XIV Latin American Symposium on Chronobiology 2017

14 TO 18 NOVEMBER 2017

PARQUE CULTURAL DE VALPARAÍSO

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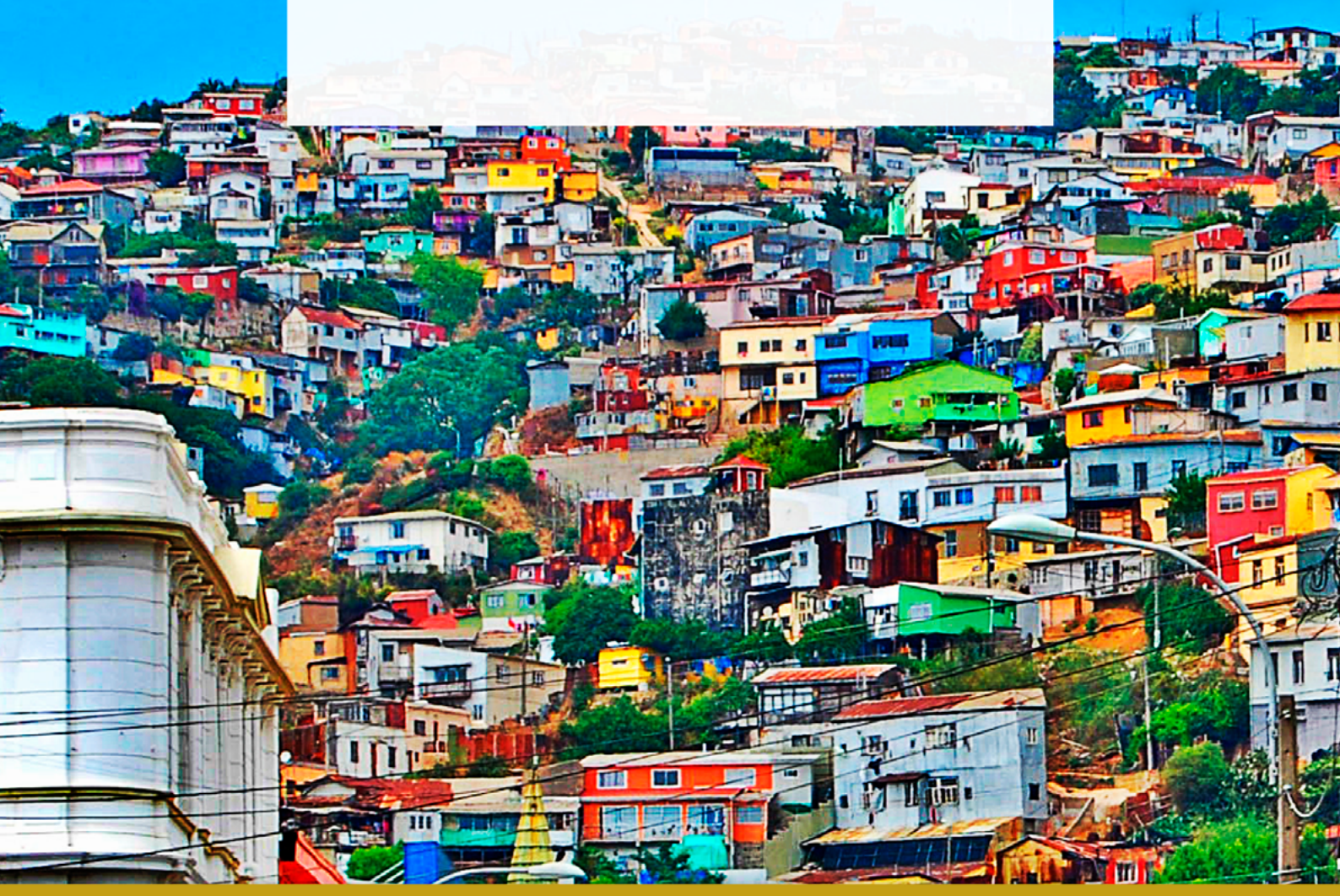

About Valparaíso

The XIV Latin American Symposium on Chronobiology 2017 - LASC 2017 will be held November 14th – 18th at the South American Port of Valparaíso, Chile. Valparaíso and its neighboring cities are home to about one million people. However, Valparaíso itself is a small city of about 200,000 inhabitants, making it easy for people to walk and use public transportation. The World Heritage List by UNESCO in 2004 included the historic quarter of Valparaíso. The hills, Cerro Alegre and Cerro Concepción, are at the heart of its historic quarter and are an important tourist attraction due to its many bars, pubs, restaurants, and hotels. The fifteen urban elevators (funiculares; a heritage from the industrial revolution) and the trolleybuses (the oldest in the world still in operation) are two unique characteristics of the city.

Valparaíso is just one and half hours from Santiago's International Airport and the city of Santiago, making national and international travel easy. From this airport, connections are possible to the major tourist attractions in the country, including Patagonia (Torres del Paine), the Atacama Desert (San Pedro de Atacama), and Rapa Nui (Easter Island).

The Symposium will take place at the Valparaíso Cultural Park (PCdV). The PCdV is a cultural center and public space of 1.5 ha (3.7 ac), which was recently created on the site of Valparaiso's former jail. It includes an auditorium with a capacity of around 300 seats, where the 1-day course and all talks will be held. Posters will be displayed throughout the entire duration of the symposium in a designated location. The PCdV is a ten-minute bus ride and a 10-30 minute walk from most Valparaiso hotels.

XIV Latin American Symposium on Chronobiology 2017 (LASC)

The Latin American Symposium on Chronobiology (LASC) is an international symposium centered on the circadian (biological) clock and sleep. It is held every two years in a different Latin American country; this is the first time that it will take place in Chile.

LASC attracts Latin American chronobiologists as well as experts from North America and from Europe. It starts with a 1-day course, and includes a Keynote address, Workshops, Symposia, short “Data Blitz” sessions during which the main findings of selected posters are briefly described, and two Public talks.

This year the field of Chronobiology was given extra visibility with the awarding of the 2017 Nobel Prize in Physiology or Medicine to chronobiologists, Jeffrey C. Hall, Michael Rosbash, and Michael W. Young. We are especially excited that Michael Rosbash will be attending LASC, where he will give a plenary talk and a Public lecture open to the general public.

Organizing Committee

John Ewer
Centro Interdisciplinario de Neurociencia de Valparaíso. Universidad de Valparaíso, Chile

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Veronica Valentinuzzi
CRILAR-CONICET, La Rioja, Argentina.

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(Tuesday November 14)

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Adrián Ocampo and **Horacio de la Iglesia**

11:00 - 11:30
Coffee break

11:30 - 12:45
Lecture 2
Human sleep and clocks
Luiz Menna-Barreto

12:45 - 14:15
Lunch (on your own)

14:15 - 15:30
Lecture 3
Circadian clocks
María Fernanda Ceriani

15:30 - 16:00
Coffee break

16:00 - 17:15
Lecture 4
Cellular basis of sleep architecture
Pablo Torterolo

17:15 - 17:30
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End of One-day course

Symposium

DAY 1 (Tuesday November 14)

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18:00 - 18:10
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18:10 - 19:10
Keynote address
“From icebergs to centimorgans to angstroms”
Joseph Takahashi, HHMI Investigator, University of Texas Southwestern Medical Center, USA.

19:10 - 21:00
Opening Reception

DAY 2 (Wednesday November 15)

9:00 - 10:00
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“Biology of bedtime: Understanding circadian rhythms and sleep”
Amita Sehgal, HHMI investigator, University of Pennsylvania, USA.

10:00 - 11:30
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Paul Taghert
Daniel Vigo
Susan Golden
Verónica Valentinuzzi

11:30 - 12:00
Coffee break

12:00 - 13:30
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Chair: **Luis Larrondo**

Joseph Takahashi
Jennifer Loros
Amita Sehgal
Charlotte Förster

13:30 - 15:00
Lunch on site

15:00 - 16:30
Workshop III
Chair: **Adrián Ocampo**

Pablo Torterolo
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Vlad Vyazovskiy
Javier Díaz

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17:00 - 17:15
Data Blitz 1

17:15 - 18:45
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Chair: **Claudia Moreno**

Adrián Ocampo
Tom de Boer
Horacio de la Iglesia
Mario Pedrazolli

18:45 - 19:30
Final discussion
(all workshops)

19:30
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(on your own)

DAY 3 (Thursday November 16)

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Jay Dunlap, Dartmouth College, USA.

10:00 - 11:00
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Luis Larrondo: “Synthetic biology: transcriptional rewiring and the emergence of a primordial visual system capable of eidetic memory”

Miriam Ben-Hamo: “A fear - entrained oscillator in the mouse”

Lia Frenkel: “Co-transmission in the circadian network: Temporal segregation of neurotransmitters as a means of daily rewiring?”

Carlos Trujillo: “Long-term effects of light gestational chronodisruption on C3 and clock genes in rat liver”

11:00 - 11:30
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11:30 - 13:15
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“Molecular Biology of Circadian Clocks”
Chair: **John Ewer**

Carrie Partch: “Dissecting the molecular basis for circadian timekeeping”

Carla Green: “An evolutionary hotspot defines functional differences between CRYPTOCHROMES”

Jennifer Loros: “Intrinsically disordered proteins in the circadian clock”

Susan Golden: “The clock of Cyanobacteria”

13:15 - 13:30
Data Blitz 2

13:30 - 14:30
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“Circadian Clocks: Cells and Circuits”
Chair: **Carola Millán**

Paul Taghert: “In vivo daily calcium rhythm activities across the *Drosophila* brain”

M. Fernanda Ceriani: “Circadian rewiring of adult networks in *Drosophila*”

Charlotte Förster: “The neuropeptide Pigment-Dispersing Factor (PDF) is part of the circadian system of the honey bee (Apis mellifera)”

Erik Herzog: “The ontogeny of circadian synchrony: Pregnancy induced changes in circadian timing”

16:15 - 16:45
Coffee break

16:45 - 18:30
Poster session I

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Public Lecture
“Circadian rhythms and health”
Céline Vetter, University of Colorado Boulder, USA.

19:30
Dinner
(on your own)

DAY 4 (Friday November 17)

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Plenary Lecture 3
“Circadian rhythms and sleep in flies: Molecules, neurons and circuits”
Michael Rosbash, HHMI Investigator, Brandeis University, USA.

10:00 - 11:00
Short talks on circadian clocks and sleep II
Chair: **Pablo Torterolo**

Victoria Acosta-Rodríguez: “Mice under caloric restriction self-impose a temporal restriction of food intake as revealed by an automated feeder system”

Malena Mul Fedele: “Blame it on the sunshine?: Differential thermoregulatory and inflammatory patterns in the circadian response to LPS-induced septic shock”

Monserrat Hevia: “Timing in the *Botrytis cinerea-Arabidopsis thaliana* interaction: a fungal circadian clock modulates virulence providing maximal pathogenic potential at dusk”

Guadalupe Cascallares: “Circadian dependence of locomotor activity statistics”

11:00 - 11:30
Coffee break

11:30 - 11:45
Data blitz 3

11:45 - 13:30
Round table discussion:
Chronobiology in Latin America
Moderator: **Mario Pedrazolli**
Participants:
Adrián Ocampo, Fernanda Ceriani, Horacio de la Iglesia, Diego Golombek, Pablo Torterolo, Verónica Valentinuzzi, Luiz Menna-Barreto, Ana Silva.

13:30
Free afternoon (Lunch bag).
See website for options.

20:00 - 23:00
LASC dinner.
To be held at Baburizza Palace.

DAY 5 (Saturday November 18)

9:00 - 10:00
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"A day in the life of a Cyanobacterium: integrating temporal and environmental information"
Susan Golden, HHMI Professor, University of California San Diego, USA.

10:00 - 11:00
Short talks on circadian clocks and sleep III
Chair: **Verónica Valentinuzzi**

Ignacio Estevan: "Staying up late is not the problem, school start times are"

Esteban Salazar: "Gestational chronodisruption impacts adrenal gland in the fetus and offspring"

Rubia Mendes: "Does the temporal pattern of adolescents" motor activity and wrist temperature vary over the week and with the school shift?"

Maria Juliana Leone: "The perfect hurricane: Chronotypes, sleep and academic performance in Argentinean adolescents"

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11:30 - 13:15
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"Human Sleep"
Chair: **Diego Golombek**

Carmen Betancur: "Unadjusted time zone and its consequences on population health"

Luiz Menna-Barreto

Ennio Vivaldi: "An EEG envelope characterization space for visualization of human sleep dynamics"

Koike Bruna: "Electrophysiological evidence that the retrosplenial cortex displays a strong and specific activation phased with hippocampal theta during paradoxical (REM) sleep"

13:15 - 13:30
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13:30 - 14:30
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14:30 - 16:15
Symposium IV
"Chronodisruption and Work Schedules"
Chair: **Horacio de la Iglesia**

Céline Vetter: "Human chronotypes and health"

Tom de Boer: "Rodents as a model for sleep and circadian rhythm disturbances: Exposure to light at night"

Claudia Moreno: "Urbanization, lighting and sleep"

Frida Marina Fischer: "Multiple job stressors of night and rotating shift workers that collectively affect health and wellbeing: basis for comprehensive interventions for their solution"

16:15 - 16:45
Coffee break

16:45 - 18:30
Poster session II

18:30 - 19:30
LASC Closing Public Lecture: What is all the fuss about circadian rhythms? / Porqué tanto alboroto sobre los Ritmos Circadianos?
Michael Rosbash, HHMI Investigator, Brandeis University, USA.
2017 Nobel Prizewinner Physiology or Medicine.

19:30 - 20:00
Business meeting.

Abstracts

Keynote address

From icebergs to centimorgans to angstroms.

***Joseph Takahashi**. Howard Hughes Medical Institute, Department of Neuroscience, University of Texas Southwestern Medical Center, USA.*

I began my career in circadian rhythms as a graduate student with Michael Menaker. At that time we were focused on the anatomical location of circadian pacemakers in vertebrates and as such our work focused on the role of the avian pineal gland and the suprachiasmatic nucleus in circadian organization of the animal. Gradually my interests became more reductionist and I focused on the chicken pineal gland because at the time it was one of the few pieces of vertebrate tissue that contained circadian oscillators and that could be studied in vitro. My lab at Northwestern used cell cultures of primary pinealocytes as a model system to study the clock because it contained photoreceptors for entrainment, circadian oscillators and a melatonin biosynthetic output pathway. Our reductionist approach eventually led us to search for cycling proteins and using state-of-the-art methods at the time, we could quantitate over a thousand proteins using 2D gels and found a number of high amplitude cycling proteins. One of these, a 56 Kd cycling protein, was identified by peptide sequencing and it was tryptophan hydroxylase (TH). This was extremely disappointing because TH is on the output pathway and its identity did not reveal anything about the circadian clock mechanism. (Iceberg) This was the turning point for me – we needed a new approach – and this led to my second career in mouse genetics.

I won't repeat the story in this abstract about the isolation of the Clock mutant mouse and the positional cloning of the causative gene (Centimorgans), but I will review this history in my talk.

Fast forward to the last five years, the clock gene pathway in mammals is well known and both continued reductionist studies (Angstroms) as well as translating and linking the circadian gene pathway to myriad physiological and biological functions such as metabolism, immune function and cancer has come to the forefront.

Public talks

What is all the fuss about circadian rhythms?/ Porqué tanto alboroto sobre los Ritmos Circadianos?

***Michael Rosbash**, HHMI investigador, Brandeis University, USA.*

Circadian rhythms and health.

***Céline Vetter**, University of Colorado at Boulder, USA.*

Our biology is tuned to the earth's 24h day, as the circadian system synchronizes to the local light/dark cycle. However, modern lifestyle can disrupt the timing, regularity, and strength of rhythmic environmental cues that the circadian system integrates to optimize physiology and behavior. Dim light during the day, light at night, more or less constant food intake, as well as activity during the biological night are all potential stressors for the circadian system, for its centers in the brain as well as for all the cellular clocks across all issues and organs. Travel across time zones and its associated jetlag symptoms illustrate the consequences we experience when the circadian system is acutely stressed (before it has fully adapted to a new light/dark cycle). Daylight Savings Time (DST), shift work and a mismatch between our biological and our social time zone (social jetlag) are examples of a chronic stress for the circadian program. We are only beginning to understand how this circadian strain, and the concurrent disruptions of sleep affect health and longevity. In her lecture, Dr. Vetter will introduce key circadian concepts, outline the prevalence of circadian strain, its consequences, and possible ways to counteract this modern epidemic.

Plenary talks

Mapping the regulatory networks governing global responses to light and time in *neurospora.*

J. Dunlap *[1], L. Larrondo[2], A. Crowell[1], J Hurley [3], J Emerson [1], J Loros[1], [1]Geisel School of Medicine at Dartmouth, USA.[2]Pontificia Universidad Católica de Chile, Chile.[3]Rensselaer Polytechnic Institute, USA.*

Eukaryotic fungal and animal clocks comprise transcription-translation based negative feedback loops that control a substantial fraction of these transcriptomes, eliciting the rhythmic changes in protein abundance that mediate circadian regulation of physiology and metabolism. Key to both circadian biology and photobiology in *Neurospora* is the transcription factor WC-1. Blue light detected by FAD stably bound by WC-1 drives a conformational change in the complex of WC-1 and WC-2 (the WCC) resulting in activation of gene expression from promoters bound by the WCC, initiating a transcriptional signaling cascade. In the circadian system this same WCC drives expression of *frq*. FRQ, an intrinsically disordered protein, forms a complex with FRH, casein kinase 1, and other proteins and after phosphorylation-mediated delays, this complex downregulates the WCC. This negative feedback loop results in rhythmic WCC activity that drives rhythms in expression of about 40% of the genome; in broad terms, daytime metabolic potential favors catabolism, energy production, and precursor assembly whereas night activities favor biosynthesis of cellular components and growth. WCC sits on top of the networks governing both light and clock regulation, controlling transcription factors that act as second order regulators, transducing regulation to banks of output ccgs including other transcription factors (TFs). Over 50 TFs have been used for chromatin immunoprecipitation at multiple times after exposure to light or across the circadian day. These data have been used to assemble the hierarchical transcriptional network governing light and clock regulation. Circadian proteomics data now extends this analysis revealing considerable post-transcriptional regulation.

A day in the life of a Cyanobacterium: integrating temporal and environmental information.

***Susan Golden**, Division of biological sciences University of California, San Diego, USA.*

Cells of diverse organisms, from cyanobacteria to humans, execute temporal physiological programs that are driven by circadian oscillators. The circadian clock of the cyanobacterium *Synechococcus elongatus* regulates global patterns of gene expression, the timing of cell division, and metabolism. We use *S.elongatus* as a model to understand how a cell keeps track of time, executes activities according to a temporal program, and synchronizes the internal clock with the external solar cycle. The components of the circadian oscillator are known (proteins KaiA, KaiB, and KaiC), their structures have been solved, and the rhythm in phosphorylation of KaiC can be reconstituted *in vitro*. One oscillator component, KaiB, undergoes a metamorphosis to a new protein fold – a rare event that is key to the slow progression of the circadian cycle. Furthermore, fold-switched KaiB initiates the “night” phase of the oscillator, and connects the oscillator to the downstream components that broadcast time to the cell. Although the oscillator can keep time *in vitro*, the situation *in vivo* is more complicated, with the clock components undergoing temporally regulated changes in intracellular localization. We are also investigating the metabolic consequences for *S. elongatus* when it is grown with or without an intact clock, in continuous light or in a diel cycle, to determine how circadian timing contributes to fitness. We found that wild-type cells turn off nighttime metabolic pathways before dawn, enabling a clock-dependent switch from primary metabolism to the synthesis of more complex molecules early in the day.

(Sponsored by grants R35GM118290 from the National Institutes of Health and MCB1244108 grants from the National Science Foundation).

Circadian rhythms and sleep in flies: molecules, neurons and circuits.

Michael Rosbash, *Kate Abruzzi, Maisa Araujo, Fang Guo, Meghana Holla, Wefei Luo, Dylan Ma, Department of Biology, Brandeis University Howard Hughes Medical Institute, USA.*

The *Drosophila* circadian clock functions within 75 pairs of brain neurons. We are expanding our understanding of their organization and functions, including their contributions to locomotor activity, their regulation of sleep and their circuitry. New methods include calcium monitoring of discrete neuronal groups, novel neuronal activity reporters and simultaneous video monitoring of behavior, all of which is done in freely-moving wake-behaving flies. The monitoring is being done over long time periods, suitable for circadian and sleep studies, and is coupled with optogenetic manipulation of individual neuronal groups. These *in vivo* approaches have been combined with neuronal purification and deep sequencing around the clock from several of these discrete neuron groups, to identify molecules and mechanisms that contribute to these behaviors in a neuron-specific manner. I may also discuss the role of the *timeless* protein in circadian transcriptional regulation as well as the contribution of the NonA protein to circadian circuitry and behavior.

Biology of bedtime: understanding circadian rhythms and sleep.

***Amita Sehgal**, Neuroscience University of Pennsylvania, USA.*

We are interested in the mechanisms that generate daily cycles of physiology and behavior, especially sleep. In addition to endogenous circadian clocks, sleep is driven by a homeostatic process that ensures sufficient amounts of sleep. Our studies of circadian rhythms and sleep use primarily a Drosophila model, which was invaluable for dissecting molecular mechanisms of the clock conserved all the way to humans. The brain clock in Drosophila is located in discrete groups of neurons that contribute in different ways to the behavioral sleep: wake cycle. However, little is known about mechanisms that transmit time-of-day signals from the clock through the rest of the brain to produce overt rhythms of sleep: wake. We recently identified an “output” circuit, which transmits circadian signals from the central clock cells, through non-clock peptidergic neurons, to motor centers in the fly. This circuit also regulates metabolic rhythms. In other work, we have identified factors that mediate homeostatic regulation of sleep, those that control sleep amount and sleep need. In particular, we are interested in molecules that induce sleep.

Symposia

The neuropeptide Pigment-Dispersing Factor (PDF) is part of the circadian system of the honey bee (*Apis mellifera*).

K. Beer[4], T. Fuchikawa[1], E. Kolbe[4], N. Kahana[2], E. Winnebeck[3], G. Bloch[2], C. ***Helfrich-Förster[4]****, [1]Biology and Geosciences Graduate School of Science. [2]Ecology, Evolution, and Behavior Hebrew University.[3]Institute for Medical Psychology Ludwig Maximilain University Munich.[4]Neurobiology and Genetics, Biology, University of Würzburg.*

Pigment-Dispersing Factor (PDF) is an important neuropeptide of the circadian clock in many insects. Here we show that it is also part of the circadian clock of honey bees. PDF co-localizes with the clock protein Period (PER) in laterally located clock neurons. As found for the fruit fly, the widespread arborisations of the PDF neurons are in close vicinity to other PER-positive neurons in the lateral and dorsal brain as well as to PER-positive glia cells, suggesting that PDF modulates the activity of the other clock cells. In addition, the intensity of PDF immunostaining oscillates in a diurnal and circadian manner in different parts of the bee brain, enabling a circadian input to the optic lobes and the central brain. This seems to be also true for nurse bees that are behaviourally arrhythmic. We observed highly synchronous PDF cycling in different brain region of nurses, whereas these oscillations appeared slightly out of phase in foragers. This suggests that the oscillation pattern changes with age or task of the bees. Finally, we were able to phase delay locomotor activity rhythms of individual bees by injecting PDF into the optic lobes at the end of their subjective day. Altogether, our results reveal PDF as an integral part of the honey bee clock that signals within the clock network and may transfer rhythmic signals to the centres of memory and brain parts needed for spatial orientation.

(Sponsored by German Israeli Foundation For Scientific Research And Development (G.I.F. Project Number 1-822-73.1/2004), The German Research Foundation (CRC 1047, Insect Timing), Israel Science Foundation (ISF; Project Number 1274/15)).

