The circadian clocks of the in vivo mouse cornea synchronize to the phase of the brain's master clock primarily through systemic cues, but ex vivo corneal clocks entrain to environmental light cycles.

Molecular circadian clocks of the cornea remain in phase with behavioral circadian locomotor rhythms in vivo but are photoentrainable in tissue culture. After full-thickness incision or epithelial debridement, expression of the opsin photopigment Opn5 is induced in the cornea in a subset of preexisting epithelial cells adjacent to the wound site. This induction coincides with conferral of direct, short-wavelength light sensitivity to the circadian clocks throughout the cornea.

Corneal circadian rhythms become photosensitive after wounding. Opn5 gene function (but not Opn3 or Opn4 function) is necessary for induced photosensitivity. These results demonstrate that opsin-dependent direct light sensitivity can be facultatively induced in the murine cornea.

Filipa Rijo-Ferreira

Takahashi Lab, Department of Neuroscience, UT Southwestern Medical Center at Dallas

Circadian rhythms in parasitic diseases: and underlying clock of malaria parasites

Our rhythmic world has been a driving force for organisms to evolve a clock to anticipate such daily rhythms. Similarly, our own circadian biology leads to body rhythms that parasites experience. Malaria is a parasitic disease whose major symptom is fever. Malarial rhythmic fevers are a consequence of the synchronous bursting of host's red blood cells (RBCs) on completion of the malaria parasite asexual cell cycle. How is this bursting synchronous across the parasite population? Are parasites following host cues or do they also have a clock to anticipate host daily rhythms? Through a combination of infection challenges where we manipulate the environment or rhythms of the host by infections of circadian mutant hosts, we probed the rhythms of the

parasites. We found that without any external cue to the parasites, their transcriptome remains rhythmic with ~60% of parasite transcripts being expressed once a day. Thus, we propose malaria parasites to have intrinsic clocks. Parasite rhythms are aligned to the host daily rhythms but are generated by the parasite, possibly to anticipate its circadian environment.

Andres Romanowski

The University of Edinburgh, School of Biological Sciences, UK

Life beyond circadian transcription: clock control of alternative splicing in Arabidopsis

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

***Melisa Malcolm**

CIQUIBIC-Dto. Qca. Biológica Dr. Ranwell Caputto, FCQ. UNC.

Processing body and stress granule rhythms in cell cultures

(Short talk selected from posters)

Stress granules (SGs) and processing bodies (PBs) are cytoplasmic membraneless organelles that are formed by liquid-liquid phase transitions. They are involved in the regulation of translation, stability and storage of mRNA and contain mRNA and several RNA binding proteins. SGs form in response to different stress stimuli, typically through phosphorylation of the eIF2alpha. PBs are constitutively present but increase in number under stress conditions. Since stress response has been shown to be circadianly regulated, we wonder whether SGs and PBs oscillate. NIH/3T3 and N2a cell cultures were synchronized with dexamethasone and harvested every 4 h for 68 h. We induced the formation of SGs with sodium arsenite (oxidative stress). We performed a double immunolabeling of SGs (eIF3 and G3BP1) and PB (GE-1/HEDLS and DDX6) by immunocytochemistry. We studied the phosphorylation temporal profile of eIF2alpha and eIF3 levels and we found no differences over time. We observed that NIH/3T3 and N2A cells show daily rhythms in SGs and PBs, respectively, for three variables: number, area, and signal intensity, with periods of approximately 24 h. These findings suggest that the molecular circadian clock controls SGs and PBs. To determine this hypothesis, we analyzed their formation in Bmal1-/- fibroblasts. Surprisingly, the rhythm persisted in these cells. The results presented here reveal new ways in which translation and cytoplasmic mRNA metabolism can be modulated over time.

Symposium 5: Circadian rhythms in human subjects

Victoria Garfield

Institute of Cardiovascular Science | University College London, UK

Sleep and diseases of ageing: what does the epidemiology say and how has genetics helped so far?

Both duration and quality of sleep have long been associated with poorer health outcomes in epidemiological studies. With a global ageing population, we now face several challenges in terms of: 1) understanding the underlying mechanisms behind some of these associations, 2) the complex interrelationships between the sleep phenotypes themselves and their relationships with poorer health, and crucially, 3) disentangling which of these associations might be causal in nature. The ability to prioritise which of these associations we need to focus on, poses yet another challenge, which relates to magnitude of effects, robustness of observational associations and plausibility of biological pathways. Genetic epidemiology has begun to shed some light on

some of these issues, but we still have a long way to go. In this talk, I will present some of the research by both our research group and others to illustrate where genetic methods are beginning to help us understand some of the underlying biology, as well as causality between sleep phenotypes and diseases of ageing.

Juliana Leone

Laboratorio de Cronobiología, Universidad Nacional de Quilmes. Buenos Aires, Argentina
Laboratorio de Neurociencia. Universidad Torcuato Di Tella. CABA, Argentina

Sleep and biological rhythms under lockdown: lessons and opportunities

Many aspects of our daily lives changed under the lockdown associated with the COVID-19 pandemic, including light exposure and daily social schedules. Consistently, our sleep and daily rhythms were expected to be affected. Here I will present results from two different but complementary studies. First, we evaluated the impact of the lockdown on sleep and biological rhythms on a participants' subset of Crono Argentina (www.cronoargentina.org). Sleep was longer and later during lockdown weekdays, social jetlag was lower but chronotype was delayed during lockdown, compared with the control condition. Second, we developed a mobile app (MiRelojInterno, www.mirelojinterno.org) that provides evidence-based and customized recommendations based on local data. The contribution of these results exceeds the generation of scientific knowledge, offering a tool designed to help users to improve and maintain healthy sleep and biological rhythms.

Elaine Marqueze

Universidade Católica de Santos, Brasil

Melatonin supplementation can decrease the circadian misalignment in night workers?

Shift workers experience chronic circadian misalignment, which can manifest itself in reduced melatonin production, and has been associated with many disorders. In addition, chronotype modulates the effect of night shift work, with early types presenting greater circadian misalignment when working night shift as compared to late types. Melatonin supplementation in humans has been used since the mid-1980s in order to treat circadian rhythm sleep disorders by shifting the timing of the circadian. Exogenous melatonin can be considered a chronobiotic capable of phase shifting the circadian timing in humans according to a phase response curve. In this discussion, we will analyze the effects of melatonin supplementation on the circadian misalignment in night workers from a clinical trial conducted among overweight night shift workers, according to chronotype, under real-life conditions.

Mario Miguel

Laboratório de Neurobiologia e Ritmicidade Biológica, Departamento de Fisiologia e Comportamento, Programa de Pós-Graduação em Psicobiologia, Centro de Biociências, Universidade Federal do Rio Grande do Norte, Brasil

The need for family-based experimental designs in chronobiology: the chronotype as an example

It is well established that the oldest chronotype questionnaire, the morningness-eveningness questionnaire (MEQ), has significant heritability, and several associations have been reported between MEQ score and polymorphisms in candidate clock genes, several of them reproducibly across populations. By contrast, there is only one report of heritability for the Munich chronotype questionnaire (MCTQ). Recent genome-wide association studies (GWAS) from large cohorts have reported multiple associations with chronotype as assessed by a single self-evaluation question. The most significant challenge in this field is the need for particular types of research, twin-based studies and family-based studies. I will present the results of heritability of chronotype from the Brazilian Baependi Heart Study, a large cohort with family-based design, in which we explored the heritability of not only the circadian but also the homeostatic drive of the sleep-wake cycle. Moreover, I will present findings that also suggest that the single chronotype question, used for large GWAS analyses, captures a larger proportion of the dimensions of chronotype than previously thought.

Symposium 6: Metabolism and non-photic inputs to the circadian clock

Victoria Acosta Rodríguez

Takahashi Lab, Department of Neuroscience, UT Southwestern Medical Center, USA

Circadian alignment of feeding regulates lifespan extension by caloric restriction

Caloric restriction (CR) has remarkable benefits on longevity, yet the mechanisms remain unclear. Under CR, mice self-impose chronic cycles of 2h-feeding/22h-fasting, raising the question whether calories, fasting or time of day are causal. To address this, we tested an AL control group and five CR protocols with different feeding time and fasting duration. After 6 weeks of baseline AL food access, C57BL/6J male mice were subjected to 30% CR with: one meal every 24h starting at the beginning of the day or night (classical protocols in which mice self-impose < 2h feeding, CR-day and CR-night), or spread out for 12h during the day or night (CR-day-12h and CR-night-12h), or evenly spread out throughout the 24h (CR-spread) in which the daily feeding pattern is abolished. We found that while CR was sufficient to extend lifespan; however, the feeding time acted synergistically. Circadian alignment of feeding with at least 12h of fasting enhanced CR-mediated benefits on survival in mice, independently of body weight. Aging promotes widespread upregulation of inflammation-related genes and downregulation of metabolic pathways in liver from ad lib fed mice; whereas CR at night ameliorates these aging-related changes. Overall, our results demonstrate that circadian interventions promote longevity and provide a novel perspective for elucidating mechanisms of aging.

Lorena Aguilar Arnal

Instituto de Investigaciones Biomédicas, Universidad Nacional Autónoma de México-UNAM

NAD+ signaling to the circadian clock: is chronotherapy a better approach to treat metabolic disfunction?

The circadian clock is a time-tracking endogenous system which anticipates and coordinates adaptation to daily environmental fluctuations. Circadian misalignment leads to obesity, which is accompanied by reduced levels of the clock-controlled metabolite NAD+. Concomitantly, increasing NAD+ levels is emerging as a therapy for diet-induced obesity and type 2 diabetes; however, the impact of circadian fluctuations of NAD+ on these therapies remains unknown. In our lab, we have characterized the metabolic consequences of circadian rhythms in NAD+ levels, exposing the time-dependent benefits of NAD+-related treatments.

Ivana Bussi

De la Iglesia Lab, Department of Biology, University of Washington, Seattle, WA, USA

Looking for the fear-entrained oscillator

Non-photic stimuli have been long shown to be able to entrain circadian rhythms. In the present talk, I will present our most recent results that demonstrate that cyclic fear acts as a synchronizer of circadian rhythms in mice. Applying a cyclic fearful stimulus can cause nocturnal mice to become diurnal and to maintain the diurnal behavior after release into constant conditions. Our results also show that the canonical circadian transcription-translation feedback loop in the SCN is necessary, but not sufficient, for the mice to entrain to cyclic fear. Using region-specific Bmal1 KO we were able to dissect the role of the SCN in the fear entrainment. We also studied the impact of fear entrainment on the release of stress-associated hormones ACTH and cortisol, as well as the expression of clock genes and key metabolic enzymes in peripheral organs such as the adrenal gland and the liver. By using ISH we studied whether the behavioral phase shift caused by fear entrainment was encompassed by a shift in the phase of expression of clock genes in the SCN and fear-related brain structures. Our results suggest that the regions in the brain that encode and modulate the behavioral responses to fear and the neural circuitry that constitutes the circadian system are linked and work in an ensemble to provide the ability to respond adaptively to 24-h cyclic threats in the environment.

Cibele Crispim

Professor Associado II, Curso de Nutrição, Faculdade de Medicina da Universidade, Brasil

Impact of mealtime on metabolism and the clock

Chrononutrition proposes that nutrients or meal timing per se could affect the circadian clock system, and that the desynchronization of biological rhythms could negatively influence timing and food choices. Several

studies from this area have suggested that time-related eating patterns – such as energy distribution throughout the day, nocturnal eating and eating frequency – may influence nutrient metabolism and be associated with metabolic and nutritional diseases. Given the growing amount of evidence linking the circadian clock system to metabolic and nutritional health, circadian organization seems to be clinically important in understanding and treating diseases such as obesity. In this way, chrononutrition emerges as an important tool to enhance the metabolic and nutritional health of particular population groups (e.g., shift workers) and in the treatment of diseases such as obesity.

*** Eva Carolina Soto-Tinoco**

Departamento de Biología Celular y Fisiología, Instituto de Investigaciones Biomédicas. Universidad Nacional Autónoma de México

The suprachiasmatic nucleus modulates the sensitivity of visceral sensory areas

(Short talk selected from posters)

The suprachiasmatic nucleus (SCN), our autonomous biological clock, synchronizes behavior with physiological parameters through hormones and the autonomic nervous system. We show that the SCN needs to receive peripheral feedback to fine-tune its output, adjusting physiological processes to the requirements of the moment. We demonstrated that the SCN is incorporated in a neuronal feedback circuit arising from brainstem visceral sensory neurons located in the nucleus of the solitary tract (NTS), and responds to blood pressure changes. Herewith, the SCN can react to hemodynamic perturbations and adapt the blood pressure response accordingly.

The SCN can also modify the sensitivity of brain areas that directly receive feedback information from the periphery. For example, brainstem and spinal cord visceral sensory neurons show a time-of-day-dependent sensitivity to incoming inflammatory information. This circadian gating process allows the organism to have an efficient inflammatory response only during the activity phase when it is most likely needed. Altogether, this data illustrates the role of the SCN as a structure that receives feedback from the periphery and in addition, modulates the sensitivity of visceral sensory areas to incoming stimuli in a circadian fashion.

POSTERS

CIRCADIAN PHYSIOLOGY AND BEHAVIOR

#A-1
Motivation behavior in mice under restricted feeding conditions

Julieta Acosta, Macarena Esquivel, Diego Golombek and Patricia Agostino
1.Laboratorio de Cronobiología, Universidad Nacional de Quilmes/CONICET, Buenos Aires, Argentina.

Presenting Author: **Julieta Acosta**

In mammals, the circadian system modulates several behavioral and physiological processes, including the response to natural rewards such as food. On the other hand, when food is temporally restricted, animals display an anticipatory food activity (FAA) that is controlled by a food-entrainable oscillator (FEO).
We have previously shown that mice under a 12:12 light/dark (LD) cycle exhibited a diurnal rhythm in motivation for food reward, becoming more motivated during the night (active phase). This rhythm was also evident under constant dark (DD) conditions, indicating the endogenous nature of this modulation.
In this work, we present evidence that motivation for food reward it´s involved in food anticipatory activity (FAA) regardless of mice being food restricted during the day or night phases of the LD cycle. Mice in a restricted feeding (RF) protocol under a 12:12 LD cycle were allowed to consume food only 3 hours during daytime or nighttime. Then, motivation behavior was assayed - through the progressive ratio (PR) schedule - in two different time points: during FAA (i.e, two hours before food availability) and in the opposite phase to wich the RF was carried out. Our results show that mice are highly motivated to work for food reward when FAA is present regardless of the time of day. These results suggest that, during FAA, components related to reward pathways might be activated and consecuently generate an increase in motivation bypassing circadian time cues.

CIRCADIAN PHYSIOLOGY AND BEHAVIOR

#A-2

Circadian disruption induced by tumor development in a murine model of melanoma

Ignacio Aiello[1], Fernanda Roman[2], Malena Iis Mul Fedele[3], Diego Andrés Golombek[1], Natalia Paladino[1]

1.1 Laboratorio de Cronobiología, Universidad Nacional de Quilmes, Buenos Aires, Argentina.
2.Departamento de Física Médica, Centro Atómico Bariloche, CNEA – CONICET, San Carlos de Bariloche, Argentina.
3.Institute for Biomedical Research (BIOMED), Catholic University of Argentina (UCA) and National Scientific and Technical Research Council (CONICET), C1107CABA Buenos Aires, Argentina.