Unadjusted time zone and its consequences on population health.

***Betancur C.**, Psiquiatría y Salud Mental, Medicina, Universidad de Concepción.*

Introduction: The effects that changes in the time zone (TZ) may have on population health, generally are not considered in the public policy when the governments makes it. This has been an important issue in Chile three years because the government has changed the official TZ from -4GMT (standar time / ST) to -3GMT (daylight saving time / DST), keeping the DST like a permanent TZ in the country in 2015 and changing to ST only three months in 2016 and 2017. With theese changes, in 2015 Chile had approximately two hours gap on its geographical TZ (-4.67GMT). This paper is a review of the available scientific information to date on the impact that change to DST and unadjusted TZ (jet lag), could have on population health.

Method: A search for information was carried out in different databases, until October 2017, using words *daylight saving time, jet lag and health*. The only criterion to select the articles was their relevance in relation to the purpose of this review.

Results: 22 selected articles that shows the change to DST increases the incidence of acute myocardial infarction, impairs performance in motor tasks, mental health and in ability to pay attention to the early hours of the morning, especially the first days on week and the jet-lag increases the prevalence of obesity and incidence of metabolic disorders and impairs school performance and sleep-wake cycle.

Conclusion: Unadjusted TZ is harmful to the population health and the Chilean government should adjust it.

Circadian rewiring of adult networks in *Drosophila.*

Fernanda Ceriani M. *Laboratorio de Genética del Comportamiento. Instituto Leloir. IIB-BA CONICET. Buenos Aires, Argentina.*

Oscillations between day and night are dominant, at times neglected, evolutionary driving forces. To cope with such challenges, biochemical timers that run with periods similar to the earth's rotation (¨circadian clocks¨) have evolved. The fruit fly *Drosophila melanogaster* has been instrumental in understanding how these timekeeping systems work at the molecular level, and to demonstrate that multiple layers of interconnected cellular mechanisms are recruited by the clock to ensure its function.

Clock neurons in the brain sustain a cell autonomous clock but rely on the communication among each other for entrainment (i.e., a response to a change in the environmental conditions) and phase adjustments. Aside from neuropeptides and classical neurotransmitters that are differentially released throughout the day, circadian remodeling of the neuronal terminals of clock neurons could contribute to the reconfiguration of the circadian network, necessary to adjust to changes in photoperiod. Such circadian structural plasticitywould provide a mechanism by which a neuron can exert sequential control of different target circuits along the day.

Rodents as a model for sleep and circadian rhythm disturbances: exposure to light at night.

***Tom De Boer**, Laboratory for Neurophysiology, Department of Molecular Cell Biology, Leiden University Medical Center, Leiden, The Netherlands.*

Light provides the main input to the master circadian clock in the suprachiasmatic nucleus. Exposure to light at night is associated with insomnia in humans. We developed a rodent model for the effect of light at night on sleep by exposing male wistar rats to 12 h normal light and 12 h 5 lux dim white light (L: Dim) (Stenvers et al 2016). In the course of L: Dim exposure the amplitude of daily sleep-wake rhythms and daily rhythms found in the NREM sleep EEG power density in the faster frequencies (16-19 Hz) decreased. In addition L: Dim induced internal desynchrony by introducing a free running rhythm with a period of approximately 25 h, next to the light entrained 24-h rhythm. This is the first rodent model of disturbed circadian control of sleep due to light at night. The data show that internal desynchrony is possible in a 24-h L: Dim cycle. The data suggest that chronic sleep disturbances due to light at night in humans may have a similar origin. We recently have extended the data to young and aging mice, showing similar results in young mice. However in older mice the influence of L: Dim on sleep is relatively mild.

Stenvers DJ, van Dorp R, Foppen E, Mendoza J, Opperhuizen AL, Fliers E, Bisschop PH, Meijer JH, Kalsbeek A, Deboer T (2016) Dim light at night disturbs the daily sleep-wake cycle in the rat. Sci Rep 6: 35662.

An EEG envelope characterization space for visualization of human sleep dynamics.

Javier Díaz, Alejandro Bassi, José Manuel Arancibia and ***Ennio A. Vivaldi,*** *Laboratorio de Sueño y Ritmos Circadianos, Instituto de Ciencias Biomédicas, Facultad de Medicina, Universidad de Chile.*

The analysis of a polysomnogram (PSG) requires a time demanding, visual pattern recognition rule-based procedure to categorize 30-second epochs according to their EEG morphological features. The discretized representation of the sequence of sleep epochs is represented by the hypnogram. We report here on a complementary method based on the coefficient of variation of the envelope (CVE) as calculated for the EEG delta band. An envelope characterization space is generated where points representing epochs are plotted. For each epoch its abscissa value is defined by the CVE of the delta band and its ordinate value by its mean amplitude. The locations of points in the plane provide an insightful visualization as epochs representing sleep states and stages are mapped into stereotyped regions. W and REM epochs cluster on the lower left area of the graph, whereas NREM sleep stage 3 (N3) conforms a cluster on the upper left area. Since N3 is associated with restorative properties of sleep, this latter dense cluster can operationally define deep sleep. This approach can be very useful in assessing sleep pathology. As a matter of fact, in patients with obstructive sleep apnea, this cluster shrinks remarkably or totally vanishes. On the other hand, fragmented sleep associated to sleep apnea is revealed as clusters in anomalous coordinates. Thus, the envelope characterization space offers a synthesis of EEG dynamics facilitating distinctions between normal patterns and patterns related to fragmented sleep associated to sleep apnea.

Multiple job stressors of night and rotating shift workers that collectively affect health and wellbeing: basis for comprehensive interventions for their solution.

F. Fischer[1], *A. Silva-Costa*[2], *R. Griep*[3], *L. Rotenberg*[4], *M. Smolensky*[5], [1]*Environmental Health, School of Public Health, Sao Paulo.*[2]*Department of Collective Health Federal University of Triângulo Mineiro .*[3]*Laboratory of Health and Environment Education Oswaldo Cruz Institute.*[4]*Laboratory of Health and Environment Education Oswaldo Cruz Institute.*[5]*Department of Biomedical Engineering, Cockrell School of Engineering, The University of Texas at Austin.*

Multiple xenobiotic, physical, and psychosocial workplace factors collectively affect wellbeing and health, particularly nightshift workers. Human circadian time structure (CTS) should be an important consideration for establishing threshold limit values and methods of employee biological monitoring. In the absence of CTS adjustment, exposures by night workers to job stresses occur during a different circadian stage than day workers. Numerous animal and human investigations document prominent circadian patterning in biological tolerance to a broad array of chemical, biological, and physical stressors. Time-qualified for biological rhythms reference values utilized in medicine today seem to be pertinent also to interpreting employee health surveillance data. Workplace psychosocial factors are additionally of great importance to employee wellbeing, with demand-control-social support and effort-reward imbalance models serving to assess detrimental outcomes. Surprisingly, there is little of knowledge of the health impact of workplace psychosocial factors on permanent and night shift workers. Some studies indicate low job control, high physical demands, low supervisor social support, and high over commitment are more problematic for night than day workers. At-work violence is another psychosocial stress especially for service employees regularly in contact with the public, e.g. police officers, security personnel, bank employees, and professional drivers. Workplace violence and consequences are probably underestimated in night workers, especially when co-existing among other stressors with known impact on health. Practical considerations and recommendations for action to mitigate the collective effect of these multiple job stressors particularly in reference to night and shift workers are presented**.**

(CNPq Grant 305123/2013-0).

An evolutionary hotspot defines functional differences between CRYPTOCHROMES.

***Carla Green**, Department of Neuroscience. University of Texas Southwestern Medical Center.*

Mammalian circadian clocks are driven by a transcription/translation feedback loop composed of positive regulators (CLOCK/BMAL1) and repressors (CRY1/2 and PER1/2). To understand the structural principles of regulation, we used evolutionary sequence analysis to identify co-evolving residues within the overall CRY/PHL protein family. We identify an ancestral secondary cofactor-binding pocket as an allosteric site in CRYs that mediates regulation through direct interaction with CLOCK and BMAL1. Mutations weakening binding between CLOCK/BMAL1 and CRY1 lead to acceleration of the clock, suggesting that subtle sequence divergences at the allosteric site can modulate clock function. Divergence between CRY1 and 2 at this site results in distinct periodic output. Weaker interactions with CLOCK/BMAL1 at this pocket are strengthened by co-expression of PER2, suggesting that PER expression limits the length of the repressive phase in CRY2-driven rhythms. Overall, this work provides a model for the mechanism and evolutionary variation of clock regulatory mechanisms.

The ontogeny of circadian synchrony: Pregnancy induced changes in circadian timing.

Erik Herzog, *Department of Biology, Washington University, St. Louis, MO, USA.*

Most, if not all, daily rhythms in vertebrate physiology and behavior depend on the hypothalamic suprachiasmatic nucleus (SCN). The ontogeny of the cells within the SCN is understudied although it has been linked to developmental events including birth. We examined circadian rhythms in maternal and fetal tissues including the SCN during pregnancy and their relationship to events leading up to parturition. Data from mice and women indicate that circadian rhythms begin in utero and follow prescribed changes in intrinsic period and synchrony. Genetic and environmental disruptions of this timing can alter the timing of birth. We conclude that communication between circadian oscillators likely underlies normal gestation and birth.

Electrophysiological evidence that the retrosplenial cortex displays a strong and specific activation phased with hippocampal theta during paradoxical (REM) sleep.

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It is widely accepted that cortical neurons are similarly more activated during waking and paradoxical sleep (PS; aka REM) than during slow-wave sleep (SWS). However, we recently reported using Fos labeling that only a few limbic cortical structures including the retrosplenial cortex (RSC) and anterior cingulate cortex (ACA) contain a large number of neurons activated during PS hypersomnia. Our aim in the present study was to record local field potentials and unit activity from these two structures across all vigilance states in freely moving male rats to determine whether the RSC and the ACA are electrophysiologically specifically active during basal PS episodes. We found that theta power was significantly higher during PS than during active waking (aWK) similarly in the RSC and hippocampus (HPC) but not in ACA. Phase–amplitude coupling between HPC theta and gamma oscillations strongly and specifically increased in RSC during PS compared with aWK. It did not occur in ACA. Further, 68% and 43% of the units recorded in the RSC and ACA were significantly more active during PS than during aWK and SWS, respectively. In addition, neuronal discharge of RSC but not of ACA neurons increased just after the peak of hippocampal theta wave. Our results show for the first time that RSC neurons display enhanced spiking in synchrony with theta specifically during PS.We propose that activation of RSC neurons specifically during PS may play a role in the offline consolidation of spatial memories, and in the generation of vivid perceptual scenery during dreaming. Key words: anterior cingulate cortex; gamma; hippocampus; retrosplenial cortex; sleep; theta

(CNRS, Societé Francaise de Recherche et Médecine du Sommeil, University Claude Bernard of Lyon, CAPES/COFECUB, CNPq and FAPESP)

Intrinsically disordered proteins in the circadian clock.

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Circadian rhythmicity, the cell-intrinsic ability of organisms to track internal time and thereby coordinate activities with the environment, has arisen at least three times in evolution. *Neurospora crassa* has been a durable system for understanding the molecular bases of these oscillators within the fungal and animal Kingdoms; Neurospora is perhaps the most intensively studied of all circadian cell types. At the core of this circadian system is the heterodimer of White Collar-1 and -2 forming the White Collar Complex (WCC) that drives the expression of the *frequency* (*frq*) gene. FRQ complexes with other proteins, including Frequency Interacting RNA Helicase (FRH) and casein kinase 1 (CK1). In a time-delayed and highly regulated manner dictated by progressive phosphorylation, the complex reduces the activity of the WCC, thus resulting in a negative auto-regulatory feedback loop. WC-1 and WC-2 interact via PAS domains as do similar positively acting clock-relevant transcription factors in animal clocks; conserved within the negative element FRQ-FRH complex is the interaction with CK1 and the extensive phosphorylation of a negative element that generates the time delay. FRQ, an intrinsically disordered protein (IDP), is stabilized by FRH which acts non-enzymatically in the clock; separately FRH has a helicase function required for cell viability. The negative elements in fungal and animal clocks are poorly conserved at the protein level, even among animals, possibly reflecting the fact that many may be IDPs for which there is no inherent structural drive for conservation of sequence. JJL is grateful to the NIH for grant R35 GM118022.

Urbanization, lighting and sleep.

***Claudia Moreno**, Health, Life cycles and Society Department, School of Public Health, University of São Paulo.*

Throughout the centuries, the adaptation to new temporal niches was possible due to the plasticity of the circadian temporal system. Nevertheless, urbanization and electrical light became a new challenge for humankind. Artificial (electrical) light has increased the time of waking that may have an effect on sleep duration, timing, and quality. Although systematic reviews have shown conflicting evidence of reduced sleep duration in past decades, a recognized cause has been reduced time spent outdoors and reduced natural light exposure. An increase of artificial light exposure in the evening delays sleep onset, thus altering sleep timing. Moreover, it has been suggested that reduced natural daylight exposure in the morning also delays sleep onset. Increased artificial light exposure as well as delayed sleep onset might lead to insufficient sleep. Studies comparing workers with electrical light at home with those without electrical light at home have helped to understand how sleep can be affected by availability of light. Urbanization brings electrical light to workplaces, homes, and increases shift work. Shift workers may be strongly affected by excessive artificial light exposure as well as natural light exposure reduction. According to the World Health Organization, 70% of the human population will be living in urban areas in 2050, which is a challenge to deal with.

Dissecting the molecular basis for circadian timekeeping.

***Carrie Partch**, Chemistry and Biochemistry University of California Santa Cruz.*

Circadian clocks synchronize physiology and behavior into rhythms that coincide with the 24-hour day to regulate global health and homeostasis in humans. Disruption of these cell-autonomous molecular clocks can lead to metabolic syndromes, cancer, and premature aging by interfering with the systemic synchronization and integration of physiology. We are interested in leveraging insight into the molecular basis of 24-hour timekeeping to develop new therapeutics that fortify circadian rhythms or adjust intrinsic timing to improve human health. At the molecular level, circadian timekeeping arises from an interlocked set of transcription-based feedback loops. Using an integrated approach encompassing structural biology, biochemistry and cell biology, we are starting to build insight into the structural basis of the dynamic protein network that generates circadian timekeeping. Here, I'll highlight some of our new findings that demonstrate how protein-protein interactions provide remarkable plasticity to the circadian timekeeping system in animals and offer new hints for structure-based drug design to control circadian rhythms.

In vivo daily calcium rhythm activities across the _Drosophila_ brain.

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24 hr in vivo imaging studies show that the *Drosophila* neuronal pacemakers exhibit staggered activity periods in spite of having synchronous molecular clocks. We hypothesize that the sequential phases of the different pacemaker groups represent deflections from a common morning activity phase and are created by inhibitory (mainly neuropeptide) signals. For example, PDF delays the daily LNd Ca surge by ~10 hours to occur towards the end of the photophase; likewise PDF delays the DN3 Ca surge by ~14 hours to occur in the early scotophase. Thus the principle contribution of PDF to this circuit is not to synchronize molecular clocks, but to set critical multi-hour delays in the activity periods of specific pacemaker cell groups. PDF signaling to suppress Ca is cell-autonomous and clock-independent, but the precise second messenger pathway is undefined. To begin characterizing daily rhythmic brain activity beyond the pacemaker network, we have studied several different classes of Ellipsoid Body (EB) cells. They exhibit biphasic spontaneous Ca transients, with peaks in the morning and evening that are coincident with the two bouts of motor activity. EB activity does not reflect motor feedback – it reflects participation in a pre-motor pathway, or in an efference copy pathway. These data suggest spontaneous daily Ca peaks are not limited to circadian pacemaker cells, but also propel outward from the pacemaker network as labeled temporal lines, to optimize the activity patterns of different neuron groups at different times of day.

Workshops

Sleep under natural and electric light.

Horacio De la Iglesia, *Department of Biology, University of Washington, Seattle, WA, USA.*

We study of sleep in the traditionally hunter-gatherer Toba-Qom of Northern Argentina. For the last four years we have studied Toba-Qom communities that either have access or no access to electricity but that are otherwise genetically and socioculturally similar. This scenario represents a unique “natural” experiment and we capitalize on our access to these communities to assess the effects of electric light on sleep and circadian rhythms. Our studies have shown that the access to electric light is associated with a delayed sleep onset and a shorter daily sleep bout. Work continues to establish the physiological basis of this effect of artificial light.

Envelope analysis links oscillatory and arrhythmic EEG activities to two types of neuronal synchronization.

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Traditionally, EEG is understood as originating from the synchronous activation of neuronal populations that generate rhythmic oscillations in specific frequency bands. Recently, new neuronal dynamics regimes have been identified (e.g. neuronal avalanches) characterized by irregular or arrhythmic activity. In addition, it is starting to be acknowledged that broadband properties of EEG spectrum (following a 1/f law) are tightly linked to brain function. Nevertheless, there is still no theoretical framework accommodating the coexistence of these two EEG phenomenologies: rhythmic/narrowband and arrhythmic/broadband. To address this problem, we present a new framework for EEG analysis based on the relation between the Gaussianity and the envelope of a given signal. EEG Gaussianity is a relevant assessment because if EEG emerges from the superposition of uncorrelated sources, it should exhibit properties of a Gaussian process, otherwise, as in the case of neural synchronization, deviations from Gaussianity should be observed. We use analytical results demonstrating that the coefficient of variation of the envelope (CVE) of Gaussian noise (or any of its filtered sub-bands) is the constant sqrt(4/pi - 1) approx 0.523, thus enabling CVE to be a useful metric to assess EEG Gaussianity. Furthermore, a new and highly informative analysis space (envelope characterization space) is generated by combining the CVE and the envelope average amplitude. We use this space to analyze rat EEG recordings during sleep-wake cycles. Our results show that delta, theta and sigma bands approach Gaussianity at the lowest EEG amplitudes while exhibiting significant deviations at high EEG amplitudes. Deviations to low-CVE appeared prominently during REM sleep, associated with theta rhythm, a regime consistent with the dynamics shown by the synchronization of weakly coupled oscillators. On the other hand, deviations to high-CVE, appearing mostly during NREM sleep associated with EEG phasic activity and high-amplitude Gaussian waves, can be interpreted as the arrhythmic superposition of transient neural synchronization events. These two different manifestations of neural synchrony (low-CVE/high-CVE) explain the well-known spectral differences between REM and NREM sleep, while also illuminating the origin of the EEG 1/f spectrum.

Human sleep and geography.

Mario Pedrazzoli, *School of Arts, Science and Humanities, Universidade de São Paulo, Brazil.*

The rotation of the Earth around its own axis and around the sun determines the characteristics of the light/dark cycle, the most stable and ancient 24 h temporal cue for all organisms. Due to the tilt in the earth's axis in relation to the plane of the earth's orbit around the sun, sunlight reaches the Earth differentially depending on the latitude. Sunshine and sunset light is incident to human circadian phases in function of the population's localization in the planet. The timing of circadian rhythms varies among individuals of a given population and biological and environmental factors underlie this variability. A series of our recent studies in different places and populations reveal that humans are sensitive to the different sunlight signals tied to differences in latitude; as a morning to evening latitudinal cline of chronotypes towards higher latitudes or different profiles of social jet lag in the same time zones.

Melanin concentrating hormone in mesopontine raphe nuclei: role in REM sleep and depression.

***Pablo Torterolo**, Physiology, School of Medicine, Universidad de la República.*

The melanin-concentrating hormone (MCH) is a neuromodulator synthesized by neurons of the postero-lateral hypothalamus. MCHergic neurons project to the serotonergic dorsal (DR) and median (MR) raphe nuclei. These nuclei have a major role both in the control of REM sleep and in the pathophysiology of Major Depression (MD).

In this lecture I will summarize and evaluate our experimental data about the functional interactions between the MCHergic systems and the raphe nuclei, in the control of REM sleep and MD.

Our main findings are the following. MCHergic receptors are present in the serotonergic neurons of the DR and MR. Microinjections of MCH into the DR promote REM sleep in the rat, while immunoneutralization of this peptide within the DR, decreases the time spent in this state. Moreover, microinjections of MCH into the DR and MR promote a depressive-like behaviour. This effect is blocked by the intra-DR microinjection of a specific MCH receptor antagonist, and prevented by the systemic administration of antidepressant drugs (either fluoxetine or nortriptyline). Using electrophysiological and microdialysis techniques, we also demonstrated that MCH decreases the activity of serotonergic DR and MR neurons.

In conclusion, there is substantive experimental data suggesting that by modulating the neuronal activity of the DR and MR, the MCHergic system plays a role in the control of REM sleep and in the pathophysiology of MD.

Heart rate variability as a tool to explore autonomic nervous system circadian rhythm.

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It is accepted that several biological (genetic, physiological), psychological (personality, mood), social (family, work) and ecological (living environment) factors, interact to allow the preservation of quality of life and health. The autonomic nervous system (ANS) is structural and rhythmically interfaced between forebrain, internal and external environments, to regulate energy, matter and information exchanges, thus expressing the bio-psycho-social nature of the individual. ANS rhythmic function can be non-invasively explored through Heart Rate Variability Analysis (HRV). Heart rate circadian rhythm is partially originated in the basal forebrain and is dependent on the sleep-wake state. This region exerts control of cardiovascular autonomic function through projections to the paralimbic cortex, amygdala, hypothalamus, and brainstem nuclei. In addition, the suprachiasmatic nuclei of the hypothalamus, regulate physiological functioning with cycles that are adjusted to day length mainly by the information of ambient light. These nuclei have projections to the paraventricular hypothalamic nucleus, which modulate ANS activity by sending input to major sites of ANS regulation. Hence, circadian HRV analysis may provide information about the state of the autonomic nervous system in several physiological settings. In addition, autonomic imbalance may configure a final common pathway to increased morbidity and mortality from a host of clinical conditions. This presentation will focus on the physiological mechanisms underlying circadian HRV rhythm, the methods for assessing it and the information that has provided about ANS activity in certain physiological and pathological situations studied by our group, including sleep-wake cycle, prolonged confinement (Mars500 project), extreme environments (Antarctica), coronary disease and cognitive impairment.

(PICT ANPCyT 2012-0984)

Local and global dynamics of cortical activity during sleep.

Vladyslav Vyazovskiy, *University of Oxford, UK.*

EEG slow waves and spindles are fundamental features of brain activity during sleep. In the last years, it has become clear that waking, NREM sleep and REM sleep are not global, mutually exclusive states. Instead, mixed states characterised by a co-existence of sleep- and wake-like patterns of brain activity, or NREM and REM sleep features, are the norm rather than the exception. In most cases, NREM slow waves do not occur simultaneously across the entire neocortex, and preceding sleep-wake history affects their spatial dynamics. Interestingly, local cortical OFF periods and slow waves are also surprisingly common during waking, especially after sleep deprivation, and even during highly active behaviours. The spatio-temporal dynamics of another hallmark of brain activity – sleep spindles – remains poorly understood. Intracranial recordings in patients with drug-resistant epilepsy revealed that spindles are local events, rarely occurring across large distributed brain networks. Also in mice, cortical spindles are often restricted to small circumscribed regions, and often remain undetectable in the 'global' EEG. Notably, the cortical micro-regions enriched with spindles during NREM sleep are characterised by the occurrence of slow waves even during REM sleep, resulting in a persistence of a unitary default NREM-like sleep state within specific local cortical areas. Our results suggest that the spatio-temporal dynamics of slow waves and spindles during sleep do not only reflect preceding sleep-wake history or experience, but manifest a fundamental organisation of subcortical-cortical circuitry, which underlies the emergence of local and global brain states.

The circadian clock network constantly monitors environmental temperature to set sleep timing.

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Animals, fungi, plants, and protists all employ circadian clocks to coordinate their behavior, physiology and metabolism with the Earth's diurnal cycle. Circadian clocks are entrained by both light and temperature cycles, and recent work suggests that daily environmental temperature oscillations contribute to human sleep patterns, however the neural mechanisms through which circadian clocks monitor environmental temperature and modulate behavior remains poorly understood. Here, we elucidate how the *Drosophila* circadian clock neuron network processes changes in environmental temperature. Using *in vivo* calcium imaging techniques, we demonstrate that the $DN1_p$s, a discrete subset of clock neurons, sensitively and constantly monitor modest changes in environmental temperature. We find that these neurons are inhibited by heating and excited by cooling, an unexpected result given the strong correlation between temperature and light in the environment and the fact that light excites clock neurons. We demonstrate that the $DN1_p$s do not sense temperature changes cell-autonomously; rather, these neurons rely on multiple peripheral thermoreceptors located in the chordotonal organs and aristae. Finally, we show that the $DN1_p$ neurons and their thermosensory inputs are required for the normal timing of behavioral rhythms in the face of gradual environmental temperature cycles. Taken together, our experiments establish that $DN1_p$s are a major gateway for temperature sensation into the circadian neural network and establish that the circadian clock neuron network continuously integrates temperature changes to orchestrate sleep and activity in the face of daily temperature cycles.

Short talks

Mice under caloric restriction self-impose a temporal restriction of food intake as revealed by an automated feeder system.

***V. Acosta-Rodriguez*[1]**, *M. De Groot*[1], *F. Rijo-Ferreira*[1], *C. Green*[1], *J. Takahashi*[1], *[1]Neuroscience University of Texas Southwestern Medical Center.*

Caloric restriction (CR) extends lifespan in mammals, yet the mechanisms underlying its beneficial effects remain unknown. CR protocols changes at least two parameters known to impact physiology and behavior: amount and timing of food intake. The implementation of feeding paradigms that control and monitor food availability is logistically difficult; therefore, we have developed an automated feeder system that controls the duration, amount and timing of food availability, and records feeding and locomotor activity in mice. Using this system, mice were subjected to various feeding schedules with or without restricting the amount. Mice subjected to CR self-impose temporal component by consolidating food intake and unexpectedly increase wheel-running activity during the rest phase, regardless of when (day or night) the food was made available. Importantly, the ability of this feeder system to simultaneously monitor locomotor and feeding behavior lead us to uncover surprising relationships between feeding, metabolism and behavior.

(Howard Hughes Medical Institute and NIH/NIA grant R01 AG045795).

A fear-entrained oscillator in the mouse.

***Ben-Hamo M.**, Ivana L. Bussi, Luis E. Salazar, Raymond Sanchez and Horacio de la Iglesia, Department of Biology and Program in Neurobiology and Behavior, University of Washington, Seattle, WA 98195.*

Under natural conditions, fearful stimuli, such as a predator, are likely to present themselves with a 24 h periodicity. We therefore hypothesized that cycling fearful stimuli presented during the active phase of the animal could lead to a shift in the temporal distribution of foraging and feeding of the animal. To test this hypothesis, we housed mice in a naturalistic cage setup consisting of a nesting area and a foraging area. The animals are forced to leave the nesting area to gain access to food and water, which is restricted to the foraging area. The foraging area is rendered dangerous by applying an aversive stimulus (an electric footshock). Using this setup we delivered footshocks in the foraging area that were randomly distributed throughout the dark phase of the LD cycle, the natural foraging and feeding time for nocturnal rodents. We show that when we deliver random nocturnal footshocks, mice avoid foraging during the night and switch the phase of their foraging and feeding behavior until most of it falls during the light phase. Interestingly, upon release into DD mice continue foraging and feeding with the same phase as under nocturnal fear conditions, indicating that nocturnal fear entrains a circadian oscillator. Our results indicate that the neural centers that code fear are part of the neural circuitry that constitutes the circadian system, and that the ability of cyclic fear to entrain circadian oscillators may be an evolutionary conserved trait.

Circadian dependence of locomotor activity statistics.

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We monitor the locomotor activity of wildtype fly Drosophila and clock mutants using custom made housing and video tracking software in order to obtain high spatial and temporal resolution. We analyze the statistics of movement and quiescence in 12 hours of light - 12 hours of dark LD and also in constant darkness DD conditions. The statistical properties reveal patterns that expose the role of the circadian clock. In particular the exponential distribution of movement and the power law quiescence distribution for the wild type present the same behavior in LD and DD. In contrast these distributions present changes in per01 clock mutant. We also analyze the interevent time distribution and again find the same behaviors in wild type for LD and DD and contrasting differences for the distributions in LD and DD in clock mutants. We consider the rate of events when the time series is analyzed using time windows of different sizes. We find strong finite size effects in wild type series, that can be rescaled using exactly the same scaling function and exponents for all flies in LD and DD. For the clock mutants the distributions do not present scaling and show different behaviors in LD and DD. We compare our results with studies in the literature involving mammals and find surprising similarities that point to a conserved mechanism.

(Supported by CONICET - Agencia Nacional de Promoción Científica y Tecnológica)

Staying up late is not the problem, school start times are.

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Eveningness has been repeatedly associated with low scholar grades, generally assessed in students attending school early in the morning. This study aims to evaluate the relationship between Morningness-Eveningness preferences and school performance in students attending school at different time slots. Participants were 224 students (142 females) from a single public high-school attending school in the morning (from 7:30 to 11:30, N=123) or in the afternoon shift (from 11:30 to 15:30, N=101). A Spanish adaptation of the Morningness-Eveningness Scale for Children was employed to obtain M-E preferences (stdα=0.71). A significant effect of attendance slot was found on M-E scores (t=3.04, p=0.003) but not on socio-demographic variables or school performance. We applied a GLM using school mean qualifications as dependent variable for each attendance shiftseparately. Age (in moths), gender, mother educational level (below or above 12 years of education), and M-E score were considered as independent variables. Both models were significant (morning: $F(4, 118)=5.00$, $p<0.001$, $R^2=0.12$; afternoon: $F(4, 96)=4.19$, $p=0.004$, $R^2=0.11$). As expected, mother's education was a predictive factor in both attendance shifts (morning: $F=7.55$, β for higher level=0.63±0.22, $p=0.007$; afternoon: $F=17.47$, β for higher level=0.94±0.27, $p<0.001$). Only in the morning shift, Morningness was associated with better mean qualifications ($F=5.50$, β=0.25±0.10, $p=0.02$). Our study not only suggests that M-E preference is influenced by social demands (i.e. school schedule), but also that M-E association with performance is modulated by the attendance shift.

(Supported by an ANII scholarship and PEDECIBA).

Co-transmission in the circadian network: temporal segregation of neurotransmitters as a means of daily rewiring?

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The central circadian oscillator of *Drosophila melanogaster* is composed of about 150 neurons. Among them, the 8 small lateral ventral neurons (sLNvs) are fundamental in various aspects of temporal organization, but by far the most studied one is in the fly daily locomotor activity. sLNvs release PDF, the neuropeptide that synchronizes circadian oscillators. While the relevance of neuropeptidergic transmission is well established, uncovering the role of fast neurotransmission is booming. After a screen aimed at unveiling sLNv neurotransmitter identity, we established that sLNvs release glycine. Glycinergic transmission inhibits sLNv post-synaptic targets. Notwithstanding, our results also suggest that sLNvs recruit excitatory cholinergic transmission for communication. Unexpectedly, acetylcholine depletion in these neurons by downregulating its synthesis, recycling or synaptic vesicle refilling, leads to a lengthening in the period of locomotor activity, as it is the case with glycinergic transmission, suggesting that period lengthening is the result of altering the hierarchical relationship among clusters The “one neuron, one neurotransmitter” dogma has repeatedly been questioned by findings within the mammalian brain, where co-transmissionseems to be the rule. In addition, it has been proposed that co-transmission would play a role in plasticity. Since reorganization of synaptic circuits by neurotransmitter switching can occur in response to changes in the environment or nutritional state, we propose that temporal segregating the synaptic vesicular content contributes to the functional rewiring of the circadian network in *Drosophila.*

(Sponsored by ANPCyT).

Timing in the *Botrytis cinerea-Arabidopsis thaliana* interaction: a fungal circadian clock modulates virulence providing maximal pathogenic potential at dusk.

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Circadian clocks confer selective advantages allowing organisms to anticipate predictable daily changes. In this way it was suggested that the outcome of a plant-pathogen interaction is controlled by an endogenous timekeeping mechanism and by light. Few reports in plants exemplify anticipatory mechanisms, as for example defense responses in *A. thaliana* by the time when the pathogen attack is most likely to happen. Nevertheless, this concept has been poorly evaluated in pathogens and the limited available data is only at a phenotypical level. Therefore, we have studied the circadian regulation in the necrotrophic fungus *B. cinerea*, demonstrating for the first time and, at a molecular level, the presence of a functional circadian clock in a pathogenic organism. In Botrytis, the circuitry of the oscillator is composed by the BcFRQ1 protein and a transcriptional complex formed by BcWCL1 and BcWCL2. Taking advantage of the *B. cinerea* clock mutants that we generated, and of available *A. thaliana* arrhythmic ecotypes, we provide compelling evidence that the outcome of the plant- fungal pathogen interaction varies with the time of day, in a manner that is largely dependent on the fungal clock.This work provides the first evidence of a microbial clock modulating and optimizing pathogenic traits at specific times of the day and opens the doors for natural pest-control strategies.

(Sponsored by MN-FISB, FONDECYT 1171151 And HHMI International Research Scholar Program).

Synthetic Biology: Transcriptional rewiring and The Emergence of a Primordial Visual System capable of Eidetic Memory.

***Luis Larrondo**, Genetica Molecular y Microbiologia, Ciencias Biologicas, Millennium Nucleus for Fungal Integrative and Synthetic Biology (MN-FISB).Pontificia Universidad Catolica De Chile.*

In the fungus Neurospora crassa, as in other model organisms, synthetic biology based-strategies have been seldom adopted for the study of circadian oscillators. Our current efforts on this matter have focused on examining the genetic plasticity of the Neurospora circadian clock through transcriptional rewiring. This design implies the addition of new positive elements (transcription factors) that are now integral part of a hybrid oscillator (HO) that mixes canonical and new components. Remarkably, this HO free-runs, has a period close to 24 h, is temperature compensated and it is entrainable by external cues. Such an approach is already revealing important insights regarding time-delay mechanisms and alternative design principles compatible with clock function. On the other hand, we have adopted optogenetic approaches to further delve into Neurospora's circadian and light-responses. In doing so, we had the ability to turn this fungus into a "live canvas" on top of which images can be projected causing a bioluminescent biological response that recreates the original image with great precision. Remarkably, since this "primordial visual system" is integrated in the Neurospora circadian regulatory network, the fungus reproduces on subsequent days -in a circadian manner- the image that it had originally "seen", creating an eidetic (photographic) memory effect. Such phenomenon, based on local discrete phase changes, not only will provide new insights on phase responses, but it also allows for the opportunity to ponder on concepts such as vision and memory.

(FONDECYT 1171151.MN-FISB and HHMI International Research Scholar program).

The perfect hurricane: chronotypes, sleep and academic performance in Argentinean adolescents.

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In Argentina, as in other places over the world, most schools start early in the morning. Chronotype changes with development and adolescence is the period where humans have later chronotypes. The interaction between social demands and chronotype is associated with different problems, including shorter sleep duration and lower academic achievement.

In this study we assessed chronotypes, sleep habits and academic performance of early (13-14 y.o., 1st year of secondary school) and late adolescents (17-18 y.o., 5th year) attending to morning, afternoon and night school shifts. Using this unique setup, we first assessed the effect of school times on chronotypes, sleep duration and social jet lag. Then, we evaluated the impact of school times, chronotypes, sleep duration, social jet lag, age, and their interactions on academic achievement.

We found that school shift modulates student chronotypes and sleep habits, with students from morning shift having earlier chronotypes, shorter sleep duration and higher social jet lag. Age modulated these effects: older students are more influenced by school times. Additionally, academic performance was affected by the interaction between school shift, sleep habits and academic subject.

To our knowledge, this is the first study that evaluates the effect of chronotypes, sleep habits and school times on academic performance of students attending to morning, afternoon and night shifts.

(Sponsored by FONCyT Argentina Grant PICT-2013-1653, James S. McDonnell Foundation, UNQ And CONICET), FONCyT Argentina Grant PICT-2013-1653, James S. McDonnell Foundation, UNQ and CONICET).

Does the temporal pattern of adolescents' motor activity and wrist temperature vary over the week and with the school shift?

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OBJECTIVE Investigate the temporal pattern of motor activity (MA) and body (wrist) temperature (WT) rhythm (acrophase, amplitude and mesor) between week-ends (WE)/week-days (WD) in students of morning/afternoon shift (MS/AS). METHODS 41 adolescents (21 MS and 20 AS) aged 12 to 14 years. The experimental protocol was applied 9 consecutive days, including 2 weekends. For AM and WT recordings we used the actimeter ACT10. RESULTS The MA average acrophase occurred earlier in the WD/MS group (13.34 ± 0.37 hours) than WE/MS (15.68 ± 0.47 hours, F = 44.13, p <0, 0001) and WD/AS (15.16 ± 0.44 hours, F = 41.73; p <0.0001) groups. The MA average amplitude in the WD/MS group was lower than WE/MS (t (20) = 3.2, p <0.01) and WD/AS (t (39) = 6.0, p <0.001) groups, and in the WE/AS group was lower than WD/AS (t (19) = 4, p <0.01). The MA average mesor in the WD/MS group was higher than WE/MS (t (20) = 5.56, p <0.001). We did not identify significant differences in the comparisons among all the groups for WT average acrophase, amplitude and mesor.The phase angle between MA and WT acrophase was higher in the WE/MS (10.97 ± 0.65 hours), WE/AS (10.57 ± 0.85 hours) and WD/AS (11.18 ± 1.45 hours) groups than WD/MS (8.14 ± 1.62 hours). CONCLUSION Together, we conclude that the MA rhythm of MS adolescents, unlike AS, has an advanced acrophase and a flattening of the rhythm pattern in the WD in relation to the WE.

(Sponsored by This Research Receives A Support By CNPq (process Nº 159827/2014-0).)

Blame it on the sunshine?: Differential thermoregulatory and inflammatory patterns in the circadian response to LPS-induced septic shock.

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Septic shock is a lethal condition caused by a pathogen-induced chain of events. In 1960, Halberg et al, reported a susceptibility rhythm to lipopolysaccharide (LPS) - induced septic shock, which showed that the same dose of LPS which is compatible with survival at ZT19 can be lethal at ZT11. Then, Hrushesky, et al (1994) observed the same effect with Tumor Necrosis Factor-α (Tnf-α) administration. Moreover, mice that lack Per2 are more resistant to this condition (Liu, et al. 2006).

In this study, we aim to further characterize the circadian response to high doses of LPS in mice. First, we measured skin temperature of animals injected with LPS at both times and we found that there was a higher decrease in mice injected at ZT11 than at ZT19. We analyzed neuronal activation by cFos immunoreactivity in the preoptic and paraventricular nucleus of the hypothalamus, brain regionsassociated with thermoregulation and neuroendocrine, autonomic and immune control, respectively. We found that at both brain regions cFos immunoreactivity was significantly higher after LPS administration at ZT11 than at ZT19.Furthermore, we found that levels of Tnf-α in serum were higher in animals injected at ZT11, whereas Tnf-α mRNA expression was higher in the liver of animals treated at ZT19. Moreover, both dendritic and macrophage cell activation in spleen was higher after treatment at ZT19, as well as peritoneal macrophage activation. These results suggest a circadian dependency of the central thermoregulatory and peripheral inflammatory response to septic-shock.

Gestational chronodisruption impacts adrenal gland in the fetus and offspring.

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Adverse prenatal conditions may modify fetal physiology, potentially increasing susceptibility to chronic diseases in adult life. Indeed, our group reported that an abnormal photoperiod alters fetal development. The fetal adrenal is a key organ which could be a target for *in utero* programming by altered photoperiod, inducing long term-effects in adrenal function. Here, we explored the effects of chronic photoperiod shifting (CPS) on fetal and adult adrenal function. Pregnant rats (day 0) were separated in two groups: control 12:12 light:dark (LD) and CPS photoperiod. At gestational day 18, dams were euthanized every 4-h, fetal adrenals were extracted to measure daily mRNA expression (qPCR and Microarray) and corticosterone content (RIA). At 90 days, male offspring (LD and CPS) were euthanized every 4-h and adrenal glands were collected for gene expression and histological studies. In a parallel cohort (n=6/group), blood was sampled around the clock to assess individual corticosterone rhythms. Adrenals from CPS fetuses presented disrupted corticosterone and gene expression daily rhythms (clock genes and steroidogenic genes). Adults gestated under CPS showed wide molecular, morphological and physiological changes, in line with major desynchronization of adrenal clock and corticosterone rhythms. For example, daily expression of clock and clock-controlled genes (including steroidogenic genes) was altered in CPS versus LD offspring.Altogether, the present resultsdemonstrate that gestational chronodisruption altered adult offspring adrenal function, which may translate in long-term abnormal stress response and metabolic adaptation, increasing the risk to develop chronic diseases, such as metabolic syndrome and cardiovascular malfunction.

(Funding: Fondecyt 1150069, 1120938 and ANILLO ACT-1116).

Long-term effects of light gestational chronodisruption on C3 and clock genes in rat liver.

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The variety effects of light pollution and chronodisruption have been clearly displayed, these include: cancer risk, behavior problems, obesity, metabolic syndrome, plenty of deleterious immunologic disfunction, cardiovascular impairs, melatonin and clock genes dysregulation. We have analyzed the effects of chronodisruption on the expression of complement system factors and clock genes in adult offspring from rats subject to constant light (LL) along the second half of pregnancy relative to animal gestated in control photoperiod (LD). The effects were evaluated in liver of adult offspring (P90) with qPCR to determine the expression of C3 and circadian expression of clock genes (Bmal1, Clock, Per2, Cry1, Rev-erb∝ and RoR∝) in Sprague Dawley rats. The adult LL rats show C3 transcripts and protein levels significantly lower than adult LD rats. The expression of clock genes Bmal1, Clock, Rev-erb∝ and RoR∝ was significantly affected in comparison to adult LD rats. We conclude that gestational chronodisruption impairs the normal expression of C3 and clock genes transcripts in liver from adult progeny in rats. In fact, these results suggest that gestational light pollution could play a critical role in developmental programming causing subsequent health disorders in the adulthood.

(FONDECYT 1150789, ANILLO ACT-1116)

Posters

Anomalies in sleep pattern and executive functions in asymptomatic offspring of patients with late-onset Alzheimer´s Disease.

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Introduction: Early neuropathological changes of late-onset Alzheimer´s disease (LOAD) involve autonomic impairment including alterations of the sleep-wake rhythm. Indeed, anomalies in sleep pattern are emerging as a potential biomarker of the disease. It is also well documented the association between such alterations and decreased performance on executive function tasks. We hypothesized that asymptomatic offspring of patients with LOAD would display circadian rhythm abnormalities along with some degree of executive impairment before the onset of the disease. Objective: An exploratory study was conducted with 35 asymptomatic middle-aged offspring of patients with LOAD (OLOAD) and 31 healthy individuals without family history of AD (CS). Methods: Measures of sleep-wake rhythm by actigraphy, circadian rhythm of body temperature and circadian heart rate variability were collected. The following executive functions were assessed: cognitive flexibility (TMT-B), abstract reasoning (WAIS-III Similarities), planning and problem-solving (Tower of London) and verbal (Verbal Fluency) and nonverbal (Design Fluency) productivity. Group comparison (T-test) showed OLOAD exhibits greater sleep duration (474±11m vs. 439±9m, p=0.018) but lower sleep efficiency than CS (96.7±0.5% vs. 97.1±0.4%, p=0.042). No other significant differences were found for HRV, temperature or executive function measures. Significant correlations between executive functions and circadian parameters were observed both in OLOAD and CS, however, no differential patterns between groups could be discerned. Conclusions: The present results support the existing evidence of sleep pattern as a potential early marker in LOAD, already present in young asymptomatic at-risk individuals with no signs of cognitive decline.

(Sponsored by ANPCyT PICT 2012-0984 And PICT-2014-0633).

Circadian modulation of motivational behavior in mice.

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The striatal dopaminergic system is particularly important in the regulation of two key behaviors: temporal processing in the seconds-to-minutes range (interval timing) and motivation for a reward. In addition, evidence suggests that the dopamine D2 receptor (DRD2) plays a main role in this regulation. We have previously reported that interval timing, as well as striatal dopamine content, is subjected to modulation of the circadian system. In the present work we present evidence of circadian modulation of motivation for food reward in young (4-months old) but not in old-aged (over 1.5 years old) C57BL/6 mice. Motivation was assayed through the progressive ratio (PR) schedule. Young mice under a 12:12 light/dark (LD) cycle exhibited a significant reduction (almost 4-fold) in motivation during the daytime. Indeed, motivation during the nighttime was increased compared to both the daytime and to constant light (LL) conditions. Aged mice, however, did not display any differences in motivation. Moreover, young mice under a normal 12:12 LD cycle exhibited a daily oscillation in the striatal DRD2 content, both at mRNA and protein level, which was coincident with the observed variation in motivation. DRD2 daily oscillation did not persist under LL conditions. Taken together, our results may contribute to improve treatment related to psychiatric disorders or drugs of abuse, taking into account potential mechanisms of circadian modulation of motivational states which might be altered under abnormal mental conditions.

Disruption of circadian rhythms during pregnancy: a study with melatonin.

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From conception to delivery, women experience dynamic changes in her body, rhythms in general, perception, social relationships and professional activity. In other words, they experience a complex and multifaceted process. Some changes in the environment and in their body functions can compromise fetus development. Melatonin, a hormone produced mainly by the Pineal Gland, regulates important physiological functions such as circadian rhythms. Findings demonstrated that melatonin plays an important role in pregnancy and delivery. In general, melatonin secretion during pregnancy helps in circadian organization, regulate fetus physiology and redox status and can regulate the placenta clock genes. Our research aim to study the potential utility of melatonin in pregnancy and its relation with disruptions of circadian rhythms. We plan to conduct a longitudinal study from the first semester of pregnancy, proceeding until infant´s first year of age. We intend to split the research into three moments: 1) analysis of the sleep-wake cycle, and the participant´s secretion of melatonin; 2) administration of melatonin in chronodisrupted mothers; and 3) comparative analysis of infant´s motor and cognitive development in both groups - with and without disruption of circadian rhythms.

Caloric restriction restores temporal patterns of DNA methyltransferases, BDNF, TrkB and Bmal1 expression in the hippocampus of aged rats.

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Caloric restriction (CR), the reduction of calories intake without causing malnutrition, is the only intervention that has shown to delay the onset and incidence of age-related diseases and, in elderly humans, to improve memory function. Previously, we observed circadian rhythms of BDNF, TrkB, Dnmts3a/3b and BMAL1 expression in the hippocampus of young rats; such oscillations were abolished in 22-m old animals. Here, we investigated temporal patterns of DNA methyltransferases (Dnmts), BDNF and TrkB expression as well as the circadian variation of the core clock protein, BMAL1, in the hippocampus of aged rats under CR. Holtzman male old rats were fed with a diet reduced by 40% in calories during the last 3 months prior to the 22 months of age. Animals were maintained under constant darkness conditions during 15 days before the experiment. Levels of Bdnf, TrkB, Dnmt1, Dnmt3a and Dnmt3b mRNA and BMAL1 protein were determined by RT-PCR and Western blot, respectively, in hippocampi samples isolated at 4h-intervals throughout a 24h period. In the present work, we observed CR restored the circadian rhythmicity of the studied parameters (Chronosfit: $p<0.05$). In addition, CR accentuated the Dnmt3a and 3b rythms' amplitude (t-Test: $p<0,05$ and $p<0,05$, respectively).CR restores the temporal patterns of molecular and epigenetic factors involved in cognitive functions in aged animals. Restoration of temporal coordination could be one of the basis of CR efficiency and could contribute to improve the cognitive performance in senile individuals.