Presenting Author: **Ignacio Aiello**

Dysfunctions in clock-controlled body functions, such as sleep disorders, as well as deregulation of clock gene expression or glucocorticoid levels has been observed in cancer patients. Moreover, these disorders have been associated with a poor prognosis. This work explored the circadian rhythms at behavioral and molecular levels in a murine melanoma model induced by subcutaneous inoculation of B16 tumoral cells. We observed that the presence of the tumors induced a decrease in the sturdiness of the locomotor activity rhythms and in the amount of night time activity together with a delay in the acrophase and in the activity onset. Moreover, these differences were more marked when the tumor size was larger than in the initial stages of the tumorigenesis protocol. In addition, serum glucocorticoids, which have strong clock-controlled rhythms, lost their circadian patterns. Similarly, the rhythmic expression of the clock genes Bmal1 and Cry1 in the hypothalamic Suprachiasmatic Nuclei (SCN, the central clock) were also abolished in mice carrying tumors. Altogether, these results suggest that tumor-secreted molecules (tumor macroenvironment) could modulate the function of the central circadian pacemaker (SCN). This could account for the worsening of the peripheral biological rhythms such as the locomotor activity or the serum glucocorticoids. The knowledge of the circadian rhythms in cancer patients could be useful to improve their quality of life. Moreover, since the deregulation

CIRCADIAN PHYSIOLOGY AND BEHAVIOR

#A-3

Light stimulation during postnatal development is not determinant for glutamatergic neurotransmission from the retinohypothalamic tract to the suprachiasmatic nucleus in rats

Javier Alamilla[1], Miriam E Reyes-Mendez[1], J. Manuel Herrera-Zamora[2], Fernando Osuna-Lopez[2], Ricardo A. Navarro-Polanco[2], Néstor Mendoza-Muñoz[3], José L. Góngora-Alfaro[4], Eloy G. [2]

1.CONACYT-UCOL
2.Universidad de Colima
3.Fac. Ciencias Químicas-UCOLL
4. CIR Hideyo Noguchi-UADY

Presenting Author: **Javier Alamilla**

The suprachiasmatic nucleus (SCN) synchronizes with environmental light through the retinohypothalamic tract (RHT). Although the SCN regulates circadian rhythms before birth, postnatal synaptic changes are needed for the RHT-SCN pathway. However, it is unknown whether visual experience affects maturation. Here, we studied the effects of constant darkness (DD) rearing on the physiology of glutamatergic neurotransmission between RHT and SCN during postnatal development.
Upon recording spontaneous and evoked excitatory postsynaptic currents (EPSCs)by electrical stimulation of RHT fibers, we found that DD animals at early postnatal ages (P3–19) exhibited different frequencies of spontaneous EPSCs and lower synaptic performance when compared with their normal light/dark (LD) counterparts. At the oldest age evaluated (P30–35), there was a synaptic response strengthening in DD rats, which functionally equaled or surmounted that of LD animals. Control experiments evaluating EPSCs in ventral SCN neurons of LD rats during day and night revealed no significant differences in spontaneous or evoked EPSCs by high-frequency trains in the RHT at any postnatal age. Our results suggest that DD conditions induce a compensatory mechanism in the glutamatergic signaling of the circadian system to increase the chances of synchronization to light at adult ages, and that the synaptic properties of RHT terminals during postnatal development are not critically influenced by environmental light.

CIRCADIAN PHYSIOLOGY AND BEHAVIOR

#A-4

Sleep habits and attention as a function of school time and the level of urbanization in technical high school students of Brazil.

Zoélia Bessa[1], Arthur Câmara[2], Pablo Valdez[3], Carolina Azevedo[4]

1.Laboratório de Cronobiologia, Programa de Pós-graduação em Psicobiologia, UFRN, Natal/Brasil
2.Laboratório de Cronobiologia, Programa de Pós-graduação em Psicobiologia, UFRN, Natal/Brasil
3.Laboratorio de Psicofisiología, Facultad de Psicología, Universidad Autónoma de Nuevo León, Monterrey, NL, México
4. Laboratório de Cronobiologia, Programa de Pós-graduação em Psicobiologia, UFRN, Natal, Brasil

Presenting Author: **Zoelia Bessa**

Adolescents' sleep patterns change along with school and free days and may interact with urban dynamics, which in turn may impair attention. This work aims to analyze the relation of school shift, sleep habits and attention in students of a technical high school in Brazil, as a function of homeplace's level of urbanization. 227 students (age: 14 to 16 y) took part (Shift: Morning n=101/Afternoon n=126; Level of urbanization: Low n=101 (Angicos 6°3'S,37°37'W)/High n=126 (Metropolitan area of Natal 5°50'S, 35°12'W). Sleep patterns were assessed by a sleep diary for 10 days. Attention was evaluated by a Continuous Performance Task. Students in the morning shift had less time in bed and earlier bed and waking times in the week, higher irregularity for waking time, time in bed, and social lag. Students from Angicos had earlier bed and waking times and are less sleepy throughout the week. Those from the morning shift had a shorter reaction time for phasic alertness and a lower percentage of correct answers for tonic alertness and selective attention. Students from Angicos had a shorter reaction time for phasic alertness; lower percentages of correct answers for tonic and phasic alertness, and selective attention; and higher percentages of omissions for phasic alertness and selective attention. Thus, in adolescents, the sleep-wake cycle varies according to the school time and level of urbanization, which can affect daytime sleepiness and attention.

CIRCADIAN PHYSIOLOGY AND BEHAVIOR

#A-5

The neural activity of the suprachiasmatic and arcuate nucleus is required during a critical window at each stage of the estrous cycle for ovulation.

Montserrat Bolaños-Hurtado[1], Angélica Flores[1], Roberto Domínguez[1], Carlos-Camilo Silva[1]

1.Chronobiology of Reproduction Research Lab, Facultad de Estudios Superiores Zaragoza-UNAM

Presenting Author: **Montserrat Bolaños-Hurtado**

Ovulation depends on neural signals occurring between 14:00 and 17:00 of proestrus. They are thought to be generated in the suprachiasmatic nucleus (SCN) and that are either generated only in a high estradiol background or have to coincide with it to stimulate the preovulatory surge of gonadotropins. To test these hypotheses, we transiently inhibited the electric activity of the SCN of freely moving-rats by microinjecting tetrodotoxin (TTX) at 14:00 of each stage of the estrous cycle. Animals were euthanized at the next expected estrus and the cyclicity and ovulation were analyzed. TTX did not modified the estrous cycle, but blocked the ovulation irrespectively of the stage when it was injected. This suggest that the SCN is indeed the locus of the neural signals described above and that they are generated at each stage of the cycle, participating in the regulation of ovulation no matter the concentration of estradiol. As the arcuate nucleus (ARC) receives efferent fibers from the SCN and modulates the secretion of gonadotropins in a low estradiol background, we hypothesized that the circadian signals generated during stages other than proestrus may be transmitted through it. We inhibited the neuronal activity of the ARC as described above and found anovulation in rats treated in metestrus, diestrus and proestrus, while acyclicity only in rats treated in metestrus. We conclude that circadian information regulates the tonic and phasic release of gonadotropins through the ARC.

CIRCADIAN PHYSIOLOGY AND BEHAVIOR

#A-6

Oxytocinergic cells of the posterior hypothalamic paraventricular nucleus participate in the food entrained clock

Mario Caba[1], Enrique Meza[1], Carolina Escobar[2], Angeles Jiménez[3], Mario Daniel Caba-Flores[1], María Luisa Moreno[4], Angel Melo[3]

1.Centro de Investigaciones Biomédicas, Universidad Veracruzana, Mexico
2.Fac. de Medicina, UNAM, México
3.Centro de Investigacion en Reproducción Animal, CINVESTAV-UAT, México
4. Instituto de Investigaciones Biológicas, Universidad Veracruzana, México

Presenting Author: **Mario Caba**

The mechanisms underlying food anticipatory activity is still not well understood. Here we explored the role of oxytocin (OT) and the protein c-Fos in the supraoptic nucleus (SON) and in the medial (PVNm) and posterior (PVNp) regions of the paraventricular hypothalamic nucleus. Adult rats were assigned to one of four groups: scheduled restricted feeding (RF), Ad libitum (AL), fasting after restricted feeding (RF-F), to explore the possible persistence of oscillations, or Ad libitum fasted (AL-F). In the SON and in the PVNm, OT cells were c-Fos positive after food intake; contrasting, OT cells in the PVNp showed c-Fos activation in anticipation to food access, which persisted in RF-F subjects. We conclude that OT and non-OT cells of the SON and PVNm may play a role as recipients of the entraining signal provided by food intake, whereas those of the PVNp which contain motor preautonomic cells that project to peripheral organs, may be involved in the hormonal and metabolic anticipatory changes in preparation for food presentation and thus, may be part of a link between central and peripheral oscillators. In addition, due to their persistent activation they may participate in the neuronal network for the clock mechanism that leads to food entrainment.

CIRCADIAN PHYSIOLOGY AND BEHAVIOR

#A-7

Melatonin prevents tumor growth through of genes implicated in processes like: circadian rhythms, cell cycle and angiogenesis

Skarleth Cárdenas-Romero[1], Oscar Daniel Ramírez-Plascencia[2], Omar Flores - Sandoval[1], Roberto Carlos Salgado-Delgado[1], Nadia Saderi[1]

1.Universidad Autónoma de San Luis Potosí
2.Beth Israel Deaconess Medical Center/Harvard Medical SchoolCenter

Presenting Author: **Skarleth Cárdenas Romero**

The hypothalamic suprachiasmatic nucleus (SCN) regulates the circadian rhythms to keep body physiology synchronized to the light-dark cycle (LD). Circadian Desynchronization (CD) has been associated to cancer and local angiogenesis. Previous studies from our laboratory showed that the CD by constant light (LL) promotes the tumor growth and vascularization in rats inoculated with glioblastoma cells, an effect that is counterbalanced by melatonin. To investigate the integrative role of this hormone, we explored the melatonin effects over the expression of clock, cell cycle and pro-angiogenic genes in the tumor tissue and the liver of rats assigned to 5 different groups: 2 of them in LD; the other 3 were kept in LL. One of LD and 2 of LL groups were injected with glioblastoma cells; moreover, a group of the LL with cancer received a daily dose of melatonin. As results, we observed that melatonin modified the rhythm of p53 and Tnfα in tumor tissue, and of Per2, p21, Cyclin E, VEGF-A, PDGF and Tnfα in the liver, as well as the daily amount of mRNA (Per2, Rorα, p53, VEGF-A, Tnfα and Ang in tumor tissue; p21, VEGF-A, PDGF, Tnfα, MT1R and MT2R in the liver). These results indicate that melatonin protects from CD and its pathological consequences by promoting the expression of suppressor genes (e.g., Rorα). Interestingly, the hepatic expression of p21, Cyclin E, VEGF-A, Tnfα, MT1R and Rev-erbα under LL with cancer occurred rhythmically, suggesting cancer as a synchronizer under LL.

CIRCADIAN PHYSIOLOGY AND BEHAVIOR

#A-8

Sleep quality of Endemic Workers in Rio de Janeiro, Brazil.

Marcus Santos[1], Victor Figueiredo[1], Gabriel Silveira[1], Ana-Paula Neves[1], Priscila Vidal[1], Luiza Dantas[2], Marcos-Rogério Silva[2], Ébio-Willis Moreira[2], Roberto-Paulo Nunes[2], Antônio-Carlos Cardoso[3], Monica Martins[3], Fátima Moreira[3], Edilene Pereira[4], Tatiana Docile[4], Aline Monte Gurgel[5], Márcia Sarpa[6], Leandro Carvalho[3], Luciana Gomes[3], Maria-Blandina Santos[3], Luiz-Claúdio Meirelles[3], Ana-Cristina Rosa[3], Eline Gonçalves[3], Isabele Costa-Amaral[3] , Ariane Larentis[3], Liliane Teixeira[3]

1.Pos graduate student atCenter for the Study of Occupational Health and Human Ecology (Cesteh), National School of Public Health Sérgio Arouca (ENSP / FIOCRUZ), Rio de Janeiro, RJ, Brazil.
2.Endemic Workers crowded in different municipalities of the state of Rio de Janeiro, Brazil
3.Researcher at the Center for Studies on Occupational Health and Human Ecology (Cesteh), National School of Public Health Sérgio Arouca (ENSP / FIOCRUZ), Rio de Janeiro, RJ, Brazil.
4. Joaquim Venâncio Polytechnic School of Health (Fiocruz), Rio de Janeiro, Brazil.
5. Researcher at Instituto Aggeu Magalhães (IAM)/ Oswaldo Cruz Foundation (Fiocruz), Recife, Brazil.
6. Researcher at National Cancer Institute José Alencar Gomes da Silva (INCA), Rio de Janeiro, Brazil.
7. Collaborating researchers in the Project "Estudo do impacto à saúde de Agentes de Combate às Endemias/Guardas de Endemias pela exposição a agrotóxicos no estado do Rio de Janeiro" coordinated by the Center for the Study of Occupational Health and Human Ecology (Cesteh), Escola Nacional of Public Health Sergio Arouca (Ensp) / Oswaldo Cruz Foundation (Fiocruz), Brazil

Presenting Author: **Marcus Vinicius Corrêa dos Santos**

Introduction: Sleep disorders affects millions of people worldwide. When these risk factors are associated with others, as pesticide exposure, they increase the health effects. Pesticides are related to acute and chronic diseases, such as neurologic disorders. In Brazil, workers known as "Endemic Disease Combat Agents" who are responsible for the vector "combat", are subjected to chronic and cumulative exposure by intensive use of pesticides, some of them banned or restricted in other countries. Among the health effects from exposure to pesticides, there is sleep disorders, which in turn lead to the development of symptoms that affect psychomotor performance, memory consolidation, creativity and decision making. Methods: This study is part of a multicenter research with "Endemic Disease Combat Agents" from Rio de Janeiro, Brazil. Sleep quality was assessed by Pittsburgh Sleep Quality Index (PSQI). Results: Preliminary results from 140 workers (mean 52 years old; SD=8.55) show that women have worse sleep quality than men but there is no significant difference in sleep latency and excessive daytime sleepiness. The difference is mainly due to sleep duration (women=5h42min and men=6h18min) and lower sleep efficiency in women (82%) than in men (92.1%). Conclusion: Changes in the work process and in the chemical-dependent vectorial "combat" are necessary, as a measure to protect the health of these workers and sleep problems that result from cumulative exposure to pesticides.

CIRCADIAN PHYSIOLOGY AND BEHAVIOR

#A-9

Cardiometabolic alterations in wistar rats under a T-22 cycle and subjected to nighttime restircted feeding

Isis Gabrielli Barbieri de Oliveira[1], Breno Tercio Santos Carneiro[2], Isabela Cristina Maioni Xavier[1], Marcos Divino Ferreira Junior[1], Keilah Valéria Naves Cavalcante[1], Rodrigo Mello Gomes[1], Gustavo Rodrigues Pedrino[1], Daniel Alves Rosa[1]

1.1Center of Neuroscience and Cardiovascular Research, Biological Science Institute, Federal University of Goiás, Brazil
2.Laboratory of Neurobiology and Biological Rhythmicity, Federal University of Rio Grande do Norte, Brazil

Presenting Author: **Isis Gabrielli Barbieri de Oliveira**

Rats chronically exposed to a symmetrical LD 11:11h (T22) express two stable circadian motor activity rhythms one with LD cycle entraining and other with in free running (>24h). Wistar rats (250-300g) distributed in four groups: rats submitted to LD 12:12 with availability of food ad libitum-T24-AL or restricted feeding-T24- RF, in which food availability just during in the dark phase; other groups of rats were under to LD 11:11 with ad libitum food T22-AL or restricted feeding -T22-RF. At the end of each experimental time (4 and 8 weeks) all groups were submitted to a glucose tolerance test (GTT), insulin tolerance test (ITT) and subsequent histology of peripheral tissues. For motor activity, nighttime restricted feeding (RF) did not affect the power of the activity rhythm in the T24 control group. All subjects under T22 presented two components for the activity rhythm, as expected for this forced desynchrony protocol. RF groups showed numerically higher power of the LD-entrained component compared to AL but not statistically significant. There was no difference among the groups in the plasma glucose responses after ITT. However, after 8 weeks of the experimental period, we observed an increase in AUC of glucose at the GTT of T24-RF when compared to the other groups. We conclude that restricted feeding nighttime affects the expression of the activity rhythm by strengthening the LD-entrained component under a T22 cycle, reducing the forced desynchrony induced by this model.