(Supported by PICT 2010-1139-ANPCyT and PROICO 2-0314-UNSL, Argentina).

Sleep-wake cycle alterations in an animal model of temporal lobe epilepsy.

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Evidence suggest that chronobiological determinants of sleep may strongly interact with the occurrence of epileptic seizures in humans. Seizures tend to occur during some stages of sleep-wake cycle (SWC) and also SWC can be modified by the epileptic activity. This research is meant to study, in the pilocarpine model of temporal lobe epilepsy, the temporal distribution of tonic clonic seizures (TCS) and the effect of epilepsy in the time distribution of stages of SWC. Additionally, we propose Envelope Coefficient of Variation (ECV), as a new method to analyze and detect abnormalities in SWC in EEG recordings of epileptic rats. Hence, epilepsy was induced in 29 rats by means of the administration of a single dose of pilocarpine. Other 13 rats was used as control group. After being implanted for chronic polysomnography, all rats were recorded during at least two undisturbed days. We found that the probability of TCS occurrence has a diurnal phase preference, with mode in first half of the light phase and nadir at midnight. Epileptic rats show more non-REM sleep than non epileptics during light, but spent less time in Wake and REM sleep. A change in the acrophase of stages was not found. Finally, according to ECV, there is a global difference between epileptic and nonepileptic rats in sleep stages that suggests a possible new target to identify epilepsy characterized by a low frequency pattern during non-REM, which is not temporal related with TCS.

(Conicyt, Biomedical Neuroscience Institute, Funcación Puelma).

Locomotor activity pattern of Culex quinquefasciatus in two cycles with gradual temperature variation.

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Circadian rhythms are under selective pressure imposed by environmental cyclical changes and present the property of entrainment to these cycles with periods near or equal to 24 hr. The main *Zeitgeber* is the light; however temperature cycles can modify the entrained pattern. Previous study in *Culex quinquefasciatus* has indicated that under a rectangular thermo/cold cycle (recTC), the locomotor activity is more restricted to the dusk-criophase, remaining stable after entrainment in light/dark cycles (LD). In *Drosophila melanogaster,* a comparison between locomotor activity pattern´s under recTC and gradual TC (gradTC) suggests significant differences and highlight the importance of a semi-natural approach in the laboratory. Therefore, we studied patterns of locomotor activity of *Cx. quinquefasciatus* under two regimes as close as possible to the daily temperature variations observed in Rio de Janeiro and Petrópolis: LDTC Rio (LD(12:12)gradTC Rio from 30ºC to 20ºC) and LDTC Pet (LD(12:12)-gradTC Pet from 20ºC to 12ºC), respectively. Our results show that in both regimes the patterns are unimodal with a peak during photophase-scotophase transition, slightly earlier than in constant temperature cycle. In LDTC Rio the activities were mainly concentrated during the dark-cold phases while in LDTC Pet was observed an anticipation and a main peak more blunted. Moreover, male and females exhibit higher levels of activity during the scotophase under LDTC Rio than LDTC Pet. Regular changes in temperature can act as powerful *Zeitgebers* in many organisms, especially in poikilothermic animals, playing a role in adapting to seasonal changes and survival throughout the day, to concentrate the activities during favorable times.

Effect of social condition on circadian activity rhythm in a diurnal primate: the common marmoset (*Callithrix jacchus*).

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Besides the stronger effect of the light-dark cycle, the social cues act as an important zeitgeber to the circadian activity rhythm (CAR) in common marmosets (*Callithrix jacchus*). To evaluate the effect of the social condition on the circadian rhythm in this diurnal primate, the characteristics of rhtyhm were compared among adult males maintained isolated, in pairs and in family groups (breeding pair and offspring of 2 juveniles). Applying a transversal approach, we monitored the motor activity of 16 males (isolated: 8; in pairs: 3 and in family groups: 5) by actiwatches for 19 days under artificial LD 12:12, and constant temperature and humidity conditions. The percentage of variance of the 24h rhythm was smaller in the males maintained in family groups (Anova; Tukey, $p < 0.05$), without differences in the total daily activity, rhythm amplitude and Intradaily Variability (IV) among conditions (Anova, $p > 0.05$). In relation to the parameters of phase, the isolated animals showed a smaller duration in the activity phase (Anova; Tukey, $p < 0.05$) and a trend to a smaller phase angle difference between the activity onset and lights-on in relation to those maintained in families (Anova, $p < 0.05$; Tukey, $p=0,06$). From these results, we suggest that the social condition influence the circadian activity rhythm in marmosets, reinforcing the importance of considering this aspect when carrying out studies with this important animal model. Additional studies with a larger number of animals are needed to confirm this evidence.

Ontogenetic evaluation of the circadian activity rhythm in a diurnal primate: the common marmoset (*Callithrix jacchus*).

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In marmosets, longitudinal approaches show that the circadian activity rhythm (CAR) stabilizes around 4 months of age, moment of the beginning of juvenile phase. From these stage, the characteristics of CAR have been described in: 1) juveniles (before and after puberty onset) x adults, and 2) adult x elderly animals. The aim of this study is to compare the characteristics of CAR among male marmosets in the juvenile (before and after puberty), adult and elderly phases. Applying a transversal approach, we monitored the motor activity of 20 males (juveniles: 6; adults: 8 and elderly: 6) by actiwatches for 20 days under CE 12:12 and constant temperature and humidity conditions. Thepercentage of variance of rhythm were similar among ages. However, the elderlies showed a higher Intradaily Variability (IV) in relation to juveniles (Tukey, $p < 0.05$), and a trend in relation to adults (Tukey, $p = 0.07$); whereas the juveniles showed higher totals of daily activity than adults and elderlies. Although no differences were observed in phase relationships between activity onset and lights-on (Anova, $p > 0.05$), the juveniles showed a smaller phase angle difference between activity offset and lights-off and a later center of gravity than adults and elderlies (Tukey, $p < 0.05$), and a longer activity phase (Tukey, $p < 0.05$). We suggest that the CAR undergoes phase changes after the stabilization at the beginning of the juvenile phase and an increase on fragmentation during aging. Additional studies with more animals are necessary to confirm these changes.

(CAPES, CNPq, Fundação de Apoio à Pesquisa do Rio Grande do Norte (FAPERN) e Universidade Federal do Rio Grande do Norte, Brazil)

Temporal sleep pattern, attention and light intensity in classrooms of adolescents of two cities in Rio Grande do Norte, Brazil.

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In adolescents, a delay in the Sleep-Wake Cycle (SWC) is associated with reduced sleep duration and attention levels. Similarly to SWC, the light-dark cycle modulates the attention. This study aims to characterize the temporal sleep pattern, attention and light intensity in adolescents' classrooms who study in the morning shift of private schools from Natal (*Nt*) (05º4742\"S; 35º1234\"W) and Santa Cruz (*SC*) (6°1346S; 36°124W) in RN. Participated in the study, 115 adolescents (*Nt*: 56 and *SC*: 59 - 41 boys), ranging from 14-18 years (average 15,5±0,7) and in the firsts years of high school. They answered: the Pittsburgh Sleep Quality Index, Puberty and Phase Preference and the Pediatric Daytime Sleepiness Scales, and a Sleep Diary for 10 days. Attention was assessed by a Continuous Performance Task and the light intensity was measured in classroom during the recess. The sample showed poor sleep quality, partial sleep deprivation and sleep irregularity in both cities. *Nt* students showed better attention performance: shorter reaction time (sustained attention), higher percentage of correct answers (tonic and phasic alertness, and sustained attention) and lower omissions (all components) ($p<0,05$). *SC*students were exposed to very low light intensities (*Nt*: 596.1 and *SC*: 121.1 lux), even for national standards (between 300 and ≥750 lux). These results corroborate to the hypothesis that the start time of classes in the morning (7am) is a strong time challenge for adolescents regardless of the urban context. However, further studies are needed to determine if classroom light intensity is an influencing factor for sleep and attention.

(Sponsored by CAPES, CNPq e Universidade Federal do Rio Grande do Norte, Brazil).

Is wrist skin temperature a good marker forhuman circadian rhythmicity?

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Melatonin, cortisol and core body temperature have been widely used as circadian markers of human rhythmicity. More recently, wrist skin temperature (ST) has been proposed as an index for circadian rhythmicity. The aim of this study was to describe rest/activity cycle and wrist ST in young healthy adults with extreme diurnal preferences (based on Horne & Östberg questionnaire–MEQ). Fifteen morning-type individuals (MEQ mean score: 68,9), mean age 23,2, were compared with sixteen evening-type volunteers (MEQ mean score: 30,7), mean age 22,1. During one week, they wore an actigraphy device (it also contained temperature sensors) in their non-dominant wrist (ActTrust, Condor Instruments, Brazil). Activity and ST were recorded using 60-s epochs. From activity data, two nonparametric variables were obtained: diurnal activity onset time (M10 onset) and nocturnal rest onset time (L5 onset). Center of gravity of ST (measure of central tendency), was also calculated. Data were compared by means one-way ANOVA (chronotype group as factor). As expected, rest/activity cycle phase differences between groups were observed. M10 onset ($p<0,001$): 08h58min (68min) for morning-type group and 10h51min (72min) for evening-type group; L5 onset ($p<0,001$): 00h00min (52min) for morning-type group and 01h55min (77min) for evening-type group. However, no differences were detected on wrist ST. Center of gravity was similar in both groups ($p=0,48$): 00h25min (±16) for morning-type group and 00h27min (±17min) for evening-type group. ST is susceptible to environmental temperature influences. Masking effects could explain the results. Based on our results, wrist ST is not a reliable human circadian marker in field studies.

REM sleep homeostasis: additive effect of homeostatic drive and photic masking during recovery after selective REM sleep deprivation.

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There is a homeostatic mechanism that preserves the daily REM sleep quota. To be truly homeostatic, the underlying REM sleep hourglass process that keeps track of the cumulated REM sleep time should promote REM sleep in response to REM sleep deficits, as occurs after selective REM sleep deprivation (RD) in humans and rodent models, and postpone REM sleep occurrence when challenged by REM sleep excesses. In the albino rat, short dark pulses (DP) transiently increase REM sleep amount by shortening the latency of NREM to REM sleep transitions, phenomenon known as photic masking. Photic masking provides a useful strategy to explore the REM sleep hourglass process in response to REM sleep excess. Male albino rats were subjected to 4 hours of RD during the rest phase (zeitgeber time, ZT, 4-7; light:dark cycle= 12:12). It was compared the subsequent REM sleep rebound when occurring in the presence or absence of 10-minute DP given in the ZT 8-9 interval. REM sleep rebound in the ZT 8-11 interval after RD was 10.9 minutes and when occurring in the presence of DP was 22.2 minutes. Whereas REM sleep rebound after RD alone fully compensated REM sleep debt within 6 hours, REM sleep rebound with DP provoked a sustained REM sleep excess that was present after 16 hours of recordings. The additive effect of photic masking suggest that REM sleep hourglass process is insensitive to the activation of REM-on neurons targeted by retinofugal projections.

(Supported by Guillermo Puelma Foundation).

A single night of sleep restriction impairs decision-making speed but not accuracy.

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Introduction: Numerous reports show that sleep deprivation impairs neurocognitive performance (executive functioning, sustained attention, and long-term memory). To our knowledge, it has not been fully studied the extent to which a single sleep restriction period can impair executive functions such as decision making. A single sleep restriction period (4h of time in bed) provides a more ecologically valid paradigm that closely resembles sleep patterns in our daily lives. Objective: To compare decision-making between sleep restricted and non-restricted subjects and to correlate decision-making outcomes with fatigue symptoms. Methods: Two experimental groups (restricted, n=10 and non-restricted, n=15) were studied. We administered a computerized decision making test (Monterde-i-Bort's TID), that assessed number of attempts (NA), number of successes (NS), success rate (SR), risk assumption (RA) and response time (RT), and a fatigue symptom survey. We used T-test to assess between group differences and Pearson's correlation tests to explore potential correlations between fatigue symptoms and decision-making outcomes. Results: Sleep restricted individuals were slower compared to non-restricted ones (restricted: 1.18±0.13s, non-restricted: 0.71±0.03s, $p<0.01$). Fatigue symptoms were correlated with NA ($r=0.424$, $p<0.05$), NS ($r=0.423$, $p<0.05$) and RT ($r=0.815$, $p<0.001$). Conclusions: A single night sleep restriction period impaired decision making RT. Increased fatigue was also associated with increased RT. Moreover, fatigue may have determined an increase in NA, and consequently in NS, but not, as expected, in SR. Overall, different dimensions of the test may provide a valid alternative for the objective assessment of sleep deprivation and fatigue symptoms.

(Sponsored by ANPCyT PICT Start-up 2013-0710, PIDDEF 2014/17n 06).

The influence of academic demand on sleep habits and cognitive performance of adolescents attending technical high school.

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In adolescents, the sleep-wake cycle (SWC) shows a sleep phase delay, contributing to reduced sleep duration on weekdays and a high discrepancy in sleep patterns between weekdays and weekends. These changes, coupled with high daytime sleepiness and sleep deprivation, are related to increased social, academic and peer pressure. Although several studies suggest that academic demand is one of the external factors that influence the SWC of adolescents, this relationship has not yet been analyzed. Therefore, this study proposes to evaluate the relationship between academic demands and sleep habits, daytime sleepiness, sleep quality and cognitive performance in Brazilian students attending technical high school, according to chronotype. The sample will be composed by 302 students who are enrolled in the technical high school in a public institution, the *Instituto Federal do Rio Grande do Norte*, located in the Northeast region of Brazil. The data collection will occur in two stages, in the first, the participants must be attending the first year in the morning or evening shift; and in the second, the same participants should be attending the second year and presenting an increase in their academic demand with curricular activities in the counterpart. The students will answer the questionnaires: \"Health and Sleep\", Pittsburgh Sleep Quality Index, Puberty and Phase Preference Scale, Pediatric Daytime Sleepiness Scale, and record the sleep patternsby Sleep diary over 10 days. Within these 10 days, cognitive tasks will be performed to evaluate the attention and working memory.

The JUPITER project: a social jetlag cohort. Results from the cross-sectional study.

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Social jetlag (SJL) (difference between midsleep point in weekends and weekdays) is associated with some adverse effects to the individual´s health. The JUPITER project (socialJetlag inUniversity: PersonalityInfluences sleepTiming, healthEstimates and academicRatings) aims to be the first social jetlag cohort study. We evaluated 1158 undergraduate students in their first year at the university. They will be re-evaluated in the second and third years. They answered questions about demographic data, their usual schedule and habits, Horne-Ostberg Morningness-Eveningness Questionaire (HO), Munich Chronotype Questionnaire (MCTQ), Big Five Inventory, Pittsburgh Sleep Quality Index Questionnaire and 36-Item Short-Form Health Survey. Body mass index (BMI) was also obtained. Academic achievement will be verified after the end of each academic year. Here we present the results of the first wave of the study. We found a positive correlation between eveningness and neuroticism, and between morningness and conscientiousness, amability and extroversion. Sleep quality explained 36% of the global health quality. Social jetlag didn´t correlate with BMI. Intermediate types classified as bimodals represented 12.2% of the sample and their sleep and health quality were not different from evening types. Those who study only at night and don´t work showed weaker correlation between MCTQ and SJL and no significant correlation between HO and SJL (unlike the others). After the second and third waves, we will be able to analyze the mediating effect of personality in the consequences of SJL.

Characterization of circadian behavior and sleep architecture in a mouse model of epilepsy.

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Dravet syndrome (DS) is a childhood epilepsy caused by a spontaneous loss-of-function mutation in the *SCN1A* gene encoding the alpha-subunit of the $Na_V1.1$ sodium channel. This channel is the primary voltage-gated Na^+ channel in adult GABAergic interneurons. Like many other types of epilepsy, DS not only involves seizures, but also several comorbidities including intellectual disability, autism spectrum disorders and sleeps disorders.

Mice carrying a heterozygous mutation in the *SCN1A* gene display the symptoms present in DS patients, representing a valid animal model of DS. Previous results from our lab have shown that DS mice exhibit lengthening of their circadian period and reduced amplitude of the circadian rhythm of wheel-running activity. In addition, DS mice show impairments in homeostatic sleep regulation, displaying higher NREM sleep fragmentation and reduced slow wave activity.

In this work, we show that DS mice also display impaired circadian regulation of sleep, including high REM sleep fragmentation. Moreover, we demonstrate that restricting the heterozygous mutation of the *SCN1A* gene to Neuromedin S (NMS) expressing SCN neurons is not sufficient to replicate the circadian features observed in DS mice. We are currently studying the expression of $Na_V1.1$ within subpopulations of SCN neurons using a co-immunostaining approach and targeting the mutation to the whole SCN through Cre-expressing adeno-associated viral injections. We aim to contribute to the understanding of the role of $Na_V1.1$ in modulating circadian and sleep behaviors, and to deepen our knowledge of the neural and molecular basis of circadian and sleep comorbidities in DS.

Dopaminergic cells seem to be part of a timing mechanism that drives motivation to nursing in the rabbit.

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Maternal care is a motivated rewarding behavior that involves activation of dopaminergic (DAergic) cells of the mesocorticolimbic system, which projects to nucleus accumbens (NA) and medial prefrontal area (mPFC). However, the specific DAergic populations of this system are poorly known as well as whether these cells are activated before the interaction with pups. Due to the prolonged separation of mother and pups required in rats to study activational responses of DA system, here we propose the rabbit as an ideal model for examining activation of DAergic cells before and in response to pups' interaction. The lactating doe visit just once a day their pups for nursing and shows high arousal. Our hypothesis is that the DAergic system would be activated by or during the expectation for nursing. By immunodetection of FOS protein we assess activational response of A10 and A10vr DAergic populations, as well as activation of NA and mPFC. Also, considering that this behavior occurs with circadian periodicity we also explored by detection of PER1 protein, possible synchronization of the preoptic area and lateral septum, which are important for maternal behavior. We found a strong activation of the two DAergic populations, NA and mPFC before the daily visit to the nest as well as a rhythm of PER1 in preoptic area and lateral septum that shifts in parallel to timing of daily nursing. Our results suggest that DAergic system is part of a timing mechanism that drives motivation to circadian periodicity of nursing.

In the heat of the night: photic and thermic synchronization of *C. elegans*.

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Circadian rhythms are driven by endogenous clocks and are synchronized by environmental cues. Although circadian responses of *C. elegans* have been extensively reported, the mechanism and pathways of synchronization of the nematode are still unknown. Here we present a novel behavioral approach to study entrainment to two of the most studied *zeitgeiber*: light and temperature, as well as the interaction between them and their possible pathways of actions. We show that the *wild-type* strain is able to synchronize to both stimuli, with a better performance when assessed under an optimal combination of light and temperature. Significantly lower performances of the mutant strain MT21793 (*lite1-gur3 ko*) and IK597 (*gcy 8, 18 and 23 ko*) were found in response to light and temperature, respectively; however, when both *zeitgeiber* were present and coordinated the mutants were able to entrain. Our results shed light on the *C. elegans'* response to different *zeitgeiber* as well as their possible synchronization pathways, the genes involved in this pathway and their relative strength. These results also complement previous studies performed in worms carrying a bioluminescence reporter (sur5: luc: gfp). This strain was recorded under similar zeitgeber conditions, with results that strengthen the previous conclusions.
We are also presenting a mathematical model to approach the population dynamics, with a Kuramoto Model of coupled phases. With this method we obtained a coupling value for each nematode strain and *zeitgeiber*. This model was also tested for the bioluminescent reporter, with similar results.

(Sponsored by Universidad Nacional de Quilmes, Conicet and Agencia Nacional de Promocion Cientifica y Tecnologica).

An exploration of circadian phenotypes, rhythmicity of mood-related behaviors and psychosocial factors in a sample of Young Colombian adults.

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The study of circadian rhythms in young adults represents a valid approach to understand the possible relationship between circadian phenotypes and relevant psychosocial factors. The aim of this study was to explore the plausible association of specific circadian phenotypes in young Colombian adults, psychosocial factors, and rhythmicity of mood-related behaviors. 450 healthy Colombian subjects were assessed with two scales to measure phenotypic patterns of circadian rhythms in humans: Epworth Sleepiness Scale and Composite Scale of Morningness (CSM). Additionally, was used the Mood Rhythm Instrument (MRI) to evaluate rhythmicity of mood symptoms. To assess some psychosocial variables and their possible association with circadian phenotypes were used the Hospital anxiety and depression scale (HADS) and the family Apgar functionality. We found a significant association between daytime sleepiness and anxiety and depressive symptoms ($p= 0.0007$; $p= 0.0003$) respectively and with specific behavioral rhythms associated with mood states (alert, self-esteem and pessimism) ($p<0.05$). Additionally, was found an interesting association between the behavioral rhythm of concentration and consumption of drinks what contains caffeine ($p=0.00003$). Our results suggest that circadian rhythms could be affected by emotional symptomatology and specific behavioral rhythms associated with mood states. Furthermore, specific rhythm moods related with cognition appear to be associated with caffeine consumption. These results need to be replicated in larger samples including a psychiatric population with the aim to identify circadian phenotypes and rhythms of behavior associated with mood states of neuropsychiatric relevance.

(Sponsored by Universidad De Ciencias Aplicadas Y Ambientales U.D.C.A).

Alteration of maternal photoperiod and long-term effects on liver clock genes of adult offspring.

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Introducción: Light pollution is one of the most pervasive forms of environmental alteration and millions of people are exposed to light at night because to work at night or work a rotating shift, phenomenon to which women in gestational age are also exposed and known as gestational chronodisruption. The evidence demonstrates a significative negative impact of circadian disruption on human health, including metabolic and cardiovascular disorders, but there is not much evidence about the gestational chronodisruption on the long-term effects on offspring and its relation with chronic diseases of the adult. The circadian clock controls a wide variety of physiological functions. In mammals, the central pacemaker is in the suprachiasmatic nucleus, which receives signals from the environment and coordinates the oscillating activity of peripheral clocks, which are located in almost all tissues and needs to be synchronized by the central clock, through the predominant external cue, the light. The circadian rhythmicity includes the mRNA expression of clock genes and clock-controlled genes.

Material and Method: Sprague-Dawley pregnant rats were exposed to normal photoperiod (LD) or to Chronic Phase Shift (CPS) during gestation and clock gene expression was determined in progeny at 90 days postnatal development.

Results: In this work, we report the results obtained from the determination in the liver by the Real Time PCR (q-PCR), about the mRNA expression of the following clock genes; *Bmal1, Clock, Cry, Per, Rev-erbα and Rorα*, in a period of 24 hours. Main findings were found in Bmal1, Rev-erbα and Rorα.

(Sponsored by FONDECYT 1150789, anillo ACT-1116. beca CONICYT, doctorado nacional, folio 21171387)

Clocks of metamorphosis: studying the circadian gating of *Drosophila* adult emergence with a single fly assay.

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The emergence of adult *Drosophila* (eclosion) is “gated” by the circadian clock: populations of flies eclose with a daily rhythmicity with a peak during the morning. Although eclosion was the first rhythm studied in *Drosophila*, the mechanism by which the clock gates it remains largely unknown. Here we present a procedure for analyzing the timing of the process that leads to adult emergence with single animal resolution. This method relies on imaging the changes in the appearance of the developing fly during the last 2 days of metamorphosis. During this period the molting fluid is resorbed, making the pupal cuticle follow increasingly closely the contours of the body. In the head the cuticle goes from being very smooth to rough, as it is increasingly pressed against the facetted eye. In initial experiments, wildtype flies of the same age were selected such that they either emerged late within one (subjective) day or early within the following (subjective) morning. We found that wildtype animals of the same age that chose an earlier gate roughened *earlier* than those that emerged during the following day, revealing for the first time that the clock regulates the timecourse of metamorphosis. Mutants without a functional clock emerge at a time that is only a function of the number of hours of metamorphosis. Importantly, our analyses shows that different genotypes that cause arrhythmicity show distinguishable phenotypes using this single-fly assay, suggesting that this approach will allow us to investigate how the circadian clock regulates the timing of eclosion.

Circadian study of Dnmt1, BDNF and TrkB expression in the cerebellum of rats subjected to caloric restriction.

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We aimed to evaluate the circadian variation of DNA methyltransferase 1 (Dnmt 1), Bdnf and TrkB expression, in the cerebellum of aged rats subjected to caloric restriction. Holtzman 3- and 22-month old male rats and 22-month old male rats under caloric restiction were maintained in constant darkness conditions during 15 days, in order to study the effects of caloric restriction on endogenous rhythms. Previously, we observed, that TrkB and Dnmt1 mRNA levels varied throughout a 24h period ($p<0.05$) in young rats. Aging increased rhythm's mesor in both cases, it also diminished TrkB rhythm's amplitude ($p<0,05$). In both groups Dnmt1 maximal expression preceded TrkB minimal expression. Old rats subjected to caloric restriction showed an increase in the rhythm's mesor and a phase advancement of TrkB mRNA levels, in comparison to both young and old rats ($p <0.05$). Additionally, old rats subjected to caloric restriction underwent a loss of circadian variation in the Dnmt1 mRNA levels in comparison to the groups of young and old rats. Previously we observed that Bdnf mRNA expression displayed a circadian rhythm ($p<0.05$) in the young rats. Aging Caused a significant alteration of the rhythm's parameters. Surprisingly, in the old rats subjected to caloric restriction we observed a loss of the circadian oscillation of Bdnf expression. Caloric restriction exerted important changes in the circadian expression of the studied factors, the impact of such modifications in the motor function of aged subjects remains to be elucidated.

Ultradian changes of cortical High Frequency Oscillations during wakefulness and sleep.

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Recently, a novel type of fast cortical oscillatory activity that occurs between 110 and 160 Hz (high-frequency oscillations (HFO)) was described. HFO are modulated by the theta rhythm in hippocampus and neocortex during active wakefulness and REM sleep. Since theta-HFO coupling increases during REM, a role for HFO in memory consolidation has been proposed. However, global properties like the cortex-wide topographic distribution, and the cortico-cortical coherence remain unknown. In the present study, we recorded the electroencephalogram during sleep and wakefulness in the rat, and analyzed the spatial extent of the HFO band power and coherence. We confirmed that the HFO amplitude is phase-locked to theta oscillations and is modified by behavioral states. During active wakefulness, HFO power was relatively higher in the neocortex and olfactory bulb compared to sleep. HFO power decreased during non-REM and had an intermediate level during REM sleep. Furthermore, coherence was larger during active wakefulness than non-REM, while REM showed a complex pattern in which coherence increased only in intra and decrease in interhemispheric combination of electrodes. This coherence pattern is different from gamma (30-100 Hz) coherence, which is reduced during REM sleep. The present data show an important HFO cortico-cortical dialogue during active wakefulness even when the level of theta co-modulation is lower than in REM. In contrast, during REM, this dialogue is highly modulated by theta, and restricted to intrahemispheric medial-posterior cortical regions. Further studies combining behavior, electrophysiology and new analytical tools are needed to plunge deeper into the functional significance of the HFO.

(PEDECIBA and CSIC. S.CZ. and A.M. currently have a postgraduate fellowship from ANII and M.C. from CAP. D.RL. Fondecyt 3120185.)

The influence of artificial light on metabolism and biological rhythms of Wistar rats.

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Sunlight is essential to entrain biological rhythms and regulate metabolism and behavior.With electricity, humans have extended the light phase to night hours. Aim: to evaluate the effects of electrical light on biological rhythms and metabolism of Wistar rats. *Study 1* encompasses changes in photoperiod that mimic seasonality. Three groups of animals: Control (CT), kept in 12:12 light/dark (LD) cycle; Long Photoperiod/Short Photoperiod (LP/SP), kept in 16.5:7.5 LD cycle for 18 days, then 17 days of gradual reductions in light time, then 18 days of shorter exposure (7.5:16.5 LD cycle); and SP/LP, with same modifications, but starting in 7.5:16.5 LD cycle. We measured activity and temperature constantly, and melatonin and cortisol twice. Correlations between activity, temperature rhythms and corticosterone levels were significantly lower in SP/LP, indicating a worseadaptability in transitioning from short to long photoperiods. *Study 2* tested differences between light qualities. Two groups stayed under 16:8 LD: standard light (SL), kept under fixed conditions (LED, 4000K); and circadian light (CL) with color temperature changes throughout the day (LED, 2700-6500K).Activity, temperature and serum melatonin were measured and visceral fat weight was assessed. CL presented better rhythmic parameters than SL i.e. lower intracycle variability, and higher amount of activity. Visceral fat negatively correlates with activity rhythm stability and positively correlates with sum of activity during rest period on SL. These reveal promising methods to improve reliability of experimental models and of environmental health research, especially considering that humans have spent increasingly more time exposed to artificial light.

(Funding: Brazilian agencies CNPq/CAPES.)

Effects of the blockade of dopaminergic D1 receptors in the preoptic area neurons on the preovulatory regulation of ovulation.

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Many neurotransmitters are involved in the regulation of the phasic secretion of the gonadotropin releasing-hormone (GnRH), which leads to the preovulatory surge of luteinizing hormone and ovulation. One of them is dopamine whose participation is controversial since there is experimental evidence suggesting that its role is inhibitory or excitatory depending on the experimental model. We analyzed if the dopaminergic receptors present on the preoptic area (POA) GnRH-neurons are involved on the regulation of ovulation. By implanting an antagonist of dopamine receptors on the left or right POA of female rats during the afternoon of proestrus ovulation did not change. Despite this, we suggested that these results are due to the non-selective blockade of dopaminergic receptors and the hour that the blockade was performed. To determine if a specific receptor is predominantly involved and if this have a circadian rhythm, a second batch of rats was unilaterally implanted with a permanent cannula directed to the right or left POA. Animals were allowed to recover and then microinjected with 0.1 µg of SCH-23390, an antagonist of the dopaminergic D1 receptor, or vehicle at 09:00 or 21:00 h of proestrous. All groups were sacrificed by a sobredoses of barbiturics, the oviducts dissected and ova shed counted using a dissecting microscope. Further analysis will be performed on the ovaries to determine the effects of these treatments on the follicular development and ovulation.

(Supported by PAPIIT IN216015).

Ovarian follicular development is altered by unpredictable feeding times.

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It is know that transmeridional traveling (jet lag), shift working, and nocturnal work has a negative effect in human reproductive function. In mammals like rat female, photoperiod modification disturbes the axis hipothalamus-pituitary-ovary. In this order we consider that food intake at an unpredictable intervals time could lead to internal desynchronization causing alterations in ovarian function. To explore this effect, 24 female rats were divided into four experimental groups: 1) AL had 24h-free access to standart rat chow; 2) RFD was exposed to a daily restricted feeding during the light phase (ZT5-ZT7); 3) RFN was exposed to a daily restricted feeding during the dark phase (ZT17-ZT19); 4) RFU was exposed to a variable daily restricted feeding (two hours different every single day). In all experimental period, the body temperature and phase of estrous cycle was determined. At the 28-day experimental, the ovaries were weighed, fixed, cut and stained to analyze the number of preantral, antral and ovarian follicles. The vaginal cytology showed a marked alteration in the regularity of the estrous cycle in the RFU group. In addition the RFU group showed a loss in the temperature circadian pattern as well as in the temperature level during the receptive phase of the estrous cycle. Microscopic analysis showed an increase in the number of antral follicles in the RFU group compared to the other groups. In conclusion, feeding at irregular hours significantly affects the function of the hypothalamic-pituitary-ovary axis, affecting the reproductive function of the female rat.

Distribution of MCH-containing fibers in the feline brainstem: relevance for REM sleep regulation.

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Neurons that utilize melanin-concentrating hormone (MCH) as a neuromodulator are localized in the postero-lateral hypothalamus and incerto-hypothalamic area. These neurons project diffusely throughout the central nervous system and have been implicated in critical physiological processes, such as sleep. Unlike rodents, in the order carnivora as well as in humans, MCH exerts its biological functions through two receptors: MCHR-1 and MCHR-2. Hence, the cat is an optimal animal to model MCHergic functions in humans, particularly regarding its role in sleep regulation. In the present study, we examined the distribution of MCH-positive fibers in the brainstem of the cat. MCHergic axons with distinctive varicosities and boutons were heterogeneously distributed, exhibiting different densities in distinct regions of the brainstem. Greater densities of MCHergic fibers were found in the dorsal raphe nucleus, the laterodorsal tegmental nucleus, the periaqueductal gray, the pendunculopontine tegmental nucleus, the locus coeruleus and the praepositus hypoglossi. Because these areas are involved in the control of REM sleep, the present anatomical data support the role of this neuropeptidergic system in the control of this behavioral state.

(Supported by the Programa de Desarrollo de Ciencias Básicas PEDECIBA Uruguay).

Distribution of hypocretinergic fibers in monoaminergic nuclei of the cat´s midbrain.

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Hypocretin (Hcrt) 1 and Hcrt 2 are two neuropeptides synthesized in a discrete group of neurons of the lateral hypothalamus, a key brain area responsible for initiating, coordinating and maintaining goal-oriented survival-type behaviors. Hcrtergic neurons project to virtually the entire central nervous system, and exert their biological function through two metabotropic receptors Hcrt-R1 and Hcrt-R2 that have broad and partially overlapping patterns of distribution. Through these receptors, Hcrts produce an excitatory effect at the presynaptic and postsynaptic sites. Hypocretinergic neurons have been implicated in the regulation of wakefulness; they are active in waking and quiescent in sleep. Previously, we reported that the highest Hcrtergic activation is achieved in wakefulness during exploratory locomotor activity and locomotion with reward. In the present study we characterize the distribution of Hcrtergic fibers in the monoaminergic nuclei of the midbrain, areas that are implicated in the regulation of locomotion, reward and sleep-wakefulness cycle.

(Supported by the Programa de Desarrollo de Ciencias Básicas (PEDECIBA)-Uruguay).

Cardiometabolic responses in rats submitted to a t-cycle of 22h.

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Disruptions of circadian rhythms is often associated with serious consequences for the organism. In this way, our aim was to understand whether chronic internal desynchronization of the locomotor activity provokes cardiometabolic dysfunction, such as, hypertension, hepatic insulin resistance and oxidative stress. Methods: Male Wistar rats were distributed into two groups: control (CTR, n= 9) submitted to 12h light/dark cycle (T24), and chronic circadian desynchronization group (DSC, n=12) submitted to 11h light/dark cycle (T22). Experimental period was 8 weeks. Results: Despite, we not observed change in baseline blood pressure (MAP) and heart rate (HR) DSC rats had decreased reflex bradycardia (2.78± 0,2 vs 1,84± 0,26 bpm/mmHg) and increased sympathetic efferent activity to the heart (36,3 ±11 vs 73,2 ±7,7 bpm). Furthermore, DSC animals shows low HDL cholesterol (28,21±1,6 vs 40,8 ±4,2 mg/dL) and elevated Castelli index I (2,77±0,23 vs 2,07 ±0,12 mg/dL) e II (1,46±0,22 vs 0,93±0,11 mg/dL). DSC group had decreased IRβ, IRS2, PI3K and Akt hepatic content, suggesting hepatic insulin resistance, associated with increased of hepatic PEPCK content. Besides, we observed reduction in SOD1 and catalase hepatic content in DSC animals in comparison to CTR. Finally, DSC group shows increased adrenal mass and hypertrophy. Conclusion: We suggest that dissociation of the circadian rhythm of locomotor activity may affect the cardiovascular function by attenuating baroreceptor sensitivity without altering baseline levels of MAP and HR. This condition is associated with the development of hepatic insulin resistance as well as oxidative stress.

(Supported by Fundação de Amparo à Pesquisa do Estado de Goiás – FAPEG; Conselho Nacional de Desenvolvimento Científico e Tecnológico – CNPq).

Transcriptional Attenuation versus Rhythmic repression: lessons from an optogenetic TTFL synthetic system in *Saccharomyces cerevisiae*.

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Synthetic biology aims to better understand the design principles of complex cellular processes, such as the ones underlying transcriptional networks. One of the most exciting transcriptional networks is the Transcriptional-Translational Feedback Loop (TTFL). Thereby, we are planning to uncover the underlying mechanisms governing the principles of the architecture of these natural systems using the photo adaptation components from *N. crassa* inside S. cerevisiae cells. We have isolated the molecular components of the *N. crassa* photoadaptation system. The basic architecture of this system is going to be like the *N. crassa* molecular clock, which is composed of a time-delayed negative feedback loop where the positive module activates the expression of a negative module that will repress its own activator. This network topology is like the *N. crassa* photoadaptation system, but it does not oscillate and instead, it leads to an attenuation response. Therefore, we are going to use these proteins from the photo adaptation system and based on the pacemaker topology- arrange them as hybrid proteins. A couple LOV domain proteins are going to interact only in the presence of a light input forming a LOV domain Gal4-like functional Transcription Factor. This activator will drive the expression of a third LOV domain protein, which is going to prevent its own expression. Hence, we predict that this novel approach will tackle biological questions inside oscillators network and contribute to the understanding of the molecular mechanisms in the photo adaptation system in Neurospora using synbio.

(Sponsored by Larrondo Lab; FONDECYT 1171151.Millennium Nucleus on Fungal Integrative and Synthetic Biology,""Iniciativa Científica Milenio NC120043. HHMI International Research Scholar program).

Exploring the contribution of Evening cells to the circadian pacemaker of *Drosophila*.

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The circadian clock of *Drosophila* is composed of 150 cells, which can be divided into 7 clusters. Under laboratory conditions, daily locomotor activity is condensed in morning and evening bouts. Each activity peak has been linked to a specific group of neurons: the PDF-positive small ventral lateral neurons (sLNv) control the morning component (M-cells), and the dorsal lateral neurons plus the 5th sLNv control the evening component (E-cells). While M-cells have long been considered as the main pacekeepers of the circadian clock, there is increasing evidence pointing to the contribution of E-cells in sustaining a coherent rhythmic output, although the mechanisms underlying their role are not fully described yet. Our first aim was to characterize the neurotransmitters secreted by E-cells, as well as their impact in the generation of a robust behavioral rhythm. To do so, we genetically manipulated the expression of different neurotransmitter transporters in a subset of E-cells, and monitored locomotor activity rhythms. Next, to characterize possible circuital mechanisms by which E-cells could mediate such influence on the behavioral output, we focused on the relation between E-cells and M-cells. Structural analysis of pre- and post-synaptic markers revealed a possible E- to M-cells communication, which was functionally corroborated using genetically encoded calcium reporters. This work contributes to shape a detailed map of the functional and structural characteristics of the pacemaker network, which is essential for understanding the neural basis of the circadian control of behavior.

(Sponsored by FIL, ANPCyT and CONICET, Argentina).

Envelope analysis of human electromyogram during sleep: useful tool to evaluate REM sleep behavior disorder.

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REM sleep Behavior Disorder (BRD) is a clinical condition of growing epidemiological interest because its association to neurodegenerative diseases such as Parkinson's Disease and other alpha-synucleinopathies. It is characterized by the absence of physiological flaccid paralysis during REM sleep. Here we evaluate muscular activity of healthy volunteers (n=8) and RBD patients (n=10) recorded during whole night polysomnograpy. Polysomnographic records of RBD patients were obtained from three sources: open databases (*physionet.org*), a collaborative project with Universitäts Klinikum Tübingen and ambulatory polysomnograpy of patients with suspected RBD. Whole night 30-second epochs of chin and dominant forearm electromyogram (EMG) were projected in a phase portrait constructed with the coefficient of variation of the envelope (CVE) and the envelope amplitude (EA). The portrait was mapped to discriminate high and low muscle tone, reported by EA, and high and low phasic activity epochs, reported by the CVE. REM sleep epochs of healthy subjects cluster around a minimal amplitude and non-pulsatile region of the phase portrait. Chin electromyogram of REM sleep epochs of RBD patients exhibit a scattered distribution with increased density in the high AE and CVE region. Forearm electromyogram of REM sleep epochs of RBD patients exhibit increment in phasic events. The envelope phase portrait of chin and forearm muscles may be a useful screening tool to identify RBD patients.

(Supported By Puelma Foundation).

The influence of a light and dark cycle on the egg laying activity of *Aedes* aegypti ***(Linnaeus, 1762) (Diptera: Culicidae).***

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The epidemiological importance of the mosquito *Aedes aegypti* as vector of multiple human pathogens has moved a growing number of studies on the physiology and behavior of its blood-feeding females. The activity of oviposition is one of the critical elements contributing to the expansion of *Ae. aegypti's* populations. Although there is a vast literature about oviposition behavior, significant knowledge about the factors that affect the activity of egg laying is still lacking. We studied, in laboratory conditions, the effect that light and dark cycles have on the efficiency of oviposition by *Ae. aegypti* females. Physiological assays were made using synchronized eggs obtained from forced egg laying. The number and viability of eggs was analyzed under three different light/dark regimes: LD12:12 (12 h of light and 12 h of dark), DD (constant darkness) and LL (constant light). Our results show that females prefer to lay their eggs in dark conditions, but maximizing the number and viability of eggs requires the occurrence of a light/dark cycle. Ongoing research on this theme has the potential of contributing to the proposition of new strategies for control based on the failure of egg laying and hatching.

Downregulation of a GABA receptor affects sleep behavior in unanticipated ways in *Drosophila.*

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Sleep is a complex and vital behavior regulated by both, circadian and homeostatic mechanisms.The neural circuits involved in sleep homeostasis are not well described yet, but it has been proposed that GABAergic inputs to the large lateral ventral neurons (lLNvs) of the adult brain of *Drosophila melanogaster* may have the role of informing those highly integrative circadian and arousal neurons about the sleep homeostat status.

Starting from this point, our aim was to study GABAergic inhibition on LNvs, its influence on sleep behavior and its role on the sleep homeostat. For this, we quantified sleep behavior by inferring it from locomotor activity under basal and sleep deprivation conditions. In addition, we studied the levels of Pigment Dispersing Factor in the axonal projections of the lLNvs in order to evidence the effect over neuronal outputs under those circumstances. Finally, we are currently performing electrophysiological recordings to identify the extent of the role of the neurotransmitter GABA in the neuronal circuit studied.

Our findings indicate that downregulation of the GABAA receptor Rdl in LNvs affects sleep behavior in the way it was reported. Moreover, we have now confirmed its previously suggested role on the sleep homeostat. However, we have surprisingly found that sleep can be differentially affected by the downregulation of Rdl in the LNvs when the genetic manipulation is performed in a constitutive or an acute fashion. This indicates that compensation played an important part on previously reported phenotypes and opens unexpected possibilities of the actual mechanism of action involved.

Role of the different eyes in the photoentrainment of the jumping spider clock.

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The phase of an animal's locomotor activity depends on the timing of the day/night cycle, which can be detected by a variety of photosensitive organs, including the retina as well as extraretinal organs and the pacemaker cells themselves. Here, we investigated the pathway that entrains the diurnal jumping spider (*Euophrys rusticana*). For this we determined the ability of L/D cycles to entrain animals in which either the entire prosome or different sets of eyes (*E. rusticana* has 4 pairs of eyes) were covered with a black polymer. We found robust entrainment when any pair of eyes was left uncovered. By contrast, covering either the entire prosome or all pairs of eyes severely affected entrainment, suggesting that eyes are necessary for entrainment but that any pair of eyes can carry out this function. We are currently determining the spectral sensitivity of the entrainment photoreceptor.

Photoperiodism in a subterranean rodent: a mathematical modelling approach.

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Many living organisms adjust their annual physiology to the seasons, by decoding day length from photoperiod, a photic environmental cue that changes consistently along the year. The tuco-tuco (*Ctenomys* aff. *knighti,* Rodentia) is a subterranean rodent from the Monte Desert, Argentina, which experiences cold/dry winters and warm/wet summers. We have previously studied the daily light exposure patterns of the species, focusing on the entrainment of their circadian system to the 24-hour day. Extending these measurements to a yearly scale, we will now study if and how tuco-tucos use photoperiod to time their physiology along the year. In a first approach, mathematical modelling will be employed to simulate the tuco-tuco's circadian oscillators and their response to different light regimens. Previous works indicate that tuco-tucos possess two oscillators controlling circadian rhythms and we hypothesize that these oscillators could be used as the basis for photoperiod reading, based on a mechanism of internal coincidence. To test this hypothesis, we will simulate a system of two coupled limit-cycle Pavlidis-Pittendrigh oscillators, exposed to different skeleton photoperiods and to the light-exposure patterns experienced by tuco-tucos throughout the year. Here, we present preliminary data to be used as a starting point. Before advancing to a complete model, we started by verifying the dynamics of a single oscillator, subjected to skeleton photoperiods, with photophases of different durations. Predictions based on the Phase Response Curve matched most of the results obtained. Having explored the limitations of a single oscillator, we will next analyze the responses of two coupled oscillators.

Characterization of the circadian expression profile of clock genes in Aag-2 cell line infected by Dengue-2 virus (DENV2).

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Aedes aegypti (Diptera: Culicidae) is a mosquito vector of several pathogens that cause important diseases such as dengue fever.Aspects of mosquitoes behavior play an important role in the dynamics of the diseases transmitted by them.Despite its medical importance, we don´t know much about the molecular control of circadian rhythms of mosquitoes. Clock genes are already known in mosquitoes, but it´s not clear how these genes interact with each other. In order to understand how the clock genes work in this species, we intend to establish the cell culture model for the circadian study. We aim to characterize the circadian expression profile of the clock genes in the Aag-2 lineage in different photoperiods (light/dark and constant darkness). For performing functional assays, the transcriptional activity of the *period* gene will be analyzed by transient transfection assays in cells. We will use a chimeric construction with the *period* gene promoter and the *luciferase* gene coding region in cells infected and non infected with DENV2. In preliminary trials, we carried out assays to collect cells maintained in incubators in light/dark cycles (LD12:12) for three days. We first collected the cells at two points over 24 hours and observed that the genes of the central clock are expressed in Aag-2 cells. Once the genes are expressed, we will elucidate the cycling profile of these genes to perform the functional assays with luciferase.

(Sponsored by CNPq, FAPERJ, FIOCRUZ and CAPES).

Spontaneous circadian rhythms in a cold-adapted natural isolate of *Aureobasidium pullulans*.

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Circadian systems enable organisms to synchronize their physiology to daily and seasonal environmental changes relying on endogenous pacemakers that oscillate with a period close to 24 h even in the absence of external timing cues. The oscillations are achieved by intracellular transcriptional/translational feedback loops thoroughly characterized for many organisms, but still little is known about the presence and characteristics of circadian clocks in fungi other than *Neurospora crassa*. We sought to characterize the circadian system of a natural isolate of *Aureobasidium pullulans*, a cold-adapted yeast bearing great biotechnological potential. *A. pullulans* formed daily concentric rings that were synchronized by light/dark cycles and were also formed in constant darkness with a period of 24.5 h. Moreover, these rhythms were temperature compensated, as evidenced by experiments conducted at temperatures as low as 10 °C. Finally, the expression of clock-essential genes, *frequency, white collar*-1, *white collar-2* and *vivid* was confirmed. In summary, our results indicate the existence of a functional circadian clock in *A. pullulans*, capable of sustaining rhythms at very low temperatures and, based on the presence of conserved clock-gene homologues, suggest a molecular and functional relationship to well-described circadian systems.

Generating a synthetic hybrid circadian oscillator through transcriptional rewiring.

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Circadian rhythms are present in diverse organisms, from bacteria to mammals. The molecular blueprints that govern them are highly conserved: a central oscillator composed by a transcriptional-translational feedback loop (TTFL), where a positive element promotes the transcription of a negative element, that when translated goes back to directly inhibit its own expression.