CIRCADIAN PHYSIOLOGY AND BEHAVIOR

#A-10

Does reduced coupling in the endogenous clocks leads to depression? Long-lasting effects of circadian dissociation in male Wistar rats

Júlio César de Oliveira-Leal[1], Karen Cristina Pugliane[2], Jeanderson Parente Soares[2], Rafael Vitor de Oliveira da Silva[2], Rochele Castelo-Branco[2], Flavio Freitas Barbosa[2], John Fontenele-Araujo[1]

1.Laboratory of Neurobiology and Biological Rhythmicity, Federal University of Rio Grande do Norte
2.Laboratory of Studies in Memory and Cognition, Federal University of Paraiba

Presenting Author: **Júlio César de Oliveira Leal**

Forced desynchronization induced by a short light-dark cycle (LD 11:11) dissociate the circadian rhythms of locomotor activity in two components. It is linked to mood disorders in animal models, although the consequences of early dissociation in adulthood are still unclear. We aim to investigate the effect of the forced desynchronization protocol in early life of rats in parameters related to depressive like-behaviors of adult rats. We allocated male Wistar rats into 3 groups: rats submitted to LD 11:11 in uterine and lactation phase (L dissociated) or in post-lactation phase (PL dissociated) and rats submitted to LD 12:12 (control). Both dissociated groups were resynchronized to LD 12:12 afterwards. We registered body mass and food consumption during post-lactation and adulthood weekly, at the point of 4 months of age, rats performed the sucrose preference test. We showed that the mother and its offspring in lactation, and individual rats in post-lactation, showed a dissociated locomotor activity rhythm. In adulthood, PL dissociated rats showed less food consumption and body mass weekly, as well as anhedonia, compared to L dissociated and control rats. Our results suggest that dissociation in the circadian rhythms in the post-lactation phase induce depression like behavior in adulthood.

Key Words: Circadian misalignment, ontogeny, behavior, affective disorders, rats.

CIRCADIAN PHYSIOLOGY AND BEHAVIOR

#A-11

Sex differences in sleep patterns, social lag and attention in adolescents

Fernanda Mayara Crispim Diogo[1], Zoelia Camila Moura Bessa[1], Sabinne Daniela Galina[1], Maria Luiza Cruz de Oliveira[1], Pablo Valdez[2], Carolina Virgínia Macêdo de Azevedo[1]

1.Laboratório de Cronobiologia e Comportamento, Programa de Pós-graduação em Psicobiologia, Departamento de Fisiologia e Comportamento, UFRN
2.Laboratorio de Psicofisiología, Facultad de Psicología, Universidad Autónoma de Nuevo León, Monterrey, NL, México

Presenting Author: **Fernanda Mayara Crispim Diogo**

Adolescents present a phase delay in sleep pattern with insufficient and irregular sleep. This scenario is harmful to academic performance and may be worse for girls due to greater sleep need. We assessed sex differences of sleep patterns, social jetlag and attention of 207 (F: 133 - 15±0.9; M: 74 - 16±0.9) high school students attending the morning shift. Sleep was evaluated by questionnaires and attention by a cognitive task. Data were analyzed using a General Linear Model. In general, girls spent more time in bed (F: 4,6; $p<0,05$; η^2: 0,02) and got up later (F: 4,1; $p<0,05$; η^2: 0,02) on weekend, had a greater irregularity in get up time (F: 4,6; $p<0,05$; η^2: 0,02) and showed higher proportion of poor sleep quality (X^2, $p<0,05$). The sexes did not differ in relation to bed and get up times, time in bed in weekdays, irregularity in bedtimes and time in bed, social jetlag, sleep quality score and sleepiness upon awakening ($p>0,05$). Regarding attention, it was only seen a tendency in girls to have a longer reaction time in phasic alertness (F: 3,4; $p=0,06$; η^2: 0,02). Therefore, girls have greater sleep compensation on weekends, reflecting less resistance to sleep deprivation during weekdays. Besides, girls tended to show a longer reaction time in phasic alertness. However, this trend showed a weak correlation index and a small effect size. Thus, further studies should be conducted in a larger sample with more balance between sexes to analyze the relation of sex with attention.

CIRCADIAN PHYSIOLOGY AND BEHAVIOR

#A-12

Evaluation of thyroid hormones actions on central circadian clock

Felipe Emrich Vieira Botelho[1], Paula Bargi-Souza[1], Dayana Silva Gonçalves-Manso[1], Rodrigo A. Peliciari-Garcia[2]

1.Department of Physiology and Biophysics, Institute of Biological Sciences, Federal University of Minas Gerais
2.Department of Biological Sciences of Federal University of São Paulo

Presenting Author: **Felipe Emrich Vieira Botelho**

In mammals, the central circadian clock is located in the suprachiasmatic nucleus (SCN) at the hypothalamus and is responsible for the synchronization of endogenous circadian rhythms according to dark/light cycle, being light the main Zeitgeber for the SCN. However, other factors/hormones can also act as Zeitgebers and mediate a synchronizing signaling effect. It has been shown that T3 alter the expression of circadian clock in heart and pituitary gland, however, whether these thyroid hormone effects are mediated through an alteration of the central clock dynamics is still unknown. To test this hypothesis, we aligned the promoter and regulatory regions of clock genes and neuronal activity markers of mice, rats and humans using the rVista2.0 software. Afterwards, the presence of sites or binding elements for thyroid hormones (T3R) and/or clock components (EBOX, RORA1, RORA2, CLOCKBMAL1) was evaluated in conserved regions using the MULTIF (Transfac professional V10.2 library). The in silico analysis evidenced the presence of possible binding sites for both thyroid hormones and clock components in target regions of the respective genes: AVP, AVPR1A, AVPR1B, DRD1A, GRP, VIP and VIPR2, suggesting that both, thyroid hormones and clock genes can simultaneously regulate the expression of main regulators of central circadian synchronization.

CIRCADIAN PHYSIOLOGY AND BEHAVIOR

#A-13

Lithium prevents mania-like behaviors induced by photoperiodic transitions in a murine model

Ellyda Fernanda Lopes Costa Costa[1], Mayara Rodrigues Barbosa Barbosa[1], Vinícius Tenório Braga Cavalcanti Pinto Pinto[1], Daniel Gomes Coimba Coimbra[1], Tiago Gomes de Andrade De Andrade[1]

1.Centro de Medicina Circadiana - Faculdade de Medicina, Universidade Federal de Alagoas, Maceió – Brasil.

Presenting Author: **Mayara Barbosa**

Epidemiological studies have shown higher occurrences of hospitalizations for manic episodes and suicide in the periods of accentuated increments in daylength. Consistently, preliminary studies performed by our group indicate that a rapid and gradual increase of the photoperiod is associated with mania-like behaviors in mice. The aim of this work was to evaluate the behavioral response of these animals with Lithium (Li) administration. Male C57BL/6J mice (n=30) were equally distributed into: (1) Equatorial (Eq) photoperiod (12:12LD), (2) DTM (Daylength Transition Model) and (3) DTM + Li. The DTM group consists of gradual (1h/day) increase of the photophase, from 8:16LD to 16:8LD. Li was administered (0,6g/L) in drinking water during the 8 days of photoperiodic transition. Behavioral tests were performed on the last day (ZT6). Data were analyzed using ANOVA (Tukey post-hoc), with a cutoff value of P < 0.05, and CI of 95%. Compared to the DTM group, the animals in the DTM+Li group showed a decreased risk exposure evaluated by percentage of entries in open arms (p=0.0001) and head dipping (p<0.0001) on the EPM; decreased exploratory behavior observed in the total number of interactions with holes (p<0.0001) in the HB; and decreased hyperlocomotion evaluated through the total number of crosses in the OFT (p=0.0001) and total immobility time in FST (p=0.0005). The findings demonstrate that administration of Li prevented the mania-like behavior observed in DTM.

CIRCADIAN PHYSIOLOGY AND BEHAVIOR

#A-14

A GABAergic circuit regulates sleep/arousal behavior in Drosophila

Florencia Fernandez Chiappe[1], Nara I. Muraro[1]

1.Biomedicine Research Institute of Buenos Aires - CONICET - Partner Institute of the Max Planck Society

Presenting Author: **Florencia Fernandez Chiappe Fernandez Chiappe**

It has been previously proposed that GABAergic inputs to the large lateral ventral neurons (lLNvs) of Drosophila may be responsible of informing those highly integrative arousal neurons about the sleep homeostat status. Meanwhile, the current paradigm proposes that the main circadian pacemaker of the Drosophila brain, the small lateral ventral neurons (sLNvs), have only minor influence in the control of sleep behavior.
Starting from this point, our aim is to describe the mechanisms of GABAergic inhibition in both sLNvs and lLNvs, their influence on sleep behavior and their role on the sleep homeostat. For this, we have performed specific genetic manipulations and quantified sleep behavior under basal and sleep deprivation conditions. Moreover, we have collected electrophysiological recordings to identify the extent of the role of the neurotransmitter GABA in the neuronal circuit studied, given that our final goal is to describe this network in detail.
Our findings confirm that the lLNvs receive information about the sleep homeostat status via the GABAA receptor Rdl through a complex neuronal circuit. They also suggest that the sLNvs are involved not only in the control of the circadian sleep timing but also, through GABAergic inputs, can regulate the quantity and quality of sleep.

CIRCADIAN PHYSIOLOGY AND BEHAVIOR

#A-15

Daily thermal cycle effects on activity, stress, and immunological capacity of Lithobates catesbeianus (Shaw, 1802), associated with high-temperature exposure

Beatriz Foganholi Fernandes[1], Lídia Sumie Yano[1], Bruno Leite Tavares[1], Gabriel Massami Izumi de Freitas[2], Stefanny Christie Monteiro Titon[3], Gisele Akemi Oda[4], Silvia Cristina Ribeiro de Souza[1]

1.Laboratory of Energy Metabolism and Seasonality / Departament of Physiology from the Institute of Biosciences / University of São Paulo
2.Amphibians Laboratory / Departament of Zoology from the Institute of Biosciences / University of São Paulo
3.Laboratory of Chronopharmacology / Departament of Physiology from the Institute of Biosciences / University of São Paulo
4. Binational Laboratory of Chronobiology Argentina-Brazil / Departament of Physiology from the Institute of Biosciences / University of São Paulo

Presenting Author: **Beatriz Foganholi Fernandes**

In nature, both photic and non-photic cycles interact to synchronize daily biological rhythms. For ectotherms that depend on water in daily activities, the cycling of water temperature - besides air - in the environment probably plays a relevant role in their physiology and behavior. Thus, this study aimed to investigate physiological effects of daily temperature cycles in Lithobates catesbeianus, by comparing frogs exposed to constant temperatures and frogs exposed to daily thermal cycles. We developed a new individual housing system consisting of buckets and circulating filtered water coupled to heaters, monitoring water quality and water and air temperatures throughout the experiment. Forty adult male frogs were purchased from a breeding ground in the summer and maintained in laboratory conditions under natural photoperiod. After seven days, groups of frogs were subjected to one of the following regimes: constant 24°C (n=21), constant 30°C (n=9), and a thermal cycle ranging from 24°C by night to 30°C by day (n=9). Frogs behavior (microhabitat choice) was video recorded, and corticosterone levels and antimicrobial capacity (stress and immunological indicators) were measured in plasma samples taken after two weeks of exposure. Generally, high temperatures associated with both constant and cyclical regimes stimulated a daily rhythm of activity (6.6 to 11.4-fold greater at day), increased stress (6.3 to 9.3-fold greater than the control), and decreased immunological capacity.

CIRCADIAN PHYSIOLOGY AND BEHAVIOR

#A-16

Daily changes in the social modulation of electric behavior variability

Valentina Gascue[1], Ana Silva[1], Adriana Migliaro[1]

1.Laboratorio de Neurociencias, Facultad de Ciencias, Universidad de la República

Presenting Author: **Valentina Gascue Gascue**

Brachyhypopomus gauderio is a nocturnal pulse-type South American weakly electric fish characterized by the constant emission of an electric discharge (EOD). The basal rate of the DOE (EOD-BR) is modulated by exploratory activities, social context and environmental clues. A daily rhythm of nocturnal increase has been shown both in laboratory conditions and in the natural habitat. Social context modulates the rhythm of EOD-BR both in amplitude and phase.

In this work we show a novel effect of social context on this daily rhythm regarding EOD-BR variability. Variability of EOD-BR is an indicator of the exploratory capacity and range of social interactions. Therefore, we studied differences in EOD-BR variability during day and night in social and isolated contexts. We found that the EOD basal rate variability is higher in the night than in the day, both in isolated and social conditions. Moreover, this increase in night EOD-BR variability was higher in animals in social context, suggesting a social modulation of electric behavior variability.

CIRCADIAN PHYSIOLOGY AND BEHAVIOR

#A-17

Temporal dissociation between activity and body temperature rhythms of a subterranean rodent (Ctenomys aff. knighti) in field enclosures

Milene G Jannetti[1], Patricia Tachinardi[1], Veronica S Valentinuzzi[2], Gisele A Oda[1]

1.Laboratorio de Cronobiologia Binacional Argentina-Brasil, Departamento de Fisiologia, Instituto de Biociências, Universidade de São Paulo, São Paulo, Brazil
2.Laboratorio de Cronobiologia Binacional Argentina-Brasil, Centro Regional de Investigaciones Cientificas y de Transferencia Tecnológica (CRILAR), Entre Ríos y Mendoza, s/n, Anillaco, La Rioja 5301 Argentina

Presenting Author: **Milene Gomes Jannetti**

Locomotor activity and body temperature (Tb) rhythms are tightly synchronized in rodents in the lab, even when submitted to different artificial light and temperature conditions. Several rodents, when transferred from field to lab, switch their activity phase from diurnal to nocturnal, but their synchrony with Tb in the field was not systematically verified. For a better interpretation of Tb field data, we compared synchrony of Tb and activity for a wild subterranean rodent (tuco-tuco; Ctenomys sp.) in the field and in the lab. 6 animals (157g±32g) with accelerometers (Axy-4, 2g, 23x12x10mm, bin interval 10Hz, range ±4G-forces, precision 8 bits; Technosmart, IT) and implanted Tb sensors (DS1925 iButton, 3.3g, 17.3x6.4mm, 5min, 0-50°C, 0.0625°C; Maxim Integrated, US) were released inside field enclosures (12mx6mx1.5m) and transferred after 20 days to lab for 10 days. Levels of synchrony were quantified using the percentage of temporal coincidence between activity and high Tb. Synchrony was consistently lower in field than in lab and Tb rhythms had higher interindividual variation than activity rhythms. Disagreement in diurnality between Tb and activity suggests that coordination of Tb rhythms must be separated from the pathway that controls activity switches. Further investigating tuco-tucos daily rhythms can help us understand the high interindividual variation in their Tb rhythms, as well as the looser relationship between activity and Tb in the field compared to the lab.

CIRCADIAN PHYSIOLOGY AND BEHAVIOR

#A-18

Acute sleep restriction induces hyperglycemia and changes SCN activity

Gabriela Hurtado-Alvarado[1], Eva C Soto-Tinoco[1], Carolina Escobar[2], Ruud M Buijs[1]

1.Hypothalamic Integration Mechanisms Laboratory, Department of Cellular Biology and Physiology, Instituto de Investigaciones Biomédicas, Univ
ersidad Nacional Autónoma de México (UNAM), Ciudad de México, Mexico
2.Faculty of Medicine, Universidad Nacional Autónoma de México (UNAM), Ciudad de México, Mexico

Presenting Author: **Gabriela Hurtado-Alvarado**

Chronic sleep restriction is a common problem in modern society. Despite chronic sleep restriction is considered a risk factor for the development of metabolic and cardiovascular diseases, there is no clear evidence about the brain-controlled mechanisms that promote such metabolic impairments. Previous studies have shown that the suprachiasmatic nucleus (SCN) is involved in the regulation of wakefulness, contributing to the sleep-wake cycle. Furthermore, circadian disturbances such as shift work also promote metabolic imbalance. Here, we propose that acute sleep restriction can modify the neuronal activity of the SCN, resulting in alterations of systemic glycemia. In order to induce sleep restriction, adult male Wistar rats were forced to be active for 2 hours at the beginning of their rest-phase by means of a slow rotating wheel. Our results indicate that 2 hours of sleep restriction at the beginning of the rest period, are enough to induce hyperglycemia and changes in the SCN neuronal activity. Our data provide novel information about the early mechanisms that drive metabolic disturbances induced by sleep restriction and that appear to be controlled by the SCN.