In *Neurospora crassa* the negative element is the protein FREQUENCY, encoded by the gene *frq*, while the positive element is the White Collar Complex (WCC), composed by White Collar-1 and White Collar-2. WC-1 is also a photoreceptor, allowing the incorporation of the light input to the clock. The rhythmic information is transmitted to the output pathways causing the oscillation of different biological process, as metabolism and conidiation. This is mediated, in part, by a hierarchical arrangement of transcription factors; which allows the rhythmic expression of clock controlled genes (*ccgs*).

To improve our knowledge of the genetic plasticity of the circadian clock, we followed a transcriptional rewiring approach, to generate new topologies of the central pacemaker. Thus, we generate a hybrid oscillator (HO) and evaluated the ability of the system to generate and sustain rhythms when its central topology has been turned into an extended TTFL that incorporates additional transcriptional steps. This Hybrid oscillator exhibits robust rhythms with a period ~24 h and temperature compensation, demonstrating the relative importance of posttranslational versus transcriptional mechanisms in the modulation of central clock properties, while providing evidence of the importance of transcriptional mechanisms in mediating phase and entrainment.

(Sponsored by FONECYT 1171151-1131030. MN-FISB NC120043).

Comparison of daily activity features of subterranean rodents (tuco-tucos) in seasonal transferences from field to laboratory.

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In desert environments, some small mammals have been shown to be diurnal in the field and nocturnal when transferred to the laboratory. Epigeous rodents also presented seasonality in their daily activity rhythms. Both features were observed in the activity pattern of subterranean rodents from Monte desert, Argentina (Ctenomys aff. knighti, tuco-tucos), but using distinct recording methods: light exposure (surface activity) in semi-natural enclosures, wheel running in laboratory and body temperature in both conditions. Here we present the first activity data obtained using accelerometers, miniature devices (0.7g; 460 days, 1Hz samples) that record activity levels even of small animals in both field and laboratory conditions. During summer and winter, accelerometers and light-loggers were tied to 9 freshly caught animals (5 males, 4 females; 178±39g). Three of them were also implanted with temperature loggers. Animals with sensors were kept inside enclosures (12m×6m×1,5m) for one month. Environmental temperature was recorded simultaneously. Recaptured animals were transferred to laboratory and kept in constant conditions for 10 days. In summer, general activity inside enclosures was observed throughout the day and presented peaks when crepuscular surface activity occurred. In winter, both activity components became diurnal. Independent of season, transition from enclosures to laboratory caused general activity to become concentrated at night. Inside laboratory, body temperature rhythm coincided with general activity rhythm, differently from enclosures. Comparison between animal and environmental records indicates that nocturnal/diurnal switch and seasonality of activity patterns can be partially due to masking by environmental temperature.

(Sponsored by CAPES, CONICET and FAPESP 2016/25058-0).

Gestational chronodisruption programs metabolic syndrome in the rat offspring.

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Introduction: Epidemiological and experimental evidence supports an association between chronodisruption and incrased incidence of chronic diseases. However, the effects of maternal chronodisruption during pregnancy and the offspring remain unclear. Here, we investigated the effects of chronic photoperiod shifting (CPS) throughout gestation on the offspring's glucose glucose homeostasis and adipose tissue physiology.

Methods: Female rats (maintained in photoperiod 12:12 light:dark; n=16) were mated and separated into two groups: A) control photoperiod 12:12 (LD; n=8) and B) chronic phase shift photoperiod (CPS; n=8), simulating a shift work schedule. At birth, mothers and their pups returned to photoperiod 12:12. On day 90 of age, male rats were exposed to a high fat diet (HFD; 45% excess calories) for 12 weeks, to carry out intraperitoneal glucose tolerance test (IGTT) and insulin tolerance test (IITT). At day 200 of age, rats were euthanized to collect blood samples and fat depots (interescapular, inguinal, perigonadal, and perirenal) for histological, molecular and functional analysis.

Results: At 200 days (after 12 weeks with HFD) we observed clear changes in the content and morphology of brown adipose tissue; weight of interescapular adipose tissue was significantly decreased in the CPS group with clear differences in the histology, while the inguinal adipose tissue was significantly increased. Thus, animals gestated under CPS conditions presented a higher response to IGTT and IITT vs LD, whit an increase of body weight without food intake increase.

Conclusion: The present findings support that gestational chronodiruption programs impaired metabolic responses in later life, increasing the risk of metabolic syndrome and T2-DM.

(Sponsored by ANILLO ACT-1116, FONDECYT-1120938, FONDECYT-11500069).

The role of masking in the impact of a running wheel during circadian disruption.

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Our prior studies have supported the hypothesis that access to a running wheel can impact response to a light cycle that induces circadian disruption. We conducted a study to determine if lifetime access to a running wheel changed the impact of wheel access on response to LD10:10. We tested both male and female mice (n=23), randomly assigning littermates to be reared with or without a running wheel. At age 7-8 mos we exposed them to LD10:10 for 5 weeks. We then tested response of these mice to a short period of constant darkness (8 days), and to a LD 3.5:3.5 cycle for 9 days designed to assess masking effects of light. Masking responses measured under LD3.5:3.5 were related to the strength of the 20 h rhythm in activity records measured during LD10:10. Lifetime access to a running wheel was associated with a sex difference that was not observed in mice naïve to the running wheel. Female mice with lifetime wheel access all showed stronger masking to light than did males with similar access. These results indicate that both sex and prior history influence masking responses, and that mice can show apparent entrainment to a 20 h light cycle mediated in part through masking effects.

Measuring circadian bioluminescence from freely behaving mice.

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Circadian disruption impacts health by affecting interactions between peripheral and central clocks. To study the dynamics at a system level, methods to measure circadian rhythms of peripheral organs in intact animals are required. We are developing a method to measure bioluminescence from tissues of freely moving mice carrying the $Per2^{Luc}$ gene. Using equipment designed by David Ferster (Actimetrics), two photomultiplier tubes are positioned above the mouse cage, housed within a light-tight box. Monitoring the level of bioluminescence from firefly luciferase in these mice reports on the circadian rhythm of expression of the PER2 protein. Delivery of a substrate for luciferase was the focus of our initial experiments. First, we compared bioluminescence from PER2: LUC in mice with either D-luciferin or the synthetic luciferin analogue CycLuc1 delivered in the drinking water. Although this was an easy method of delivery, and we could detect bioluminescence over a range of doses, the drinking rhythms complicated the measurement of circadian rhythms of gene expression. To avoid this interference, we then tested substrate delivery using osmotic mini-pumps carrying solutions of varied doses of the synthetic luciferin CycLuc1. Circadian rhythms in PER2: LUC can be measured for up to 28 days using this approach. This work thus sets the stage for the detection of circadian rhythms of gene expression in freely-behaving mice.

A neuropeptide that plays a fundamental role in the clock neurons of *Drosophila melanogaster*.

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Ventral lateral neurons (LNvs) are essential in the control of rest-activity rhythms in *Drosophila melanogaster.* A few years ago our lab reported that these clock neurons undergo circadian remodeling of their axonal projections, a phenomenon called circadian structural plasticity (Fernández et al., 2008). Axonal arborizations display higher complexity during the day than at night, and this remodeling involves changes in the degree of synaptic connectivity (Gorostiza et al., 2014). Circadian remodeling of adult projections relies on activity-dependent and -independent mechanisms and involves changes in the degree of fasciculation, neurite outgrowth and pruning (Depetris-Chauvin et al., 2011;Sivachenko et al., 2013;Depetris-Chauvin et al., 2014; Petsakou et al., 2015). Interestingly, late at night, active pruning appears to take place, and as a result primary neurites are significantly shortened, underscoring that the most dramatic remodeling takes place around dawn (Gorostiza et al., 2014). Additionally, PDF *per se* appears to play a role in circadian remodeling, since chronic yet adult-specific downregulation of *pdf* levels impairs structural plasticity (Depetris-Chauvin et al., 2014).

In this work we characterize in more detail the role of the PDF neuropeptide. Through RNAi-mediated silencing at different times along the day, we explored a time-of-day dependence to PDF contribution to this form of plasticity. Our results provide additional evidence to understand the tight relationship between these two circadian outputs.

Do the muscarinic M1 receptors of the suprachiasmatic nucleus modulate differentially the ovulation during the morning and the night?

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Ovulation depends on the convergence of endocrine and neuronal signals, which follow circadian patterns directed by the suprachiasmatic nucleus (SCN). Lesion of the SCN, the main oscillator of the mammalian circadian system, results on the suppression of cyclic release of gonadotropin releasing hormone (GnRH) and the blockade of ovulation. The SCN is a pivotal element on the neuroendocrine machinery governing GnRH preovulatory surge; additionally the participation of the cholinergic system in this regulation change throughout the hours and day of the. The systemic blockade of muscarinic receptors inhibits ovulation following a daily pattern with maximum effects when the drug is injected before the GnRH surge. It has been shown that the unilateral microinjection of pirenzepine (an antagonist of M1 receptor) on the left SCN at 09:00 h of metaestrous stimulate the ovulation ($p< 0.0357$) while in the right side of the SCN, the same treatment blocks the ovulation ($p< 0.0036$). To analyze if the M1 receptor has a pattern of circadian activity and if such activity depends on the side of the SCN, we implanted a guide cannula targeting the right or left side of the SCN in female rats. After two weeks of recovery, the animals were microinjected with 0.3 µl of pirenzepine at 21:00 h on metaestrous; in the next predicted estrus animals were sacrificed. The presence of ova in the oviducts was evaluated.

(Supported by CONACyT 236908).

Electrophysiological evidence that the Retrosplenial Cortex Displays a Strong and Specific Activation Phased with Hippocampal Theta during Paradoxical (REM) Sleep.

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It is widely accepted that cortical neurons are similarly more activated during waking and paradoxical sleep (PS; aka REM) than during slow-wave sleep (SWS). However, we recently reported using Fos labeling that only a few limbic cortical structures including the retrosplenial cortex (RSC) and anterior cingulate cortex (ACA) contain a large number of neurons activated during PS hypersomnia. Our aim in the present study was to record local field potentials and unit activity from these two structures across all vigilance states in freely moving male rats to determine whether the RSC and the ACA are electrophysiologically specifically active during basal PS episodes. We found that theta power was significantly higher during PS than during active waking (aWK) similarly in the RSC and hippocampus (HPC) but not in ACA. Phase–amplitude coupling between HPC theta and gamma oscillations strongly and specifically increased in RSC during PS compared with aWK. It did not occur in ACA. Further, 68% and 43% of the units recorded in the RSC and ACA were significantly more active during PS than during aWK and SWS, respectively. In addition, neuronal discharge of RSC but not of ACA neurons increased just after the peak of hippocampal theta wave. Our results show for the first time that RSC neurons display enhanced spiking in synchrony with theta specifically during PS.We propose that activation of RSC neurons specifically during PS may play a role in the offline consolidation of spatial memories, and in the generation of vivid perceptual scenery during dreaming. Key words: anterior cingulate cortex; gamma; hippocampus; retrosplenial cortex; sleep; theta

(CNRS, Societé Francaise de Recherche et Médecine du Sommeil, University Claude Bernard of Lyon, CAPES/COFECUB, CNPq and FAPESP.)

Circadian responses of the kissing bug *Triatoma infestans* to nocturnal and diurnal vertebrate hosts.

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Insects express diverse behavioral rhythms synchronized to environmental cycles. Whereas circadian entrainment to light-dark cycles is ubiquitous in living organisms, entrainment by non-photic cycles is critical for hematophagous bugs that depend on rhythmic hosts. *T. infestans* feed on vertebrates with different temporal patterns during each host's quiescent time. This implies that synchronization with the host's behavior is important for the insects' survival. Kissing bugs were housed in a compartment in constant dark, air-flow-connected to another compartment with a nocturnal rodent or a diurnal bird synchronized to a light-dark cycle. The activity rhythms of kissing bugs were modulated by the daily rhythms of the hosts. The effects were concentration of activity during the hosts 'sleep phase, relative coordination and decrease of endogenous period. Splitting and bimodality were additionally observed and also affected by the host presence. *T. infestans* were able to differentiate the active and inactive phases of their potential hosts, an ability that surely facilitates feeding and hinders predation risk.

(Sponsored by CONICET and ANPCYT from Argentina and NSERC from Canada).

Chronobiology and Music Therapy. A Composition of Rhythms.

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This research is an interdisciplinary dialogue between Chronobiology and Music Therapy, science that uses sounds and musical rhythms to accomplish therapeutic goals, to evaluate musical experience's considering biological rhythms and its interaction with social, parental, cultural and school rhythms.

The search for studies relating endogenous and exogenous rhythms involved factors to playing music in childhood showed unfruitfulness, so the purpose is to clarify to what extent exogenous rhythms influence children's biological rhythms and their musical experience in early ontogenetic development.

The sample consists of 38 male and female children from 3 to 6 years old wearing wrist actimeters for 4 consecutive weeks to detect motor activity, wrist temperature and light exposition; sleep and activities diaries will also be applied as the children chronotype questionnaire; a software (Genvirtual) will be used to evaluate musical experience.

Our hypothesis is that the exogenous rhythms will act as synchronizing agents of biological rhythms, which may change the perception of music, allowing Music Therapy to be planned in association with these rhythms.

mRNA cytoplasmic granule oscillations in *wt* and *Bmal1*$^{-/-}$ fibroblasts.

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Stress granules (SGs) are microscopically visible aggregates of messenger ribonucleoproteins that assemble when cells undergo stress. In these membraneless organelles the mRNA can be stored for being translated again later when stress is relieved. Processing bodies (PBs) are also cytoplasmic mRNA granules enriched in factors involved in transcript degradation, storage and translational repression. Considering that the stress response is circadianly regulated in several organisms, and that we found in circadian expression databases that some components of SGs and PBs fluctuate over time, we hypothesized that these foci oscillate. We show that the number and area of SGs induced by oxidative stress, as well as the PB number, exhibit daily oscillations in NIH3T3 cells. TIA-1, a protein with a prion-like domain that induces SG nucleation, is also expressed rhythmically. To test whether SG temporal changes were controlled by the transcriptional translational feedback loops (TTFLs) that form the molecular circadian clock, we analyzed SGs in *wt* and *Bmal1*$^{-/-}$ fibroblasts. Unexpectedly, we found oscillations in the number, area and signal intensity of SGs in both genotypes. The period and phase of the oscillations were similar in both cell lines, but the amplitude was higher in *Bmal1*$^{-/-}$ cells, suggesting that the TTFLs modulate the strength of the response at different times. We thought that the SG rhythms could be generated by redox or translational rhythms that have been shown previously in *Bmal1*$^{-/-}$ cells.

(Sponsored by The Authors Are Grateful To Dr. JS Takahashi (UT Southwestern Medical Center, USA) For The MEF Cell Lines; ANPCyT-FONCyT, CONICET, SECyT-UNC).

Effects of urbanization on health, sleep and metabolic parameters in a Amazonian population.

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Background: The urbanization process has been associated with an increase in risk factors to non-communicable diseases.

Aim: To describe the main differences in nutritional state, metabolic parameters and sleep in urban and rural residents of an Amazon community.

Methods: a cross-sectional study of rural and urban residents (22 and 20, respectively) from the municipality of Xapuri, Acre. Sociodemographic, life habits, anthropometric and metabolic parameter variables (triglycerides, total cholesterol and fractions, fasting glucose and insulin resistance) were evaluated. Sleep variables were obtained by actigraphy and daily activity protocols during 10 days. The studied groups were compared with Student's t and Mann-Whitney tests for anthropometric variables and metabolic parameters. ANOVA for repeated measures tests were performed to compare the sleep variables between groups.

Results: Urban residents showed higher averages to all anthropometric variables, fasting glucose levels, fasting insulin and insulin resistance ($p<0.05$) when compared with rural residents. The lipid profile showed no statistically relevant differences among the groups. Rural residents showed higher averages of sleep length ($p<0.01$) and earlier sleep onset ($p=0.01$). Nevertheless, there is a higher number of awakenings ($p<0.01$) and wake after sleep onset ($p=0.01$), which suggests worse sleep quality when compared to urban residents.

Conclusion: the findings show an association between urbanization and the presence of risk factors like overweight, serum lipid level alteration, and insulin resistance. However, the results are in accordance with the literature when it comes to a worse sleep quality among rural communities.

Keywords: urbanization; non-communicable diseases; metabolic parameters; sleep; rural communities.

(Sponsored by CAPES; FAPESP 2016/09582-0; PPSUS/FAPAC 00011906; University of Surrey).

Time restricted feeding without calorie restriction elicits circadian and metabolic adaptations independently of beta-hydroxybutyrate enhancement.

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Peripheral clocks are sensitive to rhythmic external cues that influence numerous rhythmic cellular functions at various stages of regulation. Time restricted feeding (TRF) with caloric restriction (TRF-CR) improves metabolic health through synchronization of the circadian clock and modulation of metabolic networks. Access to food for 2 h in rats induces food anticipatory activity (FAA) in the 2-3 h preceding food access, whose intensity correlates with the degree of caloric restriction. This behavior has been attributed to a beta-hydroxybutyrate (BHB) signaling from the liver to the central nervous system. The aim of this study was to investigate if TRF requires CR to elicit metabolic adaptations with a concomitant increase of BHB. Adult male Wistar rats with food access at daytime for 2h (TRF-CR), for 5h, without CR, (TRF) every day or *ad libitum* feeding (AL) were maintained in a 12:12 h light-dark cycles for 3 weeks. Synchronization was observed by the presence of FAA in both TRF groups with similar total activity and a shift phase of Per1 and Bmal1 genes in liver comparing with AL. Glycemia and insulin levels were similarly modulated by both TRF. Although TRF-CR displayed less white adipose tissue weight, both TRF groups induced a peak of free fatty acids before food access. Importantly, unlike TRF-CR, TRF increased the oxidized ketone body acetoacetate, with no induction of BHB. These results indicate that TRF with no CR elicits metabolic changes through synchronizing the circadian clock independently of BMB signaling.

(Sponsored by DGAPA-PAPIIT, UNAM (grant IN202515), México.)

Comparison between two one-night sleep restriction strategies.

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The structure of sleep may undergo changes in consequence of sleep deprivation, such as an increase in slow wave activity, as a response to sleep pressure accumulation. However, it is not clear if those changes occur due to sleep duration restriction only or if it could be a result of sleep onset and offset times. Thus, the aim of this study was to verify if a morning or evening sleep restriction protocol could affect sleep pressure, instead of sleep duration restriction itself. The sixty-two volunteers (mean age 22.95±4.13), all with intermediate chronotype, had their sleep-wake cycle monitored by actigraphy during the week preceding the experiment. On the day of the experiment, the volunteers had the opportunity to take a 90-min nap, while monitored by polysomnography. A simple regression analysis was applied between the actigraph variables (onset and offset time, sleep duration of the night preceding the experiment) and delta and slow oscillations bands, followed by the construction of hierarchical models for those analyses that showed significance. The results show that the sooner the sleep offset occurs, the higher is the spectral power of slow oscillations ($p<0.001$) and delta ($p=0.002$) bands, whereas onset time does not have any influence (delta: $p=0.283$; slow oscillations: $p=0.548$). In conclusion, the best strategy for intermediate chronotypes would be to delay the onset of sleep instead of advance the offset time, in order to decrease the sleep homeostatic pressure and its effect over cognitive faculties.

(Sponsored by CNPq, CAPES).

Graphical representation of oscillations of clock genes and adjustment with the Cosinor method implemented in Octave.

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The data presented here come from the offspring of female rats that were exposed to different light-dark cycles during gestation, keeping temperature constant and food ad libitum. These treatments cause variability in gene expression that needs to be properly quantified to interpret the information clearly and effectively. Several algorithms were implemented in the Octave open source software for the circadian cycles search. The mathematical model followed is Cosinor, this implementation allows us to work properly with few and unequally spaced data, like the data that we found in our terminal protocols. This basic model allows determining the period, amplitude, phase and mesor of the signal. In addition, the parameters obtained support the quality and robustness of the model.Since physiological oscillations in individuals are more complex than simple cosines, we are working on their simulation to extend the treatment to multi-frequency signals and other developments from adjustments of non-cosine waveforms.

(*Sponsored by* FONDECYT 1150789, ANILLO ACT-11169).

Studies on the molecular clock and the circadian regulation of hepatic tumor cell metabolism.

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The circadian system comprising oscillators present in organs, tissues and even in individual cells temporally controls the body physiology. The clock liver regulates the metabolism under a circadian base mainly depending on feeding times; however, it is poorly understood how liver works under a malignant growth. The aim of this project was to investigate the molecular clock work and its linkage with the lipid metabolism in cultures of the human hepatoma cell line HepG2. We performed this study under two proliferative states, partial arrest and proliferation, maintaining cells with 0 or 5% of serum respectively after synchronization with dexamethasone (100 nM). We analyzed the expression of clock genes (CGs) (*Bmal, Per, Cry*), clock controlled genes (CCGs) (*Rev-erb*) and the main enzymes involved in the glycerophospholipid (GPL) biosynthesis (*Chka*, *Pcyt2, Pemt*) at the mRNA level by qPCR, and presence and distribution of BMAL1, PER1 and CHKproteins by immunocytochemistry. The mRNAs for most genes evaluated showed temporal oscillations under proliferation while no significant differences were observed in arrested cells except for *Pcyt2.* In addition, we studied the endogenous content of GPLs in proliferation and observed significant variations in the total and individual GPL level content at different times tested. These studies suggest that an active time-dependent control of gene expression and metabolism takes place in proliferating HepG2 cells.

(Sponsored by CONICET, ANPCyT-FONCYT, SECyT-UNC).

Unpredictable feeding schedules effect on despair and experimental anxiety in the Wistar rat.

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Food is an important synchronizer of circadian rhythms and it is probable that its ingestion at unpredictable schedules causes internal desynchronization with consequences similar to those generated by the lag of the sleep-wake cycle. In this study we evaluated the unpredictable feeding schedules effect on despair and experimental anxiety in the Wistar rat. Females and males adult Wistar rats were divided into four experimental groups: 1) AL with feed*ad libitum*; 2) RFN with food availability from 21:00 to 23:00 h (ZT5-ZT11); 3) RFD with food availability from 09:00 to 11:00 h (ZT17-ZT19), and 4) RFU with access to food 2 hours a day with variable schedules each day. This experimental manipulation lasted 28 days, under a cycle of light-darkness 12:12 and water*ad libitum*. On day 29, the animals were submitted to elevated plus maze, open field test, andforced swim test. No modification was found in locomotor activity in any of the groups. The results indicate that the RFU female and male groups had higher anxiety rates. However, AFD and AFN female groups showed higher anxiety rates than AL female group. In other hand, the RFU females had a longer immobility time and shorter swim time with respect to the other groups, but the males showed no differences in any parameters of the forced swim test. It can be inferred that feeding at unpredictable schedule can generate an anxiety state in both males and females rats but induce a despair state mainly in females.

(PRODEP DSA/103.5/16/9780)

Sleep accelerates memory re-stabilization.

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Consolidated memories can be reactivated by the presentation of a memory-cue (reminder) returning to a labile state followed by a process of re-stabilization known as reconsolidation. Thus, if amnesic agents are presented inside the reconsolidation time window (when the memory is still labile) the memory is impaired. However, if they are presented outside (~6 hours after reminder presentation), it has no effect on re-stabilization. Sleep is known to support the consolidation of newly encoded memories and it is also suggested that sleep has a beneficial effect on reconsolidation. Here we ask whether sleep accelerates re-stabilization of consolidated memories protecting reactivated memories from interferences. Participants learned a list of non-sense syllable pairs on Day 1. On Day 2, they received a reminder and they were allowed to sleep a 90 min diurnal-nap or they stayed awake for the same period of time or for 10 hours. After that, they received an interference task (new list of syllables). We found that the memory performance was impaired only when the interference task was given 90 min after the reminder (inside the time window of reconsolidation). There was no impairment when it was given after 90 min sleep or 10 hours after the reminder presentation (outside the reconsolidation time window). This finding suggests that a short-nap after reactivation during wakefulness accelerates memory re-stabilization.

Blame it on the sunshine?: Differential thermoregulatory and inflammatory patterns in the circadian response to LPS-induced septic shock.

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Septic shock is a lethal condition caused by a pathogen-induced chain of events. In 1960, Halberg et al, reported a susceptibility rhythm to lipopolysaccharide (LPS) - induced septic shock, which showed that the same dose of LPS which is compatible with survival at ZT19 can be lethal at ZT11. Then, Hrushesky, et al (1994) observed the same effect with Tumor Necrosis Factor-α (Tnf-α) administration. Moreover, mice that lack Per2 are more resistant to this condition (Liu, et al. 2006).

In this study, we aim to further characterize the circadian response to high doses of LPS in mice. First, we measured skin temperature of animals injected with LPS at both times and we found that there was a higher decrease in mice injected at ZT11 than at ZT19. We analyzed neuronal activation by cFos immunoreactivity in the preoptic and paraventricular nucleus of the hypothalamus, brain regionsassociated with thermoregulation and neuroendocrine, autonomic and immune control, respectively. We found that at both brain regions cFos immunoreactivity was significantly higher after LPS administration at ZT11 than at ZT19.Furthermore, we found that levels of Tnf-α in serum were higher in animals injected at ZT11, whereas Tnf-α mRNA expression was higher in the liver of animals treated at ZT19. Moreover, both dendritic and macrophage cell activation in spleen was higher after treatment at ZT19, as well as peritoneal macrophage activation. These results suggest a circadian dependency of the central thermoregulatory and peripheral inflammatory response to septic-shock.

(Sponsored by: Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET). Agencia Nacional de Promoción Científica y Tecnológica. Universidad Nacional de Quilmes)

Deconstructing the transcriptional compensatory system of the *Neurospora crassa* circadian Clock.

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All organisms in which circadian clocks have been characterized exhibit a common molecular circuit, which is based on a positive element able to activate the expression of a negative one that then represses the action of the former, inhibiting its own expression. This system is capable of sustaining oscillations under several external perturbations, but the compensation mechanisms remain unknown. Studies in mammals, Drosophila and Arabidopsis have shown some new transcriptional inputs, supporting the central molecular circuit, by modulating the expression of some of the core-clock components (CCC). In Neurospora crassa the clock central circuit has been well characterized but the participation of other transcriptional networks (TN) in this system are still unknown. In our lab, we are trying to identify these novel TNs controlling the CCC in N. crassa. Thus, we have defined a set of transcriptional regulators (TR) that modulate circadian features. In addition, we have dissected the transcriptional units of the molecular circuit, trying to identify cis-elements impacting the robustness of the system. Finally, combining the results of both strategies with information of DNA binding preference for over 50% of the TRs available in N. crassa, we constructed a Global Transcriptional Network, with the TR interactions and their possible target genes. Arranging all the information in a comprehensive way, we are starting to decode the TNs behind the central circadian oscillator of Neurospora and the perturbations to which this compensatory system is capable of responding.

(Sponsored by FONDECYT 1171151.MN-FISB and HHMI International Research Scholar program).

Modeling the effects of translational repression on the molecular circadian clock.

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Organisms have evolved in a daily cyclic environment and, as consequence, developed circadian cell-autonomous clocks that temporally organize a wide range of biological processes. Transcriptional regulation is only one of many layers of circadian regulation of gene expression. Indeed, translation is also a highly regulated process that impacts on the molecular circadian clock dynamics. Translational activation of clock transcripts has been described for flies and mammals and we have previously reported the dynamical changes of the clockwork using a basic core clock theoretical model. Translational repression, on the other hand, is a much less understood process, mainly associated to the activity of microRNAs (miRNAs) at the initiation step of translation. In this work we use a theoretical model of the circadian molecular clock to study the effect of translational repression on the molecular clock dynamics. Because translational repression impacts on the translational kinetics and regulation by miRNAs establishes a threshold level of target mRNA below which protein production is highly repressed, our approach has been to model the translational repression by introducing a threshold to the kinetics of translation. We show how the parameters associated to translation kinetics affect the period, amplitude and waveform of oscillations. We also characterize the time delays between clock mRNA and clock protein expression. Taking into account all these results we propose that a slight translational repression favor the emergence of circadian molecular oscillations.

ZebTrack: Free Tracking Software for animal behaviour.

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Motion detection and tracking in animal behavior studies can be performed using several techniques. One of the most used is video tracking, since it provides a ground truth and can extract several animal behavior data, such as distance traveled, velocities, rest times, etc. However manual video analysis is an exhausting and error prone task. To avoid such problems there are several softwares that perform automatic video tracking. Most of them are paid softwares that cannot be customized.

This work presents an automatic, free, and open source video tracking software, developed in Matlab, called ZebTrack. It was developed to track fish, especially the Zebrafish, howeverit can be used to track several other animals. For now, ZebTrack does not work at real time, using a video recording of the experiment. The software allows the definition of polygonal areas such as a processing area, excluding areas and areas of interest. It produces a report regarding the whole experiment including data such as animal position and velocities, rest times, statistics regarding the areas of interest (number of times each animal entered the area, time spent inside the area, etc...). The produced data can also be manipulated and analysed, afterwards, in Matlab environment, or exported to Excel format. The user may add functionalities to the software as it is open source.

ZebTrack is being used successfully to track fish by psychobiology laboratory Luchiarilab, at UFRN. The software is available for free download at www.luchiarilab.com

Genetic–by-prenatal-nutrition interactions in sleep behavior and brain morphology in *Drosophila*.

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Sleep can be defined as a reversible state of inactivity, which is controlled by circadian and homeostatic factors, and is conserved across most animal species. The relation between nutrition and its effects in sleep has been studied in both vertebrates and invertebrates. In *Drosophila*, starvation leads to suppression of sleep, however it is unknown whether early-life nutrition during larval stages affects sleep variation and to what extent it is influenced by brain morphology variation. In *Drosophila*, sleep is controlled by specific structures of the brain, including integrative areas such as the mushroom bodies (MBs). Variation in MBs has been correlated with variation of sleep, suggesting a morphological basis of sleep variation. Here, we characterized sleep and brain morphology in the wild-derived *Drosophila* Genetic Reference Panel (DGRP) that were raised under prenatal nutritional restriction (NR). We observed abundant genetic variation in brain morphology and sleep. We performed a genome-wide association (GWA) and identified candidate single nucleotide polymorphisms (SNPs) that are potentially associated with natural variation in the sensitivity of sleep traits to nutrition. A subset of these SNPs is located within genes previously known to affect *Drosophila* sleep and brain development. We found significant correlations between brain morphology and sleep variation that are sensitive to NR. These results shed light on how the interaction between prenatal nutrition and the genome may result in developmental and brain functional adaptations that shape sleep behavior during adult life.

(Supported by ANILLO ACT-1401 and ICM P09-015F).

Re-evaluating the roles of Protein Kinase A (PKA) and cAMP signaling in circadian core-clock mechanisms.

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In *Neurospora crassa* the core-clock oscillator is composed of the transcription factors WC-1 and WC-2 (WCC), which positively regulate the expression of the negative element FREQUENCY. The latter, in association with an RNA helicase (FRH) inhibit WCC activity in a daily manner. Several kinases and phosphatases played critical roles in the mechanisms of WCC inactivation and FRQ maturation impacting, therefore, clock function. As part of a large genetic screen, we have re-examined the importance of one of these kinases: PKA (cAMP dependent Protein Kinase A).

In Neurospora, PKA and cAMP play a central role in the regulation of developmental processes such as conidiation, therefore overt rhythms in conidiation (which is under clock control in this organism) cannot be easily analyzed in PKA deficient strains. We have utilized bioluminescent real-time reporters to analyze clock function in different mutants associated with cAMP signaling, including PKA deficient strains. These analyses have revealed rather WT circadian rhythms in each case, indicating that the “canonical” cAMP signaling is not necessary for the generation/maintenance of circadian rhythms. Using the same approach we evaluated the effect of caffeine, theophylline and aminophylline: inhibitors of phosphodiesterase (PDE). As in previous reports, we observed that all these drugs led to significant period lengthening. Remarkably, this phenotype was still observable in all of the analyzed cAMP signaling pathway mutants, suggesting that these PDE inhibitors lead to circadian phenotypes through mechanisms different from the canonical PDE-cAMP-PKA axis.

(Sponsored by FONDECYT 1171151.MN-FISB.HHMI International Research Scholar program).

Temporal distribution of the hospitalization of patients with bipolar disorders in a psychiatric hospital.

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Bipolar disorder is a serious health condition characterized by the occurrence of episodes of depression, hypomania, mania or mixed pictures with possible seasonal presentation. This study aimed to analyze the temporal distribution of hospitalizations of patients with bipolar disorder associating it with socio-demographic, clinical, climatic and astrophysical variables. It is a descriptive, retrospective, cross-sectional, documentary and analytical study. Data from individuals with bipolar disorder were analyzed in the registry of hospitalizations of a psychiatric hospital between January 1, 1980 and December 31, 2015, totaling 36 years (432 months). During the period, 1933 hospitalizations for bipolar disorder were registered among a total of 37,151 hospitalizations in the institution. There was a significant association between the seasons of the year and the number of hospitalizations due to bipolar disorder, in general ($p = 0.001$), as well as hospitalizations for manic episodes ($p = 0.009$). Depressive episodes and other episodes were not significantly associated with the seasons. For all episodes, it was observed a lower number of hospitalizations in the summer, and an increase in admissions for episodes of mania in winter. There was also a significant low-magnitude correlation between the number of hospitalizations due to bipolar disorder and astrophysical variables, namely: solar irradiance (coefficient $R = -0.14$) and number of sunspots (coefficient $R = -0.17$) . The pattern of seasonality evidenced in this study may contribute to the design of strategies for prevention and improvement of care.

Nocturnal use effect of smartphone on sleep-wake cycle, autonomic response and morning cognitive processing of university adolescents.

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Adolescents' phase delays in relation to bed- and wakeup times combined with increased media use may result in excessive exposure to light at night, irregularity in sleep onset and offset times, sleep deprivation and poor sleep quality in adolescents who study in the morning. Studies pointed an effect of sleep deprivation over the basic cognitive processes that regulate the performance, such as attention, working memory, inhibitory control and cognitive flexibility. On the other hand, emerging researches suggest that performance and sleepiness are not only regulated by circadian and homeostatic processes, but could be influenced by a compensatory effect. During high homeostatic pressure, caused by sleep deprivation, an increase in sympathetic tonus, through the hyperactivation of *Locus Coeruleus* would act as a counter effect to the damages caused by sleep loss. This work aims to evaluate the impact caused by nocturnal use of smartphones on sleep parameters and how this influence the cognitive performance and the autonomic response to verify if there is a hyperactivation of the autonomic nerve system that possibly blocks the sleep deprivation's effects. For this, freshmen of UFRN's undergraduate courses will complete questionnaires on socioeconomic status, nighttime smartphone use, chronotype, sleep quality, daytime sleepiness with a follow-up for 10 days of sleep diary and actimeter. Finally, the autonomic responses (EEG, ECG, ERP and electrodermal activity) will be recorded during cognitive tests at the usual class start time (7:00h).

Alterations in circadian and neural functions in a *Drosophila* model of autism spectrum disorder.

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Autism spectrum disorder (ASD) is a neurodevelopmental disorder characterized by deficits in social communication and repetitive and stereotyped interests and behaviors. Circadian and sleep irregularities are also often observed in children with ASD, including abnormalities in the timing of melatonin secretion, prolonged sleep latency, decreased sleep efficiency, and reduced total sleep time, all of which worsen the behavior of children with ASD. Here, we used *Drosophila* to evaluate the function of genes associated with autism in humans by testing their role in the control of sleep and the circadian clock, and in the control of complex social behaviors such as courtship. We find that RNAi-mediated knockdown of the *Drosophila* homolog of human autism candidate genes significantly affects the amount of daytime and nighttime sleep, the duration of sleep latency, and cause a lengthening of the periodicity of the circadian rhythm of adult locomotor activity, as well as disruptions in the courtship behavioral sequence. Our result may provide insights into the behavioral and circadian abnormalities observed in ASD patients and also provides new research avenues for the field.

(Sponsored by FONDECYT POSTDOCTORAL Grant 3160177 (to Angelina Palacios-Muñoz), FONDECYT Grant 1141278 (to J.E.), CINV Millennium Institute Grant P09-022-F, supported By The Millennium Scientific Initiative Of The Ministerio De Economía, Fomento Y Turismo (to J.E.).)

Sleep differences between sated and food restricted rats after shaping an operant behavior.

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Objectives: Investigate whether food restriction protocols alters the normal sleep distribution, and compare the performance in the shaping and acquisition of an operant behavior in sated and food restricted rats.

Methods: 20 young male Sprague-Dawley rats were divided in two groups: Sated and Restricted (food deprivated up to 80% of their weight). For monitoring sleep EEG, rats underwent a surgery in which cortical and muscular electrodes were implanted. The shaping phase was carried out through a continuous reinforcement schedule for both groups. We recorded 3 hours of sleep after the shaping phase. Sleep parameters were compared between groups, and between experimental phases.

Results: Both groups learned the operant behavior, with no significant differences between them. Sated rats spent more time in REM and NREM sleep in the hours subsequent to training in comparison to the food restricted rats. Also, through the analysis of NREM's delta activity, we confirmed that delta build up shows significant differences between groups and between experimental phases. Intra-group comparison shows that delta build up after the shaping is greater than the non-training phase for the Restricted group; however, the Sated group showed a greater delta build up post-phase without training compared to shaping.

Conclusions: When sleep and learning experiments are conducted with food restricted subjects, these sleep variations should be considered. Food restriction is a widely used protocol in learning and memory research, which uses appetitive reinforcers to achieve a behavior so that the results obtained in those experiments could be masked by food restriction effects.

(Universidad de Chile, Comisión Nacional de Investigación Científica y Tecnológica (CONICYT), Instituto Milenio de Neurociencia Biomédica (BNI), Beca Puelma.)

Effect of academic demand on habits, sleep quality and cognitive performance at morning in college adolescents.

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The phase delay observed in adolescence tends to exacerbate after the admission of young people to the university, due, among other factors, to the increase in academic demand, especially at the end of the semesters. In consequence, the students who study in the morning can show irregularity in sleep and wake-up times, shorter duration and worsening of sleep quality, as well as impairments in the working memory. Given the importance of this memory for the acquisition of learning, the present study proposes to assess the influence of the academic demand during the first period, on the habits and quality of sleep and the impacts of these on the working memory at morning in college students. To characterize sleep habits and quality, participants will answer the questionnaires: \"Health and Sleep\", \"Pittsburgh Sleep Quality Index\" and \"Sleep Diary\" as subjective measures; and will use an actiwatch as objective measure for 10 days, along with Sleep Diary. The working memory will be measure through a task that evaluates both the phonological and visuospatial components (Ramirez, *et al*., 2006). The data collect will be performed in two phases: at the beginning and end of the first semester, and the cognitive performance task will be applied at 7:00h, that is the usual class starting time.

Long-term effects of gestational chronodisruption on the immune system of adult offspring.

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Our modern 24/7 society, artificial light and the re-structuring of work times has progressively detached us from normal cycle of light and dark, resulting in disruption of the circadian rhythm. Studies has demonstrated that chronodisruption has adverse effects on health. On the other hand substantial epidemiological, cellular and molecular evidences indicate that conditions during the intrauterine life plays a critical role on the homeostasis of immune system, and the environment of mother is an important factor that can influence the susceptibility to immunological disorders in the adulthood. The aim of our study was determine if gestational chronodisruption, induced by alteration of the photoperiod during pregnancy, represent another case of adverse gestational environment that alters the immune response in the progeny. We analyzed the effects of chronodisruption on the expression of clock genes in spleen and adaptative-immune response after an allergic challenge in adult offspring from rats exposed to constant light along the second half of pregnancy (LL) relative to animal gestated in normal photoperiod (LD). The expression of clock genes in the spleen of adult rats was affected by gestational chronodisruption, leading to alteration of amplitude, mesor and/or acrophase of the circadian rhythm of clock genes *Bmal1, Clock, Cry1, Rev-erb*α and *RoR*α. On the other hand, LL animals exposed to an allergic challenge during adulthood demonstrated a greater sensitivity to Ovalbumin (OVA). Levels of OVA-specific IgG and subclass IgG1 was higher in LL animals. Additionally, total IgE and OVA-specific IgE increased after the allergic challenge only in LL animals.

(Sponsored by FONDECYT 1150789, ANILLO ACT-1116, Beca Doctorado Nacional Conicyt, DID Uach, Escuela Graduados Facultad Ciencias Veterinarias.)

Light-induction of the enzyme Aralkylamine N-acetyltransferase (AANAT) in the chicken inner retina and its potential physiological role.

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The neurohormone melatonin is synthesized from serotonin through two steps of which serotonin is converted first to N-acetyl-serotonin (NAS) by the enzyme Aralkylamine N- Acetyltransferase (AANAT). AANAT is present mainly in the pineal gland, retina and other regions while NAS can activate the TrkB receptor to generate neuroprotective effects and neurogenesis. Melatonin synthesis is controlled by light (L) and the circadian clock. In photoreceptor cells, AANAT activity peaks during the dark and at subjective night while activity is significantly decreased by L exposure. By contrast, melatonin synthesis, AANAT expression and activity are high during the subjective day or L phase in chicken retinal ganglion cells (RGCs) (Garbarino et al 2004). Here we investigate the expression of AANAT and of nonvisual opsins in highly enriched RGC cultures obtained from embryos by a discontinuous BSA gradient, and exposure to different L conditions. Cultures expressed melanopsins, Opn3 and Opn5 which may confer intrinsic photosensitivity. In fact, cultures exhibited blue L induction of AANAT immunoreactivity as compared with dark or red L treated cells. In addition, expression of this enzyme was significantly increased by forskolin (10 uM), an adenylate cyclase activator, in the dark. Results suggest that AANAT is a blue L-induced enzyme in RGCs controlled by cAMP. Further studies will investigate the cascade controlling AANAT expression in RGCs and its effects on retinal cells.

(Supported by ANPCyT-FONCyT, CONICET, SECyT UNC).

Sleep quality of an adolescent population in Bariloche, Argentina.

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Sleep disorders can be a negative factor both for learning as for the mental and physical development of adolescents. Thus, measurements of the quality of adolescent sleep can be useful to inform Public Health policies. We have conducted a study in the city of Bariloche, aimed at measuring the sleep quality of an adolescent population, as part of the activities of the Semana del Cerebro (March, 2017), the local version of the Brain Awareness Week. We used the Pittsburgh Sleep Quality Index (PSQI), obtained from a questionnaire administered individually to groups of secondary students. Some adults (visitors and teachers) were also given the questionnaire, thus providing a small adult sample. Participants were 523 adolescents (age 15–19) and 204 adults (age 20-69).

The results show that sleep quality is consistently worse for women than for men, in all age groups, and is worse for adolescents than for adults. The most important component to explain this is sleep dysfunction (i.e. daytime sleepiness). As there is no significant difference between the number of hours slept, this shows that adolescents should sleep more than adults (on average). In men, there is also a significant difference in sleep latency, which measures how fast the individual gets asleep. The difference between adolescent men and women is mainly due to larger sleep dysfunctions in women. We also found that sleep disturbances (“bad dreams”) are more frequently reported by women and have a negative correlation with PSQI.

Dissecting the circuits that control circadian oviposition in *Drosophila.*

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In Drosophila the circadian clock is controlled by interlocked transcriptional feedback loops present in neuronal and non-neuronal tissues. The oviposition or egg-laying behavior is a physiological process that is under the control of the circadian clock. The periodicity in oviposition is one of the less studied rhythms in *Drosophila* and many important aspects related with the role of the molecular clock remain to be elucidated. We study the molecular clock participation on egg-laying behavior by analyzing oviposition in control flies as well as in *per* and *tim* null mutants, which lack a functional clock. In contrast to controls, no rhythmic oviposition was detected in mutant flies. To test the neuronal control of oviposition, we downregulated *per* expression exclusively on neurons. Our results showed an arrythmic oviposition phenotype when *per* was downregulated in neurons. Furthermore, to elucidate the central vs. peripheral control of circadian oviposition, we downregulated *per* expression in the brain small lateral ventral neurons. Circadian oviposition was observed on mutant flies, indicating no role of these neurons on rhythmic egg-laying behavior. Further experiments will be designed to dissect the role and hierarchy of different central and peripheral neurons.

(Sponsored by Agencia Nacional de Promoción Científica y Tecnológica).

The influence of social jetlag in body mass index.

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Obesity and its comorbidities are in alarming growing rates, becoming a public health issue. Although it's already well stablished that the unbalance between caloric ingestion and physical activity is the main responsible for weight gain, the study of both ambiental and behavioral factors that may contribute for the problem is important. Among them is Social Jetlag (SJL) The objective of this study was to evaluate the correlation between SJL and body mass index (BMI) in young adults. University freshman students of different shifts and classes from Universidade Federal do Paraná (n=1158), answered a questionnaire containing answers about Life Quality (SF-36), Chronotiype (Morningness-Eveningness Questionaire, Munich Chronotype Questionaire), Sleep quality (Pittsburgh Sleep Quality Index) and personality (Big Five Inventory).They were also weighted and measured in order to accurate determination of BMI. Social jetlag and chronotype did not correlate with BMI. Sleep quality and total life quality did not correlate with BMI. In quality of life, the "general health state" was the only one negatively related BMI (R=-0,07). Conscientiouness was the only personality factor related to BMI (R=-0,08) People with high conscientiousness scores tended to present a lower BMI, maybe due to their capacity of maintaining a healthier diet. Even though literature shows positive correlation between SJL and BMI, in this sample that result could not be confirmed. We expect to find a positive correlation after the longitudinal waves of the project.

Cyber–systems for chronobiology.

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Laboratory behavioral research usually needs a system to control remotely and automatically the environment. Most laboratories use paid corporative software to record and display experimental variables such as time, intensity of light, among others. These softwares must be installed in a computer. Moreover, the assembly and maintenance of the system requires knowledge that often is unnecessary for the researcher.

This work presents an alternative system composed by sensors and actuators that are automatically detected by a wireless server, making the assembly, maintenance and expansion of the system easier. Besides that, the system makes possible data access and control from multiplatform devices like tablets, smartphones, computers, and others, by using a simple internet browser without any special plug-ins. The sensor data, for instance, luminosity or movement, are presented in real time and any actuation over the environment can be made remotely or automatically, using predefined rules.

In general, the developed system makes easy and intuitive assembling and adding new sensors (or actuators) to the system. It makes possible a remote data analysis, fail diagnosis and environment control in an easy way.

Eveningness, excessive body weight and circadian disruption negatively influence psychomotor performance throughout rotation of shifts: an observational and prospective study.

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Background: Rotating shift workers present a low quality and duration of sleep, a poor diet and a decreased performance.

Objective: To evaluate the influence of rotation of work shifts, chronotype, BMI, Interdaily Stability (IS) and Intradaily Variability (IV) on the lapses of attention throughout the alternation of work shifts.

Methods: The study included 30 rotating male workers. The working schedule was organized in the following sequence: two first days working in the morning shift (8:00a.m- 4:00p.m.); two days in evening shift (4:00p.m. – 0:00a.m.); one day in a break after evening shift; two days in night shift (0:00a.m. - 6:00a.m.); and 72 hours of rest. The preliminary evaluations involved a questionnaire with sociodemographic characteristics and health habits; Munich Chronotype Questionnaire (MCTQshift); evaluation of height, weight and abdominal circumference. The vigilance level was measured using the Psychomotor Vigilance Test (PVT) throughout the schedule shift. The use of actigraphy registered the rest-activity rhythm and measured the IS and IV. The number of lapses progression was evaluated using Generalized Estimating Equations (GEE).