CIRCADIAN PHYSIOLOGY AND BEHAVIOR

#A-19

The impact of glial signals on neuronal structural plasticity

Juan Ignacio Ispizua[1], Maria Fernanda Ceriani[1]

1.Laboratorio de Genética del Comportamiento. Fundación Instituto Leloir, IIB-BA CONICET. Buenos Aires, Argentina.

Presenting Author: **Juan Ignacio Ispizua Ispizua**

Recently, we described that a functional glial clock is necessary for circadian plasticity in the small lateral ventral neurons (sLNvs), a group of key pacemaker neurons of D. melanogaster. Circadian structural plasticity involves rhythmic changes in the degree of arborization and fasciculation of their dorsal termini. The sLNvs express PDF, a neuropeptide relevant in the synchronization of the clock network that oscillates in phase with this remodelling process. We have previously demonstrated that circadian plasticity modifies the way the pacemaker circuitry is wired regularly, but its impact on behaviour and the molecular basis that control this process are yet to be defined. Building upon our previous results, we examine in depth the impact of neuronal-glial connectivity. Using GFP reconstitution analysis (GRASP), we found that sLNv termini contact directly with two different glial subtypes (astrocyte-like and ensheathing glia) and that these contacts are time-of-the-day dependent. Interestingly, blocking adult glio-transmission has different effects on PDF levels and plasticity depending on the type of glia recruited and the length of the treatment (12 or 24 hours). Preliminary experiments show that preventing clock oscillations in different glial subtypes affect circadian plasticity distinctively. Taken together, our results suggest a complex glial implication in the modulation of adult structural plasticity with distinct roles for different glial subtypes.

CIRCADIAN PHYSIOLOGY AND BEHAVIOR

#A-20

Daily Microglial polarization profile in the Arcuate Nucleus of male wistar rats

Rosalinda Guevara[1], Mara Guzmán[2], Rudolf Buijs[1], Natalí Guerrero[1]

1.Departamento de Fisiología, Facultad de Medicina, UNAM.
2.Instituto de Investigaciones Biomédicas, UNAM.
3.Departamento de Anatomía, Facultad de Medicina, UNAM.

Presenting Author: **Jorge Brando Márquez**

Microglia is commonly known as an immune cell of the central nervous system and though its role in mounting the innate immune response in the brain is undeniable, defining microglia by this function is not enough.It is also a versatile cell changing its function, metabolism and morphology with the developmental stage, brain region and health status of the organism, making them important for maintaining homeostasis. Recent studies have shown that microglial response to immune challenges like LPS, or its cytokine secretion is regulated in a circadian manner; adding a new factor controlling its functions. The aim of the present study is to determine if the time of the day may control microglial function in an hypothalamic nucleus involved in the regulation of energy balance, the Arcuate nucleus (ARC).Preliminary data obtained from male wistar rats in our laboratory show differences in Iba-1 immunoreactivity and cell morphology in the ARC related to the time of the day with low levels of Iba-1 and ramified cells during the resting phase of the rat, and high Iba-1 immunoreactivity with an ameboid morphology during their active phase. These differences are found without any immune stimulation, suggesting a change in microglial activity related to the time of the day therefore modulating the metabolic functions that the neurons in the ARC manage. Acknowledgments This study is supported by DGAPA-PAPIIT IN221819 to RGG, DGAPA-PAPIIT IA204121 to MAGR and DGAPA-PAPIIT IA206620 to NNGV.

CIRCADIAN PHYSIOLOGY AND BEHAVIOR

#A-21

School commuting time and sleep habits on adolescents as a function of class shift and homeplace's degree of urbanization

Arthur Medeiros Câmara[1], Zoelia Camila Moura Bessa[2], Carolina Virgínia Macedo de Azevedo[3]

1. Laboratório de Cronobiologia e Comportamento, UFRN

Presenting Author: **Arthur Medeiros Câmara**

School commuting is an important aspect of students' life and urban dynamics that may affect sleep in adolescents due to impact on routine. Impaired sleep, common at this age for its marked phase delay, may worsen health and school performance. This study aims to assess the relationship between commuting time and sleep habits as a function of school shift and level of urbanization on students from a technical high school in Brazil. 225 adolescents (from 14 to 16 y.o.) participated, of which 101 study in the morning and 124 in the afternoon, and 100 live in the Angicos region (6°3'S,37°37'W) and 125 in the metropolitan area of Natal (5°50'S, 35°12'W). Commuting time was obtained from "Health and Sleep" questionnaire, and sleep patterns via a sleep diary of 10 days. Students with higher commuting time had earlier bed and wakeup times on weekdays; higher irregularity for wakeup time; and slept more on the weekend than on weekdays. Morning shift students had earlier bed and wakeup times on weekdays; longer time in bed on weekdays; higher social lag and irregularity for wakeup time; and longer time in bed on weekend comparing to weekdays. Students from the less urbanized region had earlier bed and wakeup times; and for weekdays, longer time in bed and lower scores for sleepiness, with the latter also occurring for days off. Hence, students sleep habits are related with commuting time, homeplace's urbanization and school start time. This can affect daytime sleepiness over the week.

CIRCADIAN PHYSIOLOGY AND BEHAVIOR

#A-22

GABA neurotransmission of the suprachiasmatic nucleus is modified during rat postnatal development

Fernando Osuna-Lopez[1], Javier Alamilla[3], Miriam Reyes-Mendez[1], Manuel Herrera-Zamora[1], Eloy Moreno-Galindo[3], Jose Gongora-Alfaro[2]

1.Universidad de Colima
2.CIR HIDEYO NOGUCHI, Universidad Autónoma de Yucatán
3.UCOL-CONACYT

Presenting Author: **Fernando Osuna**

The suprachiasmatic nucleus (SCN) of the hypothalamus is the brain structure that controls circadian rhythms in mammals. The SCN is formed by two neuroanatomical regions: the ventral and dorsal. GABA neurotransmission is important for the regulation of circadian rhythms. Excitatory GABA effects have been described in both SCN regions displaying a circadian variation. Even though, there is almost no knowledge about the GABA neurotransmission during the pre- or postnatal development of the SCN. Here, we used whole-cell patch-clamp recordings to study spontaneous inhibitory postsynaptic currents (IPSCs) in the two SCN regions, at two zeitgeber times (day or night), and at four postnatal ages: P3-5, P7-9, P12-15, and P20-25. The results herein show that the three analyzed parameters of the IPSCs, frequency, amplitude, and decay time, were significantly affected by the postnatal age: mostly, the IPSC frequency increased with age, principally in the ventral SCN in both day and night recordings; similarly, the amplitude of IPSCs augmented with age, especially in the two SCN zones at night; whereas the IPSC decay time was reduced (it was faster) with postnatal age, mainly in both SCN regions during the day. Our findings first reveal that parameters of the GABA neurotransmission are modified by postnatal development, implying that synaptic adjustments are required for an appropriate maturation of the GABAergic system in the SCN.

CIRCADIAN PHYSIOLOGY AND BEHAVIOR

#A-23

Unraveling the mysteries of the colony: daily foraging and leaf-cutting rhythms in leaf-cutting ants

Mila Maria Pamplona Barbosa[1], Marcelo Arruda Fiuza de Toledo[3], Gisele Akemi Oda[2], André Frazão Helene[2]

1.Programa de Pós-Graduação em Ciências (Fisiologia Geral), Instituto de Biociências, Universidade de São Paulo, SP, Brasil
2.Departamento Fisiologia, Instituto de Biociências, Universidade de São Paulo, SP, Brasil
3.Instituto do Coração do Hospital das Clínicas da Faculdade de Medicina da USP, São Paulo, SP, Brasil

Presenting Author: **Mila Maria Pamplona Barbosa**

Understanding biological rhythms on social insects may help us understand how rhythms emerge as these systems allow us to explore them at different scales, from the individual to the colony. This work aimed to understand how a colony of leaf-cutter ants rhythmically behaved collectively regarding their leaf-cutting and foraging activity. The colony (Atta sexdens) was exposed to an LD cycle 12:12 (red lights on during all the experiment, temperature = 23°C, humidity = 60%), leaves replenishments happened once a day according to a semi-aleatory protocol. We recorded the colony foraging activity and registered how the leaf area decreased to infer leaf-cutting taxes during 30 days, including two 6 hours phase shifts (delay and advance, at the 11th and 21st days). We developed a machine learning video-tracking tool to analyze the foraging trail activity. We have observed that even though the colony is active during both the light and dark phases, most of its activity occurs during the dark phase. The video-tracking software developed in this project allowed us to count 143.904 ants after analyzing 2133 videos. Masking effects due to leaves replenishments were present thanks to the novelty effect, yet they were more intense on leaf-cutting taxes. We have also observed that the leaf consumption and the ant's flow increased after the phase shifts, showing that these parameters are sensitive to measure colony rhythmicity and efficiency after desynchronization in this type of system.

CIRCADIAN PHYSIOLOGY AND BEHAVIOR

#A-24

Effect of unpredictable feeding schedules under female physiological and behavioral rhythms

André Patiño[1], Paula Teroba[2], Elvira Morgado[1]

1.Facultad de Biología, Universidad Veracruzana, Xalapa, México
2.Facultad de Psicología, Universidad Veracruzana, Xalapa, México

Presenting Author: **André Patiño**

Modern lifestyle has modified daily routines, triggering a biological desynchronization, associated to energetic metabolism disruption. Is important to note that, female metabolism is prone to disruption in response to estrous cycle modifications. In other hand, it had been reported that restricted feeding schedules are a synchronizator of mammalian metabolism, which entrains circadian behavioral, and physiological rhythms. The aim of this investigation is to analyze the effect of unpredictable feeding schedules under physiological and behavioral rhythms, as a potential desynchronizator of metabolic circadian rhythms in female rat. To test this proposal, female rats were assigned to the following experimental groups for 28 days: ad libitum food, circadian restricted feeding schedules at rest phase, or daily unpredictable feeding schedules. At the end of feeding protocols rats were euthanized, at start of light and dark phases, and serum was obtained for corticosterone and melatonin analysis, also along the experimental days, body temperature and locomotor activity were registered when rats present diestrus phase. Unpredictable feeding schedules dissociate the circadian response of locomotor behavior and core temperature, also these females had an inverted circadian pattern of corticosterone and melatonin. We suggest that unpredictable feeding schedules dissociate physiological and behavioral rhythms in female rat.

CIRCADIAN PHYSIOLOGY AND BEHAVIOR

#A-25

Is there an association between daytime napping and cognitive function? A Mendelian randomisation study in the UK Biobank

Valentina Paz[1y2], Hassan S Dashti[3y4], Victoria Garfield[2]

1.Department of Clinical Psychology, School of Psychology, Universidad de la República, Montevideo, Uruguay.
2.MRC Unit for Lifelong Health & Ageing, Institute of Cardiovascular Science, University College London, London, United Kingdom.
3.Center for Genomic Medicine and Department of Anesthesia, Critical Care and Pain Medicine, Massachusetts General Hospital and Harvard Medical School, Boston, Massachusetts, USA.
4. Broad Institute, Cambridge, MA, USA.

Presenting Author: **Valentina Inés Paz Pérez**

Daytime napping has been associated with cognitive function in observational studies. However, it remains elusive whether napping could be beneficial or detrimental for cognition and whether these associations are causal. Using Mendelian randomisation (MR), we studied the relationship between daytime napping and cognitive outcomes. Daytime napping was instrumented using 92 genome-wide, independent genetic variants. Linear regressions with cognitive outcomes (reaction time and visual memory) were fitted in the UK Biobank (UKB) cohort (n= 378,932; mean age= 57 years old). Inverse-variance weighted (IVW) MR was implemented, with sensitivity analyses including MR-Egger and the Weighted Median Estimator (WME) for horizontal pleiotropy. No associations were found between daytime napping and reaction time (expß=1.01, 95%CI=1.00; 1.03), or visual memory (expß=0.99, 95%CI=0.94; 1.05). MR-Egger and WME approaches showed no evidence of horizontal pleiotropy. Overall, we observed no evidence of a causal association between daytime napping and reaction time and visual memory. Future studies should focus on the associations between napping and other cognitive outcomes.

CIRCADIAN PHYSIOLOGY AND BEHAVIOR

#A-26

Administration of equine chorionic gonadotrophin modifies the circadian insulin rhythm and behavior in rams but not the cortisol rhythm

Livia Pinto Santini[1], Raquel Pérez Clariget[2], Rodolfo Ungerfeld[1]

1.Facultad de Veterinaria, Universidad de la República
2.Facultad de Agronomía, Universidad de la República

Presenting Author: **Livia Pinto Santini**

Sex steroids can modulate the circadian rhythmicity of some physiological and behavioral functions of sheep. During the non-breeding season in Corriedale, the rams have a low testosterone (T) concentration, but the administration of eCG stimulates its secretion. The circadian rhythm (CR) of cortisol (C) and insulin (I) concentrations, glycemia (G), activity pattern (AP) (eating, ruminating, standing, lying, and sleeping), and surface temperature (ST) were compared in 20 Corriedale rams treated (Treated) or not treated (Con) with eCG. T and C concentration were greater in Treated than Con rams (T: 32.7 ± 0.7 nmol/L vs 5.8 ± 0.7 nmol/L; P <0.0001; C: 11.3 ± 0.6 nmol/L vs 9.9 ± 0.6 nmol/L; P =0.02). The ST was 0.9 °C lower in the Treated than in the Con rams (P =0.004). The characteristics of the CR for C, G, G:I ratio, and ST were not affected by the treatment, the acrophases (A) being at ~06:00 h, ~18:00 h, ~08:00 h, and ~11:00 h, respectively. The A of I was observed earlier in Treated than in Con rams (13:02 ± 0:56 vs 15:47 ± 0:53; P =0.04). Additionally, the A of the time spent eating was advanced in Treated rams (09:48 ± 00:16 vs 10:20 ± 00:16; P =0.04) and sleeping (21:39 ± 00:57 vs 02:02 ± 01:06; P <0.0001). Treated rams slept less time than Con rams (P =0.04). In conclusion, the administration of eCG modulated the CR of I and the AP. Nevertheless, although there was an enhanced C and reduced ST, the administration of eCG did not modify their CR.

CIRCADIAN PHYSIOLOGY AND BEHAVIOR

#A-27

Rhythmicity is reduced in oviposition and locomotor activity of mated females of Drosophila Melanogaster

Sabrina Riva[1], Maria Trinidad Breide[3], M. Sebastian Risau Gusman[1], Fernanda Ceriani[2], D. Lorena Franco[1]

1.Medical Physics Department, Centro Atómico Bariloche, Comisión Nacional de Energía Atómica (CNEA), Consejo Nacional de Ciencia y Técnica (CONICET), San Carlos de Bariloche, Río Negro, Argentina.
2.Fundación Instituto Leloir, IIBBA, CONICET , Ciudad Autonoma de Buenos Aires, Buenos Aires, Argentina.
3.Centro Regional Universitario Bariloche, Universidad Nacional del Comahue, San Carlos de Bariloche, Río Negro, Argentina.

Presenting Author: **Sabrina Carla Riva**

Oviposition in Drosophila melanogaster is one of the less studied behaviors regulated by the circadian clock. The main cause is the difficulty involved in monitoring and recording it. Because the variable studied, number of eggs, is discrete and usually small when recorded for periods of a few hours, studies are usually made at a population (or group) level. However, population level recordings are not enough to understand what happens at the individual level. To show this, we developed an automatic device that allows the simultaneous monitoring of 21 flies individually, and an analysis method that takes into account the particularities of the data. Using this we verified that, although some genotypes are rhythmic at population level, this does not necessarily imply a high degree of individual rhythmicity. We hypothesize that this may be due to the fact that the influence of the circadian clock on oviposition is partially masked by the various clues provided by the environment during egg laying. We complemented this analysis by studying the locomotor activity of mated females using video tracking. We observed that, in contrast to males and virgins, mated females have low individual rhythmicity of locomotor activity in constant darkness. Based on the above results we hypothesize that in mated females the function of the clock is more susceptible to masking by environmental cues, resulting in the generation of oviposition behaviors and locomotor activity with low rhythmicity.

CIRCADIAN PHYSIOLOGY AND BEHAVIOR

#A-28

Thyroid hormone effects on temporal gene expression of male and female mice heart

Ayla Secio-Silva[1], Rodrigo A. Peliciari-Garcia2[2], A. Guillou[3], P. Mollard[3], X. Bonnefont[3], Paula Bargi-Souza[1]

1.Department of Physiology and Biophysics, Institute of Biological Sciences, Federal University of Minas Gerais, Belo Horizonte, MG, Brazil
2.Department of Biological Sciences of Federal University of São Paulo (Diadema)
3.Department of Physiology, Institute for Functional Genomics, CNRS UMR 5203, INSERM U1191, Montpellier University, France

Presenting Author: **Ayla Secio Silva**

Triiodothyronine (T3) mediates a meaningful regulation of the heart gene expression and its circadian clock machinery. Interesting, there is an important association between clock disruption and heart failure and the prevalence of thyroid disorders is higher in women. Thus, we aimed to compare the expression of clock and functional target genes in the heart of male and female mice during the light-dark transitions (ZT0 and 12) under control, hypothyroid (H) or hyperthyroid (T3) conditions. The preliminary results show that the expression of Myh6 presented a conserved gender response, independent of the T3 condition, while Myh7 was increased in both ZTs of hypothyroid females, a pattern observed only in ZT0 for male mice. Per2 was reduced in both treatments at ZT12 for male, with no significant alterations in female. Nr1d1 was reduced in both ZTs of H males, while for T3 group this reduction was observed for both genders at ZT0. At ZT12, the hyperthyroidism does not alter the male Nr1d1 expression but seems to increase in female. The Thrb1 receptor was reduced at ZT12 in H males, which was not observed for females, but was induced by T3 at ZT0 in both genders. The Dio2 mRNA pattern seems not to be altered in H females, but reduced in males at ZT12. Therefore, it is possible to evidence distinct time-of-day-dependent gender responses in mice heart when facing different T3 availability, as encountered in hypo and hyperthyroidism.

CIRCADIAN PHYSIOLOGY AND BEHAVIOR

#A-29

Seasonal changes in daily activity patterns in a free-living subterranean rodent (Ctenomys aff. knightii)

Jefferson Tiago Silvério[1], Patricia Tachinardi[2], Verónica Sandra Valentinuzzi[3], Gisele Akemi Oda[1]

1.Instituto de Biociências, Universidade de São Paulo, São Paulo, Brazil, São Paulo, Brazil
2.Faculdade de Medicina Veterinária e Zootecnia, Universidade de São Paulo, São Paulo, Brazil
3.Centro Regional de Investigaciones Científicas y Transferencia Tecnológica de La Rioja (CRILAR), Provincia de La Rioja, UNLaR, SEGEMAR, UNCa, CONICET, Anillaco, Argentina

Presenting Author: **Jefferson Silvério**

The Anillaco tuco-tuco (Ctenomys aff. knighti) is a subterranean rodent which inhabits the Monte desert in Northwest Argentina. They exhibit highly different daily activity patterns between laboratory and natural conditions, switching from diurnal to nocturnal when transferred from the field to the laboratory. This suggests that environmental and social factors might interact with the circadian system and play a role in the modulation of their activity patterns. Since these factors undergo seasonal changes, our goal was to investigate possible seasonal changes in the daily activity patterns of free-living tuco-tucos. We trapped animals and deployed collars containing movement and light sensors, which recorded continuous body movement and light exposure. All captured animals showed diurnal activity, with individual and seasonal changes in daily pattern and overall level of activity. To further analyze their activity we used Hidden Markov Models to classify their activity in three distinct behavioral states. We also observed seasonal changes in the time spent in each state, specially for the state with higher levels of activity. Moreover, circadian rhythmicity is more pronounced in the state associated with higher levels of activity while moderate activity is spread throughout the day. Our results suggest that seasonally changing environmental, physiological or social factors are playing a role in the timing of activity and behavioral states in free-living tuco-tucos.

CIRCADIAN PHYSIOLOGY AND BEHAVIOR

#A-30

The suprachiasmatic nucleus modulates the sensitivity of visceral sensory areas

Eva Soto-Tinoco[1], Frederik N. Buijs[1], Ruud M. Buijs[1]

1.Departamento de Biología Celular y Fisiología, Instituto de Investigaciones Biomédicas, Universidad Nacional Autónoma de México

Presenting Author: **Eva Carolina Soto-Tinoco**

The suprachiasmatic nucleus (SCN), our autonomous biological clock, synchronizes behavior with physiological parameters through hormones and the autonomic nervous system. We show that the SCN needs to receive peripheral feedback to fine-tune its output, adjusting physiological processes to the requirements of the moment. We demonstrated that the SCN is incorporated in a neuronal feedback circuit arising from brainstem visceral sensory neurons located in the nucleus of the solitary tract (NTS), and responds to blood pressure changes. Herewith, the SCN can react to hemodynamic perturbations and adapt the blood pressure response accordingly.
The SCN can also modify the sensitivity of brain areas that directly receive feedback information from the periphery. For example, brainstem and spinal cord visceral sensory neurons show a time-of-day-dependent sensitivity to incoming inflammatory information. This circadian gating process allows the organism to have an efficient inflammatory response only during the activity phase when it is most likely needed.
Altogether, this data illustrates the role of the SCN as a structure that receives feedback from the periphery and in addition, modulates the sensitivity of visceral sensory areas to incoming stimuli in a circadian fashion.

CIRCADIAN PHYSIOLOGY AND BEHAVIOR

#A-31

Brain, behaviour, and melatonin: new clues into circadian regulation in the native electric fish Brachyhypopomus gauderio

Juan Vázquez[1], Laura Quintana[2], Ana Silva[1], Adriana Migliaro[1]
1.Facultad de Ciencias, UdelaR
2.Instituto de Investigaciones Biológicas Clemente Estable

Presenting Author: **Juan Vazquez Vazquez**

South American weakly electric fish emit a constant, pulse-like electric organ discharge (EOD), used in electrolocation and intraspecific communication. This organ is commanded by a brainstem pacemaker nucleus (PM) that fires one-to-one with each EOD cycle.The EOD basal rate is modulated by social and environmental cues, allowing the animal to adapt to changing conditions.
Brachyhypopomus gauderio explores, feeds, and interacts during the night. This nocturnal arousal coincides with an EOD rate increase.This increase is dependent on melatonin, the main circadian regulator of day-night rhythms.
We hypothesised that melatonin drives EOD rate by acting directly on the PM. To test this, we administered melatonin a) on PM nucleus in vitro and b) through day-time intraperitoneal injections in free swimming fish, and measured PM firing rate and EOD rate respectively. Melatonin produced a decrease, not increase, in the EOD basal rate in the PM, while day-time melatonin injections did not replicate the natural nocturnal EOD rate increase, although a dose-dependent effect on both the EOD frequency itself and its variability was observed. These results suggest that while melatonin plays an integral role in regulating EOD rate, its mechanism is less straightforward than previously thought, possibly depending on receptor availability during the day, sustained circulating levels of melatonin, and/or interactions with other systems.

CIRCADIAN PHYSIOLOGY AND BEHAVIOR

#A-32

The role of orsai in circadian rhythms

Giovanna Margarita Velázquez Campos[1], María Fernanda Ceriani[1]

1.Laboratorio de Genética del Comportamiento. Fundación Instituto Leloir- IIB-BA CONICET

Presenting Author: **Giovanna Margarita Velázquez Campos**

Rhythmic rest-activity cycles are controlled by an endogenous clock. In Drosophila, the circadian network resides in about 150 neurons organized in groups, out of which the group of ventral lateral neurons (LNv) is essential in the control of rest-activity cycles. Previous results from our laboratory suggest that chronic orsai dysregulation (osi, an important gene in lipid catabolism) within the LNvs affects circadian patterns of locomotor activity in young and aged flies. To understand osi ´s role in the adult brain, genetic tools were used to downregulate osi levels in an adult-specific way, and thus evaluate the impact of osi on the clock neurons. We show that adult-specific osi knockdown in LNv neurons lengthens the period and reduce the consolidation of circadian locomotor activity patterns in young flies. Concomitant expression of its human ortholog ETFRF1 rescues the period phenotype observed. Moreover, ETFRF1 expression in the context of OSI knockdown in aged animals results in flies with properties reminiscent of younger individuals. In addition, decreasing osi levels in this key group of circadian neurons affects the morning anticipation both in young and aged groups under daily conditions, likely through the modulation of PDF levels. Together these results suggest that osi plays a fundamental role in LNv physiology.

ENTRAINMENT

#B-33

Development of Circadian Anticipation and Addiction-Like Behaviors in Rats Exposed to Restricted Access to Alcohol

Sebastián Boy Waxman[1], María Fernanda Setien Rodríguez[1], Carolina Escobar Briones[1]

1.Laboratorio de Ritmos Biológicos y Metabolismo / Facultad de Medicina / UNAM

Presenting Author: **Sebastián Boy Waxman**

Circadian, restricted access to motivating stimuli can result in a change in general activity and body temperature patterns prior to the time of availability. It has been hypothesized that this circadian anticipation involves an increase in craving, which could promote the development of addiction. We used a model of restricted access to alcohol to explore whether anticipation of daily access to alcohol can be developed and whether this response is related to the emergence of addiction.
Four groups of rats had daily 3-hour access to an additional bottle of 10% alcohol (A) or water (C), either during their resting phase (ZT3-ZT6) or their activity phase (ZT15-ZT18). After three weeks, a series of tests were carried out to identify behaviors similar to those observed in addiction. Their activity, body temperature, and substance use were monitored throughout the study.
Only the A groups, showed an increase in activity prior to the arrival of the alcohol bottle, only the group with access to alcohol during their activity phase presented anticipatory changes in core temperature, and only the A groups exhibited 4 out of the 5 addiction-like behaviors measured. However, no correlation was found between these behaviors and the anticipation.
This means that daily scheduled access to alcohol can generate circadian anticipation and promote the emergence of addiction-like behaviors, but these two phenomena develop independently.
This study was funded by PAPIIT IG-200417 and IG-201321

ENTRAINMENT

#B-34

Photoperiodic after-effects on Ctenomys aff. knight: activity phase duration (α) and free-running period (τ) following artificial photoperiods

Giovane Carreira Improta[1], Veronica Sandra Valentinuzzi[2], Gisele Akemi Oda[1]

1.Laboratorio de Cronobiologia Binacional Argentina-Brasil, Departamento de Fisiologia, Instituto de Biociências, Universidade de São Paulo (USP), São Paulo, Brasil

2.Laboratorio de Cronobiología Binacional Argentina-Brasil, Centro Regional de Investigaciones Cientificas y Transferencia Tecnológica de La Rioja (CRILAR), Entre Ríos y Mendoza, s/n, Anillaco, La Rioja, Argentina

Presenting Author: **Giovane Carreira Improta**

Entrainment is achieved by zeitgebers setting period and phase of circadian oscillators. Even after release into constant conditions, endogenous rhythms exhibit long-term effects of the previous entrainment, the so-called after-effects. The photoperiod (ratio between photophase and scotophase) is one of the zeitgeber's properties retained in after-effects of photic entrainment, causing long-lasting effects in free-running period (τ) and activity duration (α). Our group conducts chronobiological studies on a neotropical species of subterranean rodent, known as Anillaco tuco-tucos (Ctenomys aff. knighti). Despite their subterranean habits, these rodents have demonstrated daily rhythms entrained to LD, both in the field and laboratory. Previous work also indicated seasonality in daily activity rhythms, indicating potential for measuring photoperiod. We asked if photoperiod aftereffects would be displayed by tuco-tucos. Our analysis aimed, specifically, on voluntary running-wheel activity α and τ in animals kept in DD (24 °C±2, ad libitum food), after being exposed to one artificial photoperiod (long photophase: LD 21:3, LD 18:6 LD 15:9; short photophase: LD 9:15, LD 6:18, LD 3:21; L=1000 lux). Our results indicate that the mean α for short photophase category (13.7±2.1 h) and long photophase category (11.8±1.8 h) differed significantly. τ was about 24h or longer in most individuals, shorter τ occurred only on 15,15% of observations and usually on the long photophase category.

ENTRAINMENT

#B-35

Photoperiod encoding in a subterranean rodent: Predicting the effects of daily light at irregular times with a dual-oscillator model

Danilo E. F. L. Flôres[1], Gisele A. Oda[1]

1.Laboratório de Cronobiologia Binacional Argentina-Brasil, Instituto de Biociências, Universidade de São Paulo, SP, Brasil

Presenting Author: **Danilo E F L Flôres**

Seasonal organisms may synchronize their biology to the seasons by processing photoperiod, i.e., the proportion between light and dark hours within a day. In mammals, photoperiod encoding is mediated by differential entrainment of the circadian system. Here, we investigate this process in a seasonal subterranean rodent, the Anillaco tuco-tuco (Ctenomys aff. knighti), via mathematical modeling. In nature, tuco-tucos inhabit dark tunnels and only see the light sporadically, at irregular times. We tested whether these irregular light exposure patterns can entrain the circadian system in a photoperiod-dependent fashion. To that end, we modelled a two-oscillator system exposed to simplified light schedules that mimic the natural light exposure of tuco-tucos at different seasons. Each light schedule was composed of only one or two light pulses per day, applied at random times within the light hours of the day. The two-oscillator system is based on the conceptual evening and morning circadian oscillators, and it was implemented with Pavlidis-Pittendrigh equations. Our results indicate that specific features in the light exposure patterns of tuco-tucos allow the encoding of photoperiod. Particularly, the model responds better when we replicate the contrast between mid-day exposure in winter and twilight exposure in summer. In sum, our results predict that the light exposure patterns of tuco-tucos, although irregular, can convey distinct photoperiods to their circadian system.

HUMAN CIRCADIAN RHYTHMS

#B-36

Why should Chronobiology be included in educators' training?

Rubia Carvalho Mendes[1], Luiz Menna Barreto[2]

1.Escola de Artes Ciências e Humanidades (EACH/USP)
2.Escola de Artes Ciências e Humanidades (EACH/USP)

Presenting Author: **Rubia A. Pereira Carvalho Mendes**

Chronobiologists study biological rhythms, such as the human sleep/wake cycle, a frequent topic in scientific literature. It's a multidisciplinary area, in which there are scientific studies on educational aspects. We aim to share our experience in teaching Chronobiology to educators. For this, since 2016, we have been offering extension courses on Chronobiology in "Escola de Artes, Ciências e Humanidades - EACH-USP", our methodology includes lectures, practical activities and seminar presentations. Over these years, the course was offered 11 times (8 classroom and 3 on-line), for 1 week each (20 hours) and some chronobiology researchers and students were invited to speak during the event. For evaluation of the course we used a questionnaire, critical report and selected some seminar proposals illustrating Chronobiology teaching practices. In this presentation we will share the results of the 8 versions of the course. Of the 73 educators (54 women), 35.8 ± 13.0 years, only 20.83% declared a great level of knowledge in Chronobiology before the course, 95.8% recommended it. The participants' evaluation on the course brought a new conception about the functions of sleep and with applications in personal and professional life. We propose that chronobiology may be discussed at all educational levels.

HUMAN CIRCADIAN RHYTHMS

#B-37

Circadian phase determinants in Antarctica

Julieta Castillo Stratta[1], Ana Silva[1], Bettina Tassino[1]

1.Grupo Cronobiología, CSIC, Universidad de la República
2.Facultad de Ciencias, Universidad de la República

Presenting Author: **Julieta Castillo Stratta Castillo Stratta**

The onset of the endogenous melatonin rhythm in dim light (DLMO) is a reliable marker of the human circadian clock. Although light in the evening delays the circadian phase, light in the morning advances it. In this study we aimed to identify the determinants of DLMO in response to an abrupt change of light exposure during a short summer trip to Antarctica. We compared two conditions in 11 university students: control, during the austral fall equinox in Montevideo, Uruguay; and a 10-day trip to King Georges Island, Antarctica during the austral summer. Circadian preferences were assessed by the Morningness-Eveningness Questionnaire (MEQ). All participants wore actigraphs to determine light exposure and sleep timing. To estimate circadian phase by DLMO, hourly (18:00-24:00) saliva samples were collected under light < 30 lux, on the last night of each sample period. We calculated for each participant the hourly average of light exposure across each sample period in both conditions in two 3h-phase-relevant time windows centered by individual DLMO (zeitgeber time=0). Although DLMO on average did not differ between the two locations, individual DLMO change between both conditions (ΔDLMO) strongly correlated with the changes in the light exposure in both the evening (R=0.75, p=0.008 and the morning (R =-0.80, p=0.003). In addition, ΔDLMO also correlated with participants' circadian preferences (R=0.66, p=0.026), and with changes in sleep timing (R=0.69, p=0.018).

HUMAN CIRCADIAN RHYTHMS

#B-38

Physical activity as modulator of dancers' circadian phase

Natalia Coirolo[1], Bettina Tassino[1], Ana Silva[1]

1.Grupo Cronobiología, Comisión Sectorial de Investigación Científica, Universidad de la República, Montevideo, Uruguay
2.Sección Etología, Facultad de Ciencias, Universidad de la República, Montevideo, Uruguay
3.Laboratorio de Neurociencias, Facultad de Ciencias, Universidad de la República, Montevideo, Uruguay

Presenting Author: **Natalia Coirolo**

Dancers are a valuable model to evaluate the influence of physical training on the biological clock. Professional dance training in Uruguay is organized in two shifts: morning (8:30-12:30) and night (20:00-24:00). We evaluated the influence of shifts on the circadian phase and their determinants in 18 dancers (7 morning shift, 11 night shift) during 18 days from data obtained by self-reports, actigraphy, and melatonin levels. We found no significant differences between shifts in daily intensity of activity. The activity-rest rhythm acrophase was later in night than in morning dancers in workdays. Individual dim light melatonin onset (DLMO) was calculated as indicator of circadian phase. Hourly saliva samples (18:00–24:00) were collected in dim light (<30 lx) the last night of the recording period. DLMO was significantly later in night than in morning dancers ($p<0,01$), and in night dancers associated with chronotype ($R=0,78$, $p<0,005$) and marginally with sleep timing ($R=0,56$, $p=0,07$). During the circadian phase-sensitive time window around the DLMO (-2h-+1h), physical activity, but not light exposure, was significantly higher in night than in morning dancers. Moreover, an increase in the intensity of physical activity during this time window, but not of light exposure, was significantly associated with a delay in the circadian phase ($R=0,57$, $p=0,02$). This work allows to dissect the impact of light and exercise as independent circadian zeitgebers in a real-life situation.

#B-39

Chronobiology and Occupational Health: A promising society?

J. Mathías Cosentino[1y2], Ignacio Estevan[1], Bettina Tassino[2]

1.Programa de Neuropsicología y Neurobiología, Facultad de Psicología, Universidad de la República.
2.Sección Etología, Facultad de Ciencias, Universidad de la República

Presenting Author: **José Mathías Cosentino**

Occupational Health seeks to promote physical, mental and social well-being of workers. Rotating shift work is a challenge for most people, and its association with irregular, short, and poor-quality sleep has major negative health consequences. However, adverse effects can be modulated by individual characteristics, such as, chronotype, estimated by the timing of sleep on free days. Our main objective was to assess whether the working shift influence in workers' sleep varies with the reported chronotype. For this, 104 workers affected by a 8-hour three-shift rotation system (morning, night, afternoon) completed a sociodemographic questionnaire and a reduced version of the Munich Chronotype Questionnaire for shift-workers. It was found that chronotype modulates the effect of shift type on the sleep duration. Increasing eveningness was associated with longer main sleep duration during series of night and afternoon shifts (night: $r=0.48$, $p<0.001$; afternoon: $r=0.51$, $p<0.001$), but negatively associated with chronotype during series of morning shift ($r=-0.48$, $p<0.001$). The strategies to compensate for sleep debt (longer sleep duration on free days and naps on workdays) were more used by workers with later chronotypes, resulting in a longer overall sleep duration (night: $r=0.46$, $p<0.001$; afternoon: $r=0.49$, $p<0.001$). These novel results will allow an individualized intervention by Occupational Health in the search for the prevention of chronic diseases associated with sleep deficit.

#B-40

Sleep patterns in childhood in Uruguay: preliminary results

Dimara Curbelo[1], Olivera Andres[1], Estevan Ignacio[2], Cecilia Rossel[3], Ana Silva[1], Bettina Tassino[1]

1.Facultad de Ciencias, Universidad de la República
2.Facultad de Psicología, Universidad de la República
3.Departamento de Ciencias Sociales, Universidad Católica del Uruguay

Presenting Author: **Dimara Irma Curbelo Machado**

Quality and duration of sleep have effects on different dimensions of child well-being, such as mental health, psychomotor development and nutritional status. Despite its relevance, there are no studies in Uruguay that allow to estimate the impact of sleep habits on these variables. This project aims to contribute to filling this gap, providing novel evidence on the chronobiological characterization and sleep patterns in childhood, and their relationship with sociodemographic parameters. An interdisciplinary team was formed to process data from a longitudinal survey on Nutrition, Development and Health in childhood (ENDIS) and contribute to the design of evidence-based public policies through two components: (a) a quantitative analysis of the ENDIS database to explain children's sleep patterns by family and socioeconomic context and address the consequences of these patterns on different dimensions of well-being and (b) an experimental assessment of the daily sleep-wake cycle, physical activity level and light exposure patterns by actimetry. In this instance, we will present preliminary results of the ENDIS parameters, along with preliminary results of a pilot experiment of actimetric recordings in 5 children aged 7 to 8 years. The results of this project will contribute to the development of objective measures to be applied in future longitudinal surveys, and will provide elements for the design of public interventions on health, child education and child-rearing practices.

HUMAN CIRCADIAN RHYTHMS

#B-41

The loss of social zeitgebers during the COVID-19 lock down in Mexico affected daily habits and day-light exposure in young adults favoring depression and anxiety

Carolina Escobar[1], Natali Nadia Guerrero Vargas[1], Manuel Angeles Castellanos[1]
1.Departamento de Anatomía, Facultad de Medicina UNAM

Presenting Author: **Carolina Escobar**

In March 26 2020 started the official lockdown due to the COVID-19 pandemia in Mexico. From May 6 to May 22, 2020, 6-8 weeks after a strict lockdown condition, we distributed a questionnaire via social media that was answered by more than 3000 adults along the country. The questionnaire explored information about daily habits before and during the lockdown for sleep schedules, meals, exercise and light exposure, work and/or study conditions. It also included two brief questionnaires exploring depressive and anxiety states (PH9 y GAD7, respectively). The participants were 74% females and 26% males, in a range of 18-87 years of age.
Because of the forced lockdown and loss of work / study schedules, participants had delayed their sleep midpoint. However, the number of sleep hours and perception of sleep quality improved. The exposure to natural daylight was significantly reduced representing only a 45% from the previous regular weekdays, while the use of the cellular phone during the night was increased by 50%. Changes in daily habits were associated with higher values in the depression and anxiety scales and affected especially adults below 30 years of age.
Present data indicate the relevance of social Zeitgebers for adaptation to daily cycles and for circadian synchrony. They also point out the tight association between circadian disruption and mood disorders.

This study was partially supported by PAPIIT IG-201321 and División de Investigación, Facultad de Medicina UNAM.

HUMAN CIRCADIAN RHYTHMS

#B-42

Bidirectional association between light exposure and sleep in adolescents

Ignacio Estevan[1], Bettina Tassino[2], Céline Vetter[3], Ana Silva[4]

1.Instituto de Fundamentos y Métodos, Facultad de Psicología, Universidad de la República
2.Sección Etología; Facultad de Ciencias, Universidad de la República
3.Department of Integrative Physiology; University of Colorado Boulder
4. Laboratorio de Neurociencias; Facultad de Ciencias, Universidad de la República

Presenting Author: **Ignacio Estevan**

During adolescence, changes in both circadian and homeostatic factors are related to the delayed sleep timing observed in adolescence, which is related to restrictions in their sleep duration. In addition, the circadian factor allows light exposure and sleep pattern to be related. We recorded 15 high-school students for 11 vacation days and 12 school term days using GENEactiv accelerometers. Using a repeated-measures analysis, we explored the day-to-day bidirectional association between light exposure and sleep behavior across a period with extreme variability in social pressures. When the previous day light was 10 times more intense the sleep onset was more than 30 min earlier and the sleep duration was almost 20 min longer. In addition, when sleep ended 1 h later the light intensity had a reduction of more than 20%. Sleep onset and offset were both later during vacation than on school days (almost 2 h and 4 h, respectively), and free days (almost 1 h), while sleep duration was almost 2 h longer on vacation and free days than on school days. On the other hand, light exposure intensity was two times higher during vacation days when adjusted by sleep timing. Although we found that light exposure was associated with longer sleep duration, the influence of school start times was greater and ended up prevailing, which explained the short sleep durations observed on school days.

HUMAN CIRCADIAN RHYTHMS

#B-43

Circadian rhythm alterations in body temperature as a surrogate marker of the severity of depressive symptoms and disability in fibromyalgia

Betina Tocchetto[1], Leticia Ramalho[1], Maxciel Zortea[1], Samara Bruck[1], Rafaela Tomedi[1], Wolnei Caumo[1]

1.Laboratory of Pain & Neuromodulation, Hospital de Clínicas de Porto Alegre (HCPA), Porto Alegre, Brazil

Presenting Author: **Betina Franceschini Tocchetto**

Introduction: Fibromyalgia (FM) is a syndrome characterized by generalized musculoskeletal pain, fatigue, non-restorative sleep, depressive symptoms (DS) and correlates of autonomic dysfunction. It is suggested the presence of melatonin secretion rhythm disruption in FM and Major Depressive Disorder (MDD). MDD individuals also present body temperature rhythm disruption. Objective: To assess the association between peripheral body temperature (PBT) rhythm according to the severity of DS. Methods: Cross-sectional study. 58 women, aged 30-65 years, with FM diagnosis were included. 24h PBT was assessed by actimetry. DS groups were divided based on the Hamilton Depression Rating Scale score. Secondary outcomes were assessed by Pittsburgh Sleep Quality Index, Central Sensitization Inventory, and Profile of Chronic Pain: Screen. Results: Univariate analysis showed a higher PBT in moderate to severe DS group (p=0.013) and considering 4 periods of a 24h cycle, the same group showed a higher temperature just in the evening (p=0.002). A generalized estimating equation revealed a difference between the groups (χ2 = 337.79, DF = 7; p <0.001), when analyzing PBT rhythm in each 6h, starting 6am. Evening PBT was positively correlated with greater disability due to pain and higher central sensitization score (P<0.05 for both outcomes). Conclusion: FM women with more severe DS present PBT rhythm alterations and it is related to the severity of clinical symptoms.

HUMAN CIRCADIAN RHYTHMS

#B-44

Relationship between chronotype and the sleep-wake cycle and attention in adolescents in the morning shift

Silva Júnior, E.L.R. 1; Bessa, Z.C.1; Galina, S. D.1; Oliveira, M. L. C.1; Valdez,P.2; Azevedo, C.V.M; Louzada, F. M. 3

1.Universidade Federal do Rio Grande do Norte
2.Universidade Federal do Paraná
3.Universidad Autónoma de Nuevo León

Presenting Author: **Emanuel Júnior**

Adolescents tend to eveningness. This tendency along with early school start times induce a reduction in sleep time and an increase in social jet lag, which can lead to worse cognitive and academic performance. Therefore, this study aimed to assess the relationship between the chronotype and the sleep-wake cycle and attention in adolescents attending a morning shift in schools of Natal. 207 adolescents of high school participated in the study. Sleep patterns were evaluated from sleep diaries for 10 days. Midsleep on free days sleep corrected was used as a chronotype indicator and as a basis for calculating social jet lag. Attention was assessed by the Continuous Performance Task in the morning during school days. Evening types go to bed and wake up later during the week, with higher irregularity in bedtime and waking times and higher social jet lag. However, morning types had more time in bed at the weekend. Regarding attention, longer time in bed at the night before the task predicted a higher percentage of correct responses for phasic alertness. Chronotype did not predict the indicators of attention components. Although the chronotype was related to the sleep and wake cycle in adolescents, further studies are needed to investigate the relationship between eveningness and attention.

HUMAN CIRCADIAN RHYTHMS

#B-45

Association between sleep duration and quality with physical activity in adolescents.

Ana Paula Leão Maia Fonseca[1], Maria Luiza Cruz de Oliveira[2], Sabinne Danielle Galina[2], Carolina Virginia Macêdo de Azevedo[2], Rute Marina Santos[3], Rute Marina Santos[4]

1.Universidade Lusófona de Humanidades e Tecnologias, Lisboa-Portugal
2.Laboratório de Cronobiologia e Comportamento. Programa de Pós-graduação em Psicobiologia Universidade Federal do Rio Grande do Norte, Natal, Brasil
3.Research Centre in Physical Activity, Health and Leisure, Faculty of Sports, University of Porto
4. General Directorate-General of Health - National Program for Physical Activity Promotion, Portuguese Ministry of Health, Lisbon, Portugal

Presenting Author: **Ana Paula Leão Maia Fonseca**

Background: Evidence shows that adolescents today tend to sleep less compared to previous generations and the health benefits of regular physical activity are well documented. Despite the consensus that both adequate quality and duration of sleep and physical activity are essential for maintaining health, only recently there has been a growing interest in research on the impact of physical activity on sleep in adolescents. Thus, this study aimed to investigate the association between sleep duration and quality with physical activity, in Brazilian adolescents.
Methods: The sample consisted of 150 adolescents (95 girls) aged of 16.1±0.8 years. Sleep duration and quality were assessed with actimetry, and physical activity using the Health and Sleep questionnaire.
Results: 63.6% of the participants engage in some type of physical activity. Approximately 95.4% of girls and 89.7% of boys did not comply with recommendations for moderate and/or vigorous physical activity/week (60 min/day). The averages of sleep onset and offset times, sleep duration, WASO and the number of nighttime awakenings differed between weekdays and weekends, being later and higher on weekends. The start, finish and duration/week of physical activity were predictors of irregular bedtime: the sooner the physical activity starts and ends, and the longer its duration/week, the smaller was the irregularity in the sleep onset but not with the indicators of sleep quality, assessed through actimetry.

HUMAN CIRCADIAN RHYTHMS

#B-46

The influence of sleep quality in retrieval and recognition of the episodic memory: An exploratory study.

Rael Lopes Alves[1], Letícia Ramalho[2], Maxciel Zortea[2], Wolnei Caumo[2]

1.Post-graduation Program in Medicine: Medical Sciences, Federal University of Rio Grande do Sul (UFRGS)
2.Laboratory of Pain & Neuromodulation, Clinical Hospital of Porto Alegre (HCPA)

Presenting Author: **Rael Lopes Alves**

Introduction: Fibromyalgia (FM) is a musculoskeletal and generalized chronic pain, associated with a Descending Pain Modulatory System dysfunction that is related to sleep disturbances, cognitive impairment, and mood disorders. The influence of pain in sleep quality and its association with episodic memory is not yet understood.

Aim: Evaluate the influence of disability due to pain on sleep quality and its relationship with episodic memory.

Methods: A cross-sectional case-control study. Were included 45 women aged 30 to 65 divided in 2 groups: a group of 30 FM patients and a group of 15 healthy subjects (HS). Episodic memory was evaluated with the Rey Auditory-Verbal Learning Test (RAVLT), specifically the retrieval and recognition index of memory (A9-A8). The sleep quality was measured by the Pittsburgh Sleep Quality Index (PSQI).

Results: The groups did not show significant differences in age (FM=47.5±10.7; HS=44,8±10,0) and years of study (FM=13.87±4.53; HS=15.40±4.92). The hierarchical linear regression model was performed. Data showed that retrieval memory and recognition was explained by the groups and sleep quality (F2,42=6.723; p=0.003; R2=0.243). Retrieval memory and recognition are predicted by Sleep Quality (β=341; t=2.471; p=0.018) and Groups (β=284; t=2.057; p=0.046). Conclusion: These results show that the bad quality of sleep affects the retrieval and recognition process of the episodic memory more than disability promoted by chronic pain in FM patients.

HUMAN CIRCADIAN RHYTHMS

#B-47

Does the sleep-wake cycle influence the visuospatial memory of patients with chronic migraine?

Mírian Celly Medeiros Miranda David[1,2], Marina Souza Barbosa de Mattos[2,3], Nataly Ferreira dos Santos[2], Jandirlly Julianna de Souza Souto[4], Sarah Aline Curioso de Freitas Brito[3], Etcheverry Silva Leite[3], Gilma Serra Galdino[3], Patrick Giordanni Gomes Sampaio[5], Gilberto Ramos Vieira[2,6], John Fontenele Araújo[7],Carlúcia Ithamar Fernandes Franco[3]; Rhowena Jane Barbosa de Matos[1,2,8*]

1.Program in Neuropsychiatry and Behavioral Sciences, Federal University of Pernambuco, Recife, Brazil.
2.Neural Plasticity, Environment and Behaviors Research Group, Federal University of Recôncavo da Bahia, Santo Antônio de Jesus, Brazil.
3.Department of Physiotherapy, State University of Paraíba, Campina Grande, Brazil.
4. Program in Social Psychology, Federal University of Paraíba, João Pessoa, Brazil.
5. Faculty of Medical Sciences of Campina Grande, UNIFACISA, Campina Grande, Brazil.
6. Physical Education and Sports Science Nucleus, Academic Center of Vitória, Federal University of Pernambuco, Vitória de Santo Antão, Brazil.
7. Department of Physiology, Federal University of Rio Grande do Norte, Natal, Brazil.
8. Health Sciences Center, Federal University of Recôncavo da Bahia, Santo Antônio de Jesus, Brazil.

Presenting Author: **Mírian Celly Medeiros Miranda David**

Aim: To investigate the interaction between the sleep-wake cycle and the visuospatial memory of individuals with chronic migraine (CM). Methods: Actimetry was performed on 46 individuals, who composed the experimental groups with CM (WM, N=23) or without CM (control: C, N=23), for 15 days. The 30-day headache and sleep diaries were requested. Subjects were submitted to the Morningness and Eveningness Questionnaire; Pittsburgh Sleep Quality Index; Epworth Sleepiness Scale; and Rey-Osterrieth Complex Figure Test (ROCFT). The Mann-Whitney test and a multiple linear regression were applied, with p<0.05. The study was approved by the Ethics Committee of UFPE (protocol: 92552318.6.0000.5208). Results: 96% of the sample were female, 58.7% had an intermediate chronotype, and the average age of C group corresponded to 27.4±6.3 years, while the WM was 27.8±6.4 years old. WM individuals had worse sleep quality when compared to C group (p<0.001), but with no difference in daytime sleepiness. As for the ROCFT, WM individuals had longer reaction (p<0.001), copying (p=0.02) and evocation time (p=0.04), however without differences in the drawings. The relative amplitude (p=0.04) and the rhythmic percentage (%V, p=0.03) of the activity-rest rhythm were determinant for the reaction time of WM individuals. Conclusion: The sleep-wake cycle is one of the modulating aspects of the reaction time delay among WM group, however, there was no association for visuospatial memory and copying abilities.

HUMAN CIRCADIAN RHYTHMS

#B-48

The impact of social jetlag and training shifts on sleep quality and depressive symptoms

Valentina Paz[1], Natalia Coirolo[2], Micaela De Mori[2], Agustín Laguardia[2], Betania Martínez[2], Luna Perla[2], Bettina Tassino[2], Ana Silva[2]

1.Facultad de Psicología, Universidad de la República, Montevideo, Uruguay.
2.Facultad de Ciencias, Universidad de la República, Montevideo, Uruguay.

Presenting Author: **Valentina Inés Paz Pérez**

Social schedules (e.g. school shifts) could lead to a discrepancy between social and biological time (a concept referred to as social jetlag; SJL). This misalignment has been related to poor sleep quality and depressive symptoms. In this study, we took advantage of the extreme training shifts of END-SODRE, a school for professional training in dance in Uruguay (morning-shift: 8:30-12:30; night-shift: 20:00-24:00), to investigate the impact of SJL and training shifts on the student's (n=77) sleep quality and depressive symptoms. Participants completed the Munich Chronotype Questionnaire to assess SJL, chronotype and sleep duration, the Pittsburgh Sleep Quality Index for sleep quality and the Beck Depression Inventory for depressive symptoms. Participants reported high levels of SJL (2.02±1.52), late chronotypes (06:11±02:05), an adequate sleep duration (7.28±1.19), poor sleep quality (6.56±2.81) and mild depressive symptoms (7±4.85). Interestingly, morning-shift participants reported significantly higher SJL than night-shift ones. SJL mediated the association between sleep quality and depressive symptoms; the higher the SJL, the worsen the sleep quality as depressive symptoms increase. Finally, sleep quality worsened as SJL increases, but only in night-shift participants, while depressive symptoms did not depend on SJL. Our findings suggest that the training shifts have an impact on SJL and that this misalignment affects distinctively sleep quality and depressive symptoms.

HUMAN CIRCADIAN RHYTHMS

#B-49

The relationship between incidents reported by medical residents with their sleep and circadian patterns

Malena Lis Mul Fedele[1], Maria del Pilar López Gabeiras[2], Hernán Seoane[1], Giannina Bellone[1], Guido Simonelli[1], Joaquín Diez[1], Joaquín Cagliani[1], Luis Larrateguy[1], Kumiko Eiguchi[4], Diego Andrés Golombek[3], Daniel Cardinali[1], Daniel Pérez Chada[2], Daniel Eduardo Vigo[1]

1.Chronophysiology Lab, Institute for Biomedical Research (UCA-CONICET), Buenos Aires, Argentina
2.Austral University, Buenos Aires, Argentina
3.Chronobiology Lab, Department of Science and Technology, National University of Quilmes, Buenos Aires, Argentina
4. Ministry of Health of Buenos Aires city, Buenos Aires, Argentina

Presenting Author: **Malena Mul Fedele**

Nowadays, many operations and services require continuous activity. One of them are medical residents, whose work schedules often include extended shifts and nocturnal work. These schedules can lead to a reduction in resident's mental and physical performance capability which can affect their safety and well-being, and have potentially adverse implications on patient care.
The aim of the present study was to explore the presence of sleep and circadian alterations in Argentinian medical residents and its relationship with incidents at the hospital. We carried on a subjective study in a group of 661 medical residents, and then an objective study in a group of 37 of them. The group that informed incidents, reported sleeping less per working day and we found that sleep debt was about 30 minutes greater than the ones who reported not having incidents. This group also reported higher scores of anxiety, depression and burn out. Regarding the objective measures, we found that residents that reported incidents worked more hours of extended shift and that they slept more hours during the day and less hours during the night than those who didn't. Finally, we also studied the temperature rhythm and found that those who reported incidents had a lower amplitude and percentage of rhythmicity than those who didn't, suggesting circadian misalignment. These results suggest the development of new methods to monitor circadian misalignment, to prevent incidents in health care facilities

HUMAN CIRCADIAN RHYTHMS

#B-50

School timing and age effects on chronotype and sleep on adolescents: a longitudinal study

Guadalupe Rodríguez Ferrante[1], Andrea Paula Goldin[4], Diego Andrés Golombek[2], Mariano Sigman[3], María Juliana Leone[4]

1.COICET-UNQ-UTDT
2.Universidad Nacional de Quilmes, CONICET, Laboratorio de Cronobiología, Departamento de Ciencia y Tecnología, Buenos Aires, Argentina
3.Facultad de Lenguas y Educación, Universidad Nebrija, Madrid, Spain
4. Universidad Torcuato Di Tella, CONICET, Laboratorio de Neurociencia, Buenos Aires, Argentina

Presenting Author: **Guadalupe Rodriguez Ferrante Rodriguez Ferrante**

Chronotype is the expression of the circadian timing under light-dark conditions and it is modulated by several factors, including age and social cues. Although in humans chronotype reaches a peak of lateness at the end of adolescence, most students have to wake up early in the morning to go school, which leads to chronic social jetlag and sleep deprivation. We longitudinally evaluate the effect of age and school timing (ST) on adolescents' chronotype and sleep habits at two different time points: at the start (13-14 y.o.) and again at the end (17-18 y.o.) of high school (n=259). Students were randomly assigned to one of three STs (morning, afternoon or evening) in their first year. Importantly, we also assess the effect of the basal chronotype and ST on the developmental change in chronotype. We observe that students' chronotype is partially aligned with their ST but that is insufficient for students to reach healthy sleep habits. In particular, morning-attending students show higher levels of SJL and shorter sleep duration on weekdays than students attending later STs. Interestingly, the developmental change in chronotype depends on ST and on basal chronotype. Our results add evidence to local knowledge, which is important because of the extremely late chronotype of Argentinean adolescents. Importantly, the novel relation between the developmental change in chronotype and the basal chronotype might be crucial to develop interventions to improve adolescents' sleep health.

HUMAN CIRCADIAN RHYTHMS

#B-51

The emergence of chronobiology from the perspective of historical and dialectical materialism

Cristiane Juciara Siniscalchi[1], Mario Pedrazzoli[1]

1.Universidade de São Paulo

Presenting Author: **Cristiane Juciara Siniscalchi Siniscalchi**

To explain how the science of biological time, human circadiam rhythms, named chronobiology, was engendered, during the 1960s, both social and economic aspects, recognized as fundamental in the externalist conception of the history of science, were analyzed, as well as the internal dynamics of scientific making, hypotheses and methods were considered, present and determinant in the internalist conception. To overcome the dichotomy between these interpretations of history, the relationship of reciprocity between external and internal factors in the production of scientific knowledge was observed. The methodological procedures included reading scientific, historical and biographical articles, consulting digital documents and bibliography specialized in science and history. From these sources, the observed evidence showed the influence of the Cold War on the scientific production of chronobiology, as NASA and NATO funded experiments and laboratories for research into biological rhythms in humans. F. Halberg, J. Aschoff and C. Pittendrigh, founding scientists of this field, showed in some historical narratives, reports on the trajectory of their lives and the emergence of chronobiology, which allowed us to observe the intrinsic relationship between external and internal aspects of the history of this scientific knowledge. The present approach make possible to break down the linearity of the history of science. A narrative on history and science was proposed.

HUMAN CIRCADIAN RHYTHMS

#B-52

RNAseq analysis of the prefrontal cortex of mice exposed to a daylength transition model

Vinicius Tenório Braga Cavalcante Pinto[1], Mayara Rodrigues Barbosa[1], Daniel Gomes Coimbra[1], Tiago Gomes de Andrade[1]

1.Centro de Medicina Circadiana - Faculdade de Medicina, Universidade Federal de Alagoas, Maceió – Brasil.

Presenting Author: **Vinícius Tenório Braga Cavalcante Pinto**

Several epidemiological studies have described a seasonal variation of suicide rates and mania symptoms with peaks in spring and early summer. We previously demonstrated that mice exposed to a photoperiodic transition protocol present a mania-like behavior that is prevented by lithium administration. In this work, we evaluated transcriptome alterations in the prefrontal cortex (PFC) of this model. We used thirty mice (C57BL/6) divided into: Equatorial photoperiod (12:12LD) group (n=15); DTM (Daylength Transition Model) group (n=15), initially subjected to 12:12LD regime before stabilization at short photoperiod (8:16LD) for 8 days, then an increasing photoperiodic condition (1h/day) until reach 16:8LD where the samples were collected (ZT06). PFC were pooled in three replicates (n=5 for replicate) for each group for RNAseq. Sequences were annotated using Kallisto and the differentially expressed genes (DEGs) were determined using Deseq2 (Fdr<0.05). We identified 102 DEGs (49 up-regulated in DTM). Some of these DEGs were previously associated with neuropsychiatric disorders, including Schizophrenia, Autism, Parkinson and Alzhheimer. Serpinh1, a repressed gene in DTM was previously identified to be downregulated in the brain of suicidal patients. These results indicate that important neurological changes occur at the molecular level in the prefrontal cortex of a potential model for bipolar disorder and suicidal behavior induced by photoperiodic transitions.

HUMAN CIRCADIAN RHYTHMS

#B-53

Rest-Activity rhythm throughout one year of Antarctic isolation

Camila Tortello[1], Juan Manuel Lopez[2], Estefanía Sala Lozano[3], Maria Soledad Rivero[2], Guido Simonelli[4], Diego Andrés Golombek[5], Daniel Eduardo Vigo[1], Santiago Andrés Plano[1]

1.Institute for Biomedical Research, UCA-CONICET
2.Argentine Joint Antarctic Command
3.Argentine Antarctic Institute
4. University of Montreal
5. National University of Quilmes

Presenting Author: **Camila Tortello**

In Antarctica, people are exposed to long periods of absence of natural light, isolation, and confinement that pose risks to the circadian clock. In this extreme context the main zeitgebers, such as, light and social cues change drastically challenging the human ability to adapt to these harsh conditions which can impact health and performance. A total of sixteen (N=16) army military personnel from the Argentinian Antarctic station Belgrano II were assessed throughout a year. The rest-activity rhythm was evaluated by wrist accelerometers 24 hours a day, 7 days a week from March 2019 to January 2020. Cosinors and Waveforms were performed to estimate the activity circadian pattern. Multilevel models revealed a monthly 5.11-minute Acrophase advance (γ00= 840.52, SE= 15.58, CI 95%= [809.98, 871.07], t(16)= 53.94, p< .001), a 5.16-minute decrease in the Activity Interval each month (γ00= 928.09, SE= 12.26, CI 95%= [904.06, 952.14], t(46)= 75.67, p< .001) and a 11-count monthly Amplitude increase (γ00= 589.62, SE= 33.72, CI 95%= [523.53, 655.72], t(29)= 17.48, p< .001). Time did not influence the Mesor values as it was not a predictor for the model (γ00= 816.41, SE= 23.96, IC 95%= [769.44, 863.38], t(16)= 34.07, p< .001). Results showed that the rest-activity period decreased throughout the campaign with an advance of its maximum level month by month. Social cues determined by confinement and isolation seem to modulate rest-activity rhythm.

HUMAN CIRCADIAN RHYTHMS

#B-54

{mctq}: An R Package for the Munich ChronoType Questionnaire

Daniel Vartanian[1], Ana Amélia Benedito-Silva[1], Mario Pedrazzoli[1], , , , , , ,

1.Interdisciplinary Sleep Research Group (GIPSO), School of Arts, Sciences and Humanities (EACH), University of Sao Paulo (USP)

Presenting Author: **Daniel Vartanian**

{mctq} is an R package that provides a complete and consistent toolkit to process the Munich ChronoType Questionnaire (MCTQ), a quantitative and validated method to assess peoples' sleep behavior presented by Till Roenneberg, Anna Wirz-Justice, and Martha Merrow in 2003. The aim of {mctq} is to facilitate the work of sleep and chronobiology scientists with MCTQ data while also helping with research reproducibility.
Although it may look like a simple questionnaire, MCTQ requires a lot of date/time manipulation. This poses a challenge for many scientists, being that most people have difficulties with date/time data, especially when dealing with an extensive dataset. The {mctq} package comes to address this issue. {mctq} can handle the processing tasks for the three MCTQ versions (standard, micro, and shift) with few dependencies, relying much of its applications on the {lubridate} and {hms} packages from tidyverse. We also designed {mctq} with the user experience in mind, by creating an interface that resembles the way the questionnaire data is shown in MCTQ publications, and by providing extensive and detailed documentation about each computation proposed by the MCTQ authors.
The first stable version of {mctq} is available for download on GitHub (https://github.com/gipso/mctq). The package is currently under a software peer-review by the rOpenSci initiative. We plan to submit it to CRAN soon after the review process ends.

HUMAN CIRCADIAN RHYTHMS

#B-55

Characterization of circadian rhythms and body mass index (BMI) in individuals with different genotypes for gene PER3: comparison between daytime and nighttime college students

Juliana Viana Mendes[1], Maria Augusta Medeiros Andrade[1], Daniel Vartanian[1], Ana Amélia Benedito-Silva[1], Mario Pedrazzoli[1]

1.School of Arts, Sciences and Humanities, University of São Paulo

Presenting Author: **Juliana Viana Mendes**

Introduction: Biological rhythms are partly a result of the interaction of the dark/light environmental cycle and genetic characteristics. However, the temporal organization of contemporary society can lead to new environmental clues to human health and productivity. A possible conflict between inherited biological rhythms and social timing emerges when the temporal organization of work and study lead to circadian disruption. The present study aims to investigate the possible associations between chronotype, school shift, polymorphisms in the PER3 clock gene and the consequences for the energy metabolism of the temporal challenge of the evening study. Methods: 513 undergraduates (18-30 years old) volunteers had their saliva and Munich Chronotype Questionnaire collected. Genotyping for PER3 gene VNTR polymorphism was conducted. Objective rest/activity parameters have been obtained through the wrist actigraphy from a subsample of students with different genotypes. Preliminary results: Based on the partial results, it was possible to observe that the students showed great variability related to their self-reported sleep habits. But mainly a very late and irregular profile of sleep at all. This result is interesting in the sense of enabling more accurate analysis, and searches for associations between circadian genotypes, sleep phenotypes and BMI.

MOLECULAR BIOLOGY

#B-56

The Drosophila clock regulates ecdysone action to control the daily rhythm of adult emergence

Liliana Bustos-González[1], John Ewer[1]

1.1Centro Interdisciplinario de Neurociencia de Valparaíso, Universidad de Valparaíso, CHILE
2.2Doctorado en Neurociencia, Universidad de Valparaíso.

Presenting Author: **Liliana Bustos**

In insects, the daily rhythm of adult emergence or eclosion depends on the coupling between the central clock and a peripheral clock contained in the Prothoracic Gland (PG), an endocrine gland whose only known function is the production of the molting hormone, ecdysone (E). In some insects the rhythm of eclosion is achieved through the circadian control of E levels. However, the mechanism by which the clock regulates E signaling in Drosophila remains to be explored. To investigate how the fly clock regulates E action to control the timing of eclosion we: a) asked whether E levels are relevant for the rhythm of eclosion; b) Disrupted E signaling in relevant targets for emergence; c) Evaluated whether the clock regulates the subcellular localization of the E receptor (EcR); Finally, d) we downregulated candidate genes involved in transducing E actions. We found that increasing E levels prior to eclosion produced a dosage-dependent delay in the time of emergence but did not disrupt its circadian rhythmicity, suggesting that the clock acts downstream of E. Consistent with this hypothesis, interfering with E signaling in the PG eliminates the rhythm of emergence. Additionally, the subcellular localization of the EcR in the PG showed a daily variation. Finally, the downregulation of genes directly induced by E eliminated the rhythm of eclosion. Our results suggest that the circadian clock may impose a daily rhythm to the pattern of emergence by regulating E transduction in the PG.

MOLECULAR BIOLOGY

#B-57

Pinealectomy differentially modulates circadian clock expression in rat white adipose depots

Tatienne Costa[1], Paula Bargi-Souza[2], Sandra Andreotti[3], Fábio Lima[4]

1.Institute of Biomedical Science, University of São Paulo, SP, Brazil; Federal University of Tocantins, Palmas, TO, Brazil
2.Institute of Biological Science, Federal University of Minas Gerais, Belo Horizonte, MG, Brazil
3.Institute of Biomedical Science, University of São Paulo, SP, Brazil
4. Institute of Biomedical Science, University of São Paulo, SP, Brazil

Presenting Author: **Tatienne Costa**

The circadian expression of several genes involved in most physiologic processes of white adipose tissue is controlled by clock genes and modulated by melatonin. In this sense, this work aimed to investigate the influence of pinealectomy in clock gene transcript contents in periepididymal (PE), retroperitoneal (RP) and subcutaneous (SC) fat. For this, male Wistar rats were divided into Sham-operated (Control) or Pinealectomized (Pinx) groups. Four weeks after surgery, the animals were euthanized every 4 h up to 24 h and PE, RP and SC fat were collected for RT-qPCR analysis. In control animals, Bmal1 and Nr1d1 expression showed a circadian pattern in all fat depots. Clock (SC), Per2 (PE and RP) and Cry1 (RP) also exhibithed a daily oscillation in control group. The circadian pattern of Bmal1 and Clock in SC and Per2 in PE depots was disrupted in Pinx animals, while the acrophase of Bmal1 in PE was delayed and its mesor increased. The pinealectomy did not alter Nr1d1 circadian expression among depots, as well as, Per2, Cry1 and Bmal1 in RP. These findings demonstrate the existence of a circadian clock in PE, SC and RP fat and evidence that the oscillatory pattern of circadian clock in each adipose depot presents a differential responsiveness to pineal hormones.

HUMAN CIRCADIAN RHYTHMS

#B-58

Microglia changes in the aging pineal gland: a view from the endolysosomal system

Carlos Leandro Freites[1], Estela Maris Muñoz[1]

1.Laboratory of Neurobiology: Section of Chronobiology, IHEM-UNCuyo-CONICET, Mendoza, Argentina.

Presenting Author: **Carlos Leandro Freites**

Microglia are multifunctional immune cells within the brain. In the circadian pineal gland (PG), these phagocytes interact with other cell populations and constituent elements to finely modulate their development and function. As long-lived cells, microglia participate in changes that occur during normal aging, such as alterations in the architecture and functionality of subcellular organelles. Herein, we studied the expression pattern of the lysosomal and associated compartments marker ED1/CD68 in the PG from old male Wistar rats by using multiple immunofluorescence staining followed by quantitative confocal microscopy. Our results showed that the pineal Iba1+ cell population is composed of different subtypes according to the arrangement and cytoplasmic distribution of ED1, in both 3- and 18-month-old samples. However, our quantitative analysis revealed significant changes in the relative density of each phenotype with respect to age. To evaluate the functionality of the different ED1+ structures, we studied the expression of the lysosomal protease cathepsin D. Immunoreactivity for this enzyme in the aged PG suggests that microglia with ED1+ bodies might retain certain proteolytic capacity. Our results indicate that the pineal microglia themselves undergo many changes across their lifespan, including their phagocytic capacity. Microglia, as a plastic and resilient cell population, may continue to influence PG homeostasis and function even as the PG ages.

MOLECULAR BIOLOGY

#B-59

Processing body and stress granule rhythms in cell cultures

Melisa Malcolm[1], Laura Gabriela Pennazi[1], Eduardo Garbarino Pico[1]

1.Universidad Nacional de Córdoba, Facultad de Ciencias Químicas, Departamento de Química Biológica Ranwel Caputto, Córdoba, Argentina
2.CONICET, Universidad Nacional de Córdoba, Centro de Investigaciones en Química Biológica de Córdoba (CIQUIBIC), Córdoba, Argentina

Presenting Author: **Melisa Malcolm**

Stress granules (SGs) and processing bodies (PBs) are cytoplasmic membraneless organelles that are formed by liquid-liquid phase transitions. They are involved in the regulation of translation, stability and storage of mRNA and contain mRNA and several RNA binding proteins. SGs form in response to different stress stimuli, typically through phosphorylation of the eIF2alpha. PBs are constitutively present but increase in number under stress conditions. Since stress response has been shown to be circadianly regulated, we wonder whether SGs and PBs oscillate. NIH/3T3 and N2a cell cultures were synchronized with dexamethasone and harvested every 4 h for 68 h. We induced the formation of SGs with sodium arsenite (oxidative stress). We performed a double immunolabeling of SGs (eIF3 and G3BP1) and PB (GE-1/HEDLS and DDX6) by immunocytochemistry. We studied the phosphorylation temporal profile of eIF2alpha and eIF3 levels and we found no differences over time. We observed that NIH/3T3 and N2A cells show daily rhythms in SGs and PBs, respectively, for three variables: number, area, and signal intensity, with periods of approximately 24 h. These findings suggest that the molecular circadian clock controls SGs and PBs. To determine this hypothesis, we analyzed their formation in Bmal1-/- fibroblasts. Surprisingly, the rhythm persisted in these cells. The results presented here reveal new ways in which translation and cytoplasmic mRNA metabolism can be modulated over time.

MOLECULAR BIOLOGY

#B-60

Rhythms in lipid metabolism of Hepatocellular Carcinoma Cells, insights of a tight connection with the molecular clock

Natalia M Monjes[1], Mario E Guido[1]

1.Centro de Invertigaciones en Química Biológica de Córdoba, CIQUIBIC-CONICET / Dpto de Química Biológica Ranwel Caputto, UNC

Presenting Author: **Natalia Maribel Monjes**

The liver is a crucial organ for the general physiology of the organism acting as a major metabolic integrator. It is a central hub for lipid and energy homeostasis, being involved in triglyceride (TG) and glycerophospholipid (GPL) metabolism. Different factors cause a metabolic disorder which promote an abnormal lipid accumulation in organelles named lipid droplets (LDs) -hepatic steatosis- which is the metabolic syndrome manifestation, and it can progress to an hepatocellular carcinoma (HCC), the most common primary liver malignancy worldwide. The circadian clock present in the liver and in immortalized cell lines temporally regulates physiological processes, driving transcriptional and metabolic rhythms. Here we investigated in HepG2 cells, a human HCC-derived cell line, metabolic rhythms and their link with the circadian clock in control (B-WT) and Bmal1-knocked down (B-KD) cells. In B-WT cells we observed rhythms in expression of key lipid synthesizing enzymes (Chokα, Pcyt2 and Lipin1), in the metabolisms of particular GPLs (phosphatidylcholine PC and phosphatidylethanolamine PE), and in LD and TG content. Strikingly, when the circadian clock was disrupted (B-KD), the lipid metabolism was severely altered with a significant decrease in PC/PE ratio, TGs and LD content, as well as in rhythms of Chokα content. Results suggest a very strong cross-talk between the molecular clock and the lipid metabolism, which exhibits an exacerbated pathological.

HUMAN CIRCADIAN RHYTHMS

#B-61

Evaluation of thyroid hormone actions on GH secretion in the absence of a functional circadian clock

Dayana Silva Gonçalves-Manos[1], Anne Guillou[2], Patrice Mollard[2], Xavier Bonnefont[2], Paula Bargi-Souza[1]

1.Department of Physiology and Biophysics, Institute of Biological Sciences, Federal University of Minas Gerais, Belo Horizonte, MG, Brazil
2.Department of Physiology, Institute for Functional Genomics, CNRS UMR 5203, INSERM U1191, Montpellier University, France

Presenting Author: **Dayana Silva Gonçalves Manso**

The circadian and ultradian patterns observed in anterior pituitary hormone secretion daily profile are crucial for the homeostasis. The synthesis and secretion of Growth hormone (GH) are altered by thyroid hormones. Therefore, the aim of this work was to evaluate the T3 effect on the pulsatility of GH secretion and its dependence on a functional clock. Wild-type (WT) and Cry1-/-Cry2-/- double knockout mice (KO) were subjected to euthyroid, hypothyroid (0.1 % MMI and 1 % PCL in drinking water) and hyperthyroid (L-3,5,3' triiodothyronine, 5µg/20g BW, ip at ZT11) conditions. GH blood concentrations were measured by ELISA using tail-tip blood samples collected every 10 min during 6 h (ZT0-6). The treatment effectiveness was confirmed by Tshb expression in anterior pituitary and by T4 and T3 serum concentrations. The absence of a functional clock in KO mice was confirmed by the desynchronization of locomotor-activity pattern in D:D conditions. Hypothyroidism increases the frequency of GH pulses but reduces its content, which, in turn, is increased in hyperthyroid mice. The amplitude of GH pulses was increased in Euthyroid KO mice and preserved in hypothyroid and hyperthyroid KO mice, while the GH pulses frequency was reduced and increased, respectively. Thus, our preliminary data suggest that there is a mutual correlation between circadian clock and thyroid hormone actions to regulate the pulsatile GH secretion pattern in mice.

MOLECULAR BIOLOGY

#B-62

The circadian clock of glioblastoma: prognosis, novel chronoparmacological strategy and other fine herbs

Laura Lucia Trebucq[1], Georgina Alexandra Cardama[2], Pablo Lorenzano Menna[2], Diego Andres Golombek[1], Luciando Marpegan[3], Juan Jose Chiesa[1]

1.Laboratory of Chronobiology, University of Quilmes, Buenos Aires Argentina.
2.Laboratory of Molecular Oncology, University of Quilmes, Buenos Aires, Argentina.
3.Laboratory of Medical Physics, National Commission of Atomic Energy, Bariloche, Argentina.

Presenting Author: **Laura Trebucq Trebucq**

Glioblastomas (GBM) account for almost half of the malignant central nervous system tumors. Novel drugs and therapeutic approaches are necessary in order to improve patients' survival. The novel drug 1A-116 specifically blocks the interaction of the Rho GTPase Rac1 with some of its activators (such as Tiam1) inhibiting cell motility and proliferation of GBM. We studied the therapeutic response to 1A-116 at different circadian times in vitro and in vivo. In addition, to study the effect of the tumor's circadian clock in mice survival, we conducted in vivo experiments with WT or Bmal1 knock down tumors (LN229E1).
Cell cultures displayed circadian oscillations in BMAL1, PER1 and TIAM1 protein expression. We found that the effectivity of 1A-116 is rhythmic and that this rhythmicity is lost in LN229E1. In nude mice bearing tumors, we found median survival of 68 days in mice treated with 1A-116 at ZT3, increasing to 73 days in mice treated at ZT12. Implantation of LN229E1 cells in mice resulted in a median survival of 62 days, while for WT xenografts the median survival was of 67 days.
These results suggest that a chronopharmacological approach based on the drug 1A-116 is a feasible strategy to improve GBM treatment outcomes and that it's efficacy, as well as Rac1 pathway, are under circadian control. The circadian disruption elicited by LN229E1 cells resulted in a poorer outcome in nude mice, suggesting that the circadian clock has a protective role in tumor development.

MOLECULAR BIOLOGY

#B-63

The circadian clock as a novel therapeutic strategy for the treatment of glioblastoma

Paula M Wagner[1], César G Prucca[1], Beatriz Caputto[1], Mario E Guido[1]

1.CIQUIBIC-CONICET, Facultad de Ciencias Químicas, Universidad Nacional de Córdoba, Córdoba 5000, Argentina

Presenting Author: **Paula Micaela Wagner**

The circadian system temporally regulates diverse cellular processes in organs, tissues, and even individual cells, including tumor cells. The potentiality of the function of the circadian clock on cancer cell modulation offers a new target for novel treatments. Here, we investigate a chrono-chemotherapeutic administration of SR9009 (agonists of REV-ERBs) and Bortezomib in different glioma models. First, T98G glioblastoma cultures exhibited a differential temporal susceptibility to SR9009 treatment with the lowest levels of viability during a time window going from 18 to 30 hours after synchronization. Moreover, when Bortezomib and SR9009 were given together at lower doses in T98G cells, the viability was significantly reduced compared with each drug alone. On the other hand, in vivo studies evidenced a total tumor growth inhibition (TGI) when Bortezomib was applied at a high dose at the beginning of the day or night in a murine glioma model. On the contrary, at a low dose of Bortezomib, the nocturnal treatment showed a more significant effect on tumor volume than day-time treatment, exhibiting a TGI of 70% for the night administration and only 18% for the day treatment. Our observations strongly suggest that the chemotherapeutic treatment efficacy is subject to tight temporal control of the circadian clock. Understanding tumor regulation from a chronobiological viewpoint will further help to design new treatments that maximize therapeutic benefits at precise day-times