Results: We found a significant effect of the shift (less lapses on day 1 than other days of schedule shift), chronotype (early and intermediate presented less lapses than the late), BMI (eutrophic presented a smaller number of lapses than excessive body weight), IS (synchronized presented a smaller number of lapses than not synchronized) and IV (little fragmented presented a smaller number of lapses than higher fragmented.

Conclusion: BMI, chronotype, lower synchronization and higher fragmentation influence on lapses.

Adolescent working memory assessment: Using an Android platform.

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Working memory (WM) is a complex cognitive component that allows store information during a short-time range in the brain. The prefrontal cortex plays a critical role in WM performance. This brain region is affected by sleep restriction. Several learning processes are linked to WM information storage, which exhibit a circadian pattern. The aim of this study was to develop an Android device App to evaluate WM in adolescents in their natural home environment to investigate effects of sleep restriction. Two WM tests, which have been widely used, were implemented: Auditory Sternberg Test (AST) and Visual n-Back Test (VBT). In a preliminary study, 43 students were submitted to both tests. In AST, hits and reaction time were obtained. In VBT, omissions, reaction time and false alarms were obtained. The results indicate that this is a useful tool in sleep and chronobiological field studies.

Time-of-day effects on declarative memory performance in children.

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Many factors can modulate the influence of sleep on memory consolidation. The expectancy that the information will be used in the future is one of them. Furthermore, human cognitive performance fluctuates over the day, but the literature on daily performance rhythms in children is scarce. Thus, this study examined whether learning a declarative memory task is affected by time-of-day in school-aged children; and if the future relevance of this learning is differently affected. Children were submitted to a modified version of the Visuospatial 2D Object-Location Task, which included cards tagged by their relevance. They were distributed into groups according to the time of day that they performed the learning session: 8 A.M (22 children, age mean 8.6 ± 1.4) and 16 P.M (16 children, age mean 8.4 ± 1.4). The performance was assessed by the number of correctly recalled cards during learning and the results were submitted to analysis of factorial Anova. There was a significant effect of time-of-day ($F = 4.1$; $p = 0.04$) and Fishers post hoc test showed that performance at 16 P.M was better than at 8 A.M ($p = 0.04$). However, there was no effect of tagging ($F = 0.2$; $p = 0.6$) or interaction between time-of-day and tagging ($F = 1.4$; $p = 0.2$). These findings suggest that learning a declarative memory task is influenced by the time-of-day that the children performed the task, independently if the information was tagged by their future relevance.

New functions for a core clock protein: examining the role of frequency in circadian regulation, nutritional sensing and stress responses in the plant pathogen *Botrytis cinérea.*

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Circadian clocks are molecular devices that allow organisms tons have been reported to vary daily in *Arabidopsis thaliana*. Although the plant–pathogen interaction is a two-sided story, nothing was known regarding circadian regulation of pathogenic traits. Thus, we characterized a functional circadian clock in the phytopathogenic fungus *Botrytis cinerea*. By using different plant and Botrytis clock-null mutants, we demonstrate that the interaction between this pathogen and its host varies with the time of day, being the *B. cinerea* circadian clock key in regulating this outcome. In Neurospora, the FREQUENCY (FRQ) protein is the main component of the circadian oscillator, role that is also conserved for the Botrytis orthologue BcFRQ1. Surprisingly, in this fungus this protein serves extra-circadian roles, as it plays a critical function in asexual/sexual decisions. Nevertheless, developmental phenotypes triggered by the absence of BcFRQ1 can be reverted by nutritional cues, placing this protein at the crossroad between circadian and metabolic regulation. Such studies can help understanding the evolutionary origins and specialization of core clock proteins.

(Sponsored by Millennium Nucleus for Fungal Integrative and Synthetic Biology, Nº 120043)

Variation in Stability and Synchronization correlated with Food Intake: exploratory analyses of healthy subjects with different levels of desynchronization.

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The expression of biological rhythms is characterized by temporal combinations of elements presented at different times of the day. According to this temporal scenario the expression of rhythms may represent synchronized or desynchronized internal states. Several evidences in the literature relate obesity and its associated disturbances with sleep disorders as well as with misalignment of the rest-activity cycle. This suggests that the disorders of these biological rhythms could accompany changes in dietary patterns and promote the development of pathologies. This research was carried out with twenty-seven eutrophic university students (aged 18-30 years). During 8 days, the participants completed self-reported food consumption and sleep logs, and their skin temperature, activity and light exposure were monitored. The alimentary parameters were obtained through the “Food Processor Nutrition Analysis Software” and measures of portion weight, calories, macronutrients and sugars consumed along the days were extracted. We analyzed nutrition measures and actimetry variables such as interdaily stability (IS), intradaily variability (IV), the most active 10h period (M10), the least active 5h period (L5) and relative amplitude (RA). In addition, we analyzed six non-traditional indexes of periodicity and synchronization between pairs of auto and cross-correlation functions of the actimetry variables. We found negative correlations between stability and synchronization indexes (as well as sleep quality measures) with food intake.From such results we suggest that internal desynchronization and low sleep quality may be involved in the promotion of increase in food intake. This misalignment of the biological rhythm could be the foundation of some feeding disorders.

NPYR2 polymorphism and circadian rhythms phenotypes in a sample of Brazilian university students.

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Introduction: Neuropeptide Y (NPY) plays a prominent role in modulation of circadian rhythms through a direct action on the Suprachiasmatic Nuclei (SCN). The NPY-induced phase advance seems to be mediated by the NPYR2 receptors. Therefore, genetic alterations in the *NPYR2* gene could influence the modulation of circadian rhythms via NPY.

Objective: To conduct an association study between a polymorphism in *NPYR2* gene (-244A/G) with diurnal preference and sleep habits in a Brazilian sample population (Alagoas State - latitude 9°S).

Methods: A sample of 820 undergraduate students (556 females), aging from 18 to 30 years (mean= 21.36 and SD= ±2.74), answered the Portuguese version of the MEQ and MCTQ questionnaires. All subjects were genotyped using Real-Time PCR and 7% of the extreme morning and evening types, together with the same size group of intermediates (n= 56 each) were selected for genetic association.

Results: Means of sleep duration on weekdays were significantly different for -244A/G polymorphism, with G Carriers (A/G-G/G genotypes) sleeping more (*M*= 6:59 and *SD*= 1:30) than Non-G Carriers (A/A genotype; *M*=6:23 and *SD*=1:26) ($t(166)= 2.55$, $p= .012$,$d= .40$). No association was found between -244A/G polymorphism and diurnal preference, at genotypic ($\chi^2(4)= 5.61$, $p=.230$) or allelic level ($\chi^2(2)= 1.62$, $p= .444$).

Conclusion: Our study demonstrated that carriers of a G allele presented a longer sleep duration**.** A possible explanation for this result could be related to the fact that G allele can express higher levels of NPYR2, resulting on the advance of circadian rhythms and differences in sleep phenotypes.

(Sponsored by Aline C P Silva Was Supported By CAPES Doctoral Fellowship.)

Tetrodotoxin-blockade of the suprachiasmatic nuclei disrupts ovulation. A study throughout the rat estrous cycle.

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In rodents, ovulation occurs spontaneously every estrous cycle at a particular time. There is evidence linking the circadian system to the delimitation of a time window where the rising levels of estradiol can induce ovulation. The classic experiments by Everett and Sawyer showed that injection of barbiturates during proestrous afternoon delays ovulation by exactly 24 hours if the injection occurs between 14:00-16:00. On the other hand, lesions of the suprachiasmatic nuclei (SCN) results on the inhibition of phasic release of gonadotropins and hence in the failure of ovulation. These evidences suggest the generation of a neural circadian signal, probably at the SCN, which converges with proestrous estradiol levels to trigger gonadotropin secretion. There is also evidence showing that barbiturate injections on the other stages of the cycle inhibit ovulation. Unfortunately, nothing is known about the role of the SCN on the regulation of ovulation on these stages. We implanted bilateral cannulas targeting the SCN of adult female rats. After a recovery time of 15 days rats showing at least two consecutive 4-day estrous cycles were microinjected with either saline or a solution containing 100ng/µL of tetrodotoxin during the critical period of each stage of the cycle. Microinjections were carried out while rats were awake and free-moving. Animals were sacrificed at the predicted day of estrous and the number of ovulating females, as well as the number of oocytes shed, was analyzed. Intact rats were used as absolute controls. Study supported by CONACYT grant 236908 and Posgrado en Ciencias Biomédicas, UNAM.

(Supported by CONACYT grant 236908 and Posgrado en Ciencias Biomédicas, UNAM).

Circadian regulation in filamentous cyanobacteria, what do we know?

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Cyanobacteria are the oldest known organisms with a functional circadian mechanism. KaiA, KaiB and KaiC are the nucleus of this regulation (central clock), which together with input (entraining) and output (genetic control) pathways, coordinate a response to environmental light-dark cycles. Initially the light was thought as KaiABC clock's modulator factor, but later was demonstrated the role of the redox state and the ATP availability on it. It has been conserved throughout cyanobacterial group, including both unicellular and multicellular organisms. However, most of the studies have been on unicellular being the knowledge on multicellular cyanobacteria such as *Anabaena sp.*PCC7120 (hereafter *Anabaena*) very limited. A comparative analysis of *Anabaena*'s circadian components with the unicellular model *S. elongatus* PCC7942 showed a high amino acid sequence identity for most components, except for KaiA that lacks an N-terminal region in multicellular organisms involved in the environmental synchronization process through the redox species detection, which suggests differences in their regulation. However, our experiments have shown a similar circadian control, in both organisms, where the growth rate is modified every 12 hours (subjective-night: decreases and subjective-day: increases). Moreover, this behavior depends on the cellular redox state, because methyl viologen adding (inhibits NADPH and ATP production) generates a forced circadian resetting, independent of light stimulus. Thus, it suggests that circadian regulation is similar in both organisms. Furthermore, our preliminary results require further studies on the influence of circadian clock on cell division in multicellular cyanobacteria.

(Sponsored by Acknowledgments to Fondecyt Project 1161232 And Conicyt Doctoral Fellowship)

School times: The School schedules and the Teacher´s everyday life.

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INTRODUCTION: The long teachers workday, inside and outside the classroom, makes the environment conducive to the analysis of their Sleep / wake cycle (SWC) as well as your job satisfaction.OBJECTIVE: Evaluate the drowsiness of a group of teachers, who work in the State of São Paulo, in two moments: weekdays and weekends and check their satisfaction with the work. METHODS: Twenty-four subjects (5 men and 19 women) were analyzed. We used the Karolinka Sleepiness Scale (KSS) for 23 days (4 times a day), including 3 weekends and the Occupational Stress Indicator (OSI) to verify the satisfaction index with the work. RESULTS: On the weekends, sleepiness varied throughout the day [F (3.66) = 5.1; p <0.01]. The mean drowsiness assessed at 13.33 ± 1.4 hours (KSS = 4.2 ± 1.95) and at 17.4 ± 1.25 hours (KSS = 4.6 ± 1.32) was lower than to 21.35 ± 1.14 hours (KSS = 5.9 ± 2). On weekdays no difference was observed [F (3.66) = 2.01; p> 0.05]. From OSI we observed that the variables that caused the most dissatisfaction with the work were participation in decisions (56%), remuneration (44%) and structure (40%). While the variables that caused the most satisfaction with work were relationship (68%), subject (52%) and motivation (40%). CONCLUSION: we suggest that the teaching activity reflects on the teachers SWC organization throughout the week and that the factors that lead to the professional satisfaction of the teacher are related to their daily work: interpersonal interaction and subject taught.

Effects of the microbiome on *Drosophila melanogaster* sleep.

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Over the past decade, there has been a growing awareness of the impact of the microbiome on the development, metabolism, and behavior of their host. Although the proportion of microorganisms changes with the host's environment, the dominant phyla are highly conserved across species. In humans and in the fruit fly,Drosophila melanogaster, some of most abundant phyla are Firmicutes and Proteobacteria.
In this work, we investigated the effects of the microbiome onDrosophila sleep. For this, we generated germ-free (axenic) flies by dechorionating the eggs before hatching, and analyzed the sleep of the resulting adults using the Trikinetics monitors of locomotor activity. Our results show that axenic flies bearing different alleles of theperiod gene (per[+],per[S], per[L] and per[0]) have more consolidated sleep than their control with microbiota, as they sleep longer and express a smaller number of sleep episodes. They also show differences in the total amount of activity during the day and night. Finally, we evaluated sleep rebound of flies after sleep deprivation, and found differences between axenic and control flies.

These results suggest a relationship between microbiota and sleep, which may be helpful for understanding sleep disorders associated with changes in microbiome such as occur following stressful situations and antibiotic treatments.

(FONDECYT 1141278 and ICM grant P09-022-F Centro Interdisciplinario de Neurociencia de Valparaiso to John Ewer).

Chronobiological overview of Uruguayan youngsters.

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We present the largest circadian characterization of Uruguayan youth reported so far (n=1197, age=18.82±0.63). As part of a national longitudinal study (ELBU, acronym for \"*Estudio Longitudinal del Bienestar en Uruguay*\"), children that were first graders at elementary schools in 2004 have been monitored with the aim of building a solid database of various socioeconomic factors and living conditions to contribute to the better understanding of development problems in Uruguay. For the first time in 2016 (4th ELBU survey), when the study population reached the age of majority, a reduced Munich Chronotype Questionnaire was included in the global ELBU survey. This study confirms the late preferences and desynchronization of Uruguayan youth. The midsleep point of free days corrected for sleep debt on work days, proxy of individuals 'chronotype, was of 5.62h±2.34. Although the average sleep duration was near to 9h (8.58h±2.11 and 8.74h±3.29 for workdays and free-days, respectively), sleep habits exhibited a social jet lag (misalignment between individual biological clock and social time) of above 2h. In addition, young Uruguayan women exhibited significant earlier chronotypes, longer sleep durations, and lower social jet lag than men, as previously reported for other studied populations. Among the diverse health and sociocultural factors reported in ELBU, body mass index, current educational and/or working status, screen time, and even the number of friends declared showed association with chronotype. We present preliminary results and perspectives to discuss different approaches for the modelling of this big and comprehensive database.

(Sponsored by ANII, CSIC, PEDECIBA (Uruguay)).

Evaluation of the work context, sleep schedules and quality of sleep, and daytime sleepiness in high school teachers in Rio Grande do Norte, Brazil.

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Teacher work is related to work overload and inadequate working conditions. In addition, problems related to sleep are observed in teachers. Therefore, the aim of this study was to evaluate the working context, sleep schedules and quality, and daytime sleepiness of high school teachers of public schools in RN/Brazil. The sample was 46 teachers (♀ = 48% / ♂ = 52%), with 40±9.1 year-old. The work context was evaluated by the Work Context Evaluation Scale divided by 3 factors (F1 = work condition, F2 = work organization, F3 = socio-professional relationship). The bedtime, wake-up time, time in bed and sleep quality were obtained from the Pittsburgh Sleep Quality Index, and the diurnal sleepiness from the Epworth Sleepiness scale. The comparison according to work context was performed by Mann-Whitney test ($p<0.05$). Among the teachers, 67.4%, 63% and 45.7% evaluated the F1, F2 and F3 factors as critical or severe, respectively. In addition, 67.4% presented poor sleep quality and 43.5% excessive daytime sleepiness. The bedtime was 11:27±1:16 pm, wake-up time was 6:00±1:00 am, and the time in bed was 6:08±0:53h. There was no difference in sleep shedule and quality, and daytime sleepiness of teachers according to F1 and F2 factors of the work context. However, it was observed that teachers who evaluated socio-professional relationships as critical or severe presented poorer sleep quality 8.05±3.2 than those who evaluated as satisfactory 6.38±2.7 ($p<0.05$). However, it is necessary to increase the sample to better analyze the work context as a factor that influences teachers sleep.

Systemic study of biological rhythms in mental disorders.

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The chronobiologic system controls the circadian cycles. This system has been studied meticulously; clock genes, proteins, photic and non-photic neuronal pathways, the split behavior and the opposite activity of the right and left suprachiasmatic nuclei, etc.

Circadian cycles seem to be particularly relevant in mental health. Sleep-wake, endocrine, motor and autonomic abnormal cycles, have a direct relation with mental disorders, however, the biological mechanisms involved are still unknown. Most of the investigations have been centered in genetic mutations of the chronobiologic system. Now, we propose to incorporate a systemic approach, a wider view of the whole physiology in vivo, not just a molecular view. For that, certainly, we have to develop new non-invasive technics. It is necessary to investigate new forms to understand the circadian system; for example, studying the effects of different kinds of photic and non-photic stimuli, and of symmetric and asymmetric synchronizers in living humans.

Mental health is a world problem. Its prevalence is increasing in all countries. The finding of effective techniques to resynchronize the circadian system would be a different and interesting way to deal with mental health disorders.

Abnormal right/left sympathetic asymmetry in major depression.

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The chronobiologic system controls the circadian rhythms in humans. Mood disorders like depression, show abnormal circadian cycles of sleep-wake, endocrine, motor, and autonomic nervous system; however, the chronobiologic mechanisms involved are still unknown. Previous studies in animals suggest that each side of the chronobiologic system governs the ipsilateral sympathetic activity. To have an appreciation of the sympathetic activity in right-handed humans, we measured the electrodermal activity (EDA) in both wrists in 6 healthy and 6 major depression subjects. All healthy subjects showed higher right side activity compared to left (R>L). In all depressed patients, we found the contrary (R<L). The abnormal asymmetry of the sympathetic activity found in major depression, confirm previous studies published by other authors. These findings open the question whether right and left sympathetic activity represents or not the function of each side of the chronobiologic system in humans. Also, raise the question if abnormal asymmetries of the chronobiologic system could be the underlying physiopathologic mechanism in depression´s circadian disorders. This exploratory research allows introducing new approaches to mental and chronobiologic disorder´s study in living humans.

Chronotype-dependent changes in sleep habits associated with dim light melatonin onset in the Antarctic summer.

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The dim light melatonin onset (DLMO) is the most reliable measure of central circadian timing in humans. DLMO thus depends on both light exposure and individual chronotype preferences, though only a few studies have been able to demonstrate these modulations so far. The impact of light and chronotype preferences on the sleep habits of a group of university students was compared between the semester start in the fall equinox 2016 (Montevideo, Uruguay, 34° 54' S; 56° 11' W, LD 12:12) and the 2016 Uruguayan Summer Antarctic School (King George Island, 62° 11' S; 58° 52' W, LD 20:4). Chronotype preferences of the study population (n=20) were tested by Munich Chronotype Questionnaire and Morningness-Eveningness Questionnaire (MEQ). The midsleep point of free days corrected for sleep debt on work days, proxy of individuals' chronotype, was around 6 am. MEQ scores ranged from 25 to 65 with predominant late values. Sleep log records showed a chronotype-dependent change in sleep onset: while early chronotypes delayed their sleep onset in Antarctica versus Montevideo, late chronotypes did not. Actimeter records (n=12) showed that students were significantly more exposed to natural daylight (in intensity and duration) in Antarctica versus Montevideo. Salivary melatonin measurements by ELISA (Salimetrics) paralleled both observations. First, basal melatonin levels (n=12) were significantly lower in Antarctica with respect to Montevideo. Second, the DLMO shifts (n=12) between Montevideo and Antarctica had positive values (later onset in Antarctica) for early chronotypes whereas the opposite was observed for late chronotypes.

(Partially supported by CSIC_I+D_2016 and EVIIA Facultad de Ciencias, UdelaR).

Analysis of the influence of temperature cycles on locomotor activity and expression of circadian clock genes of Aedes aegypti.

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Hematophagous mosquitoes are vectors of important tropical diseases pathogens. Mosquitoes activity rhythms and blood feeding are crucial to the transmission of these pathogens. Despite the evident influence of temperature cycles for *Ae. aegypti*biology, nothing was known about the expression of genes in the body clock of these vectors. In this work we describe the expression of circadian clock genes in the body of *Aedes aegypti* mosquitoes subjected to temperature cycles, as well as to light/dark cycles or even in the absence of environmental cues. We observed significant differences in gene expression analyzed in the body compared to what is known in the head. Our results suggested that a fully functioning clock in the body depends on a synergy between temperatures and light/dark cycles, but that temperature cycles would have a greater influence. We also simulated in laboratories semi-natural regimes as close as possible to what could be found in the municipalities of Rio de Janeiro and Petrópolis (RJ, Brazil), locales that differ considerably in their thermal amplitudes. When we compare these conditions, we noticed that mosquitoes exposed to the thermal amplitude of Petrópolis concentrates its activity in the middle of the afternoon. This behavior is very similar to what happens with Drosophila in temperatures regimes colder than 25°C. Finally, we aim to investigate how viral infection could modulate the locomotor activity in colder places where arboviruses are not epidemic.

Acknowledgments: We thank Robson Costa for technical assistance.

Objective chronobiology-based markers of depression: perspectives in diagnosis and treatment of mood disorders.

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Background: Despite prevalence and impairment caused by mental illness, its diagnosis still lacks of validity. We aimto demonstrate chronobiological markers of diagnosis and treatment of major depressive disorder (MDD), through 1)actigraphy-based differentiation of MDD subtypes, and 2)predictive value of urinary 6-sulphatoxymelatonin (aMT6) in clinical response to fluoxetine.

Method: S*tudy 1.* CORE questionnaire assigned participants to melancholic (MEL) or non-melancholic (N-MEL) group, according to subjective psychomotor disturbance. Participants underwent 7 days of actigraphy. S*tudy 2*. Urine of women diagnosed with MDD was collected 24 hours before and after the first fluoxetine tablet. Samples were separated in four times: morning (6:00 - 12:00), afternoon (12:00 - 18:00), night (18:00 - 0:00) and dawn (0:00 - 6:00).

Results: *Study 1.*Nocturnal motor activity was significantly higher in N-MEL. ROC curve shows that average night activity discriminate participants with 71% sensitivity and 100% specificity (area under the curve, AUC = 0.84). *Study 2.* Respondents increased (0.2649ng/mg), and non-responders decreased (-0.4040ng/mg) dawn aMT6s. ROC curve shows that dawn ΔaMT6 could discriminate responders with 75% sensitivity and 100% specificity (AUC = 0.92).

Discussion: Wrist actigraphy contribute to the objective differentiation of MDD subtypes. Changes in aMT6 levels following a single dose of fluoxetine could predict a 28-day treatment response. These results are in line with the NIMH-RDoc, which presents chronobiological variables as essential biological markers for the personalized diagnosis and treatment of mental illness. This data highlights that aMT6 should also be included in this expanding diagnostic method in Psychiatry.

*(**Funding:** Brazilian agencies CNPq/CAPES).*

Interval timing and circadian rhythms: Antarctica as a model of desynchronization.

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Introduction: Circadian rhythms seem to play a role in the modulation of time perception. Light is one of the main synchronizers of the circadian clock and studies are now focusing on its effect on time estimation. For example, constant light or dark conditions modify time perception in mice. In humans, diurnal variation in time productions correlate with circadian variations in core body temperature in well controlled lab studies. Antarctic provides a real-world and unexplored context to study the effect of desynchronization of the circadian clock on time perception.

Objective: To explore interval timing in the crew of Belgrano II Argentine Antarctic station as a model of desynchronization of the circadian clock.

Methods: A total of 13 subjects were assessed for interval timing in short (3s) and medium (6s) duration stimuli. Measures were taken during the morning and evening, five times along the year. Differences were evaluated using repeated measures ANOVA.

Results: Our results show significant variations for 3s during the morning (2595±123ms; 3022±161ms, 2987±197ms, 3229±203ms, 3449±249ms, p=0,004); 6s during the morning (5510±290ms; 5605±203ms, 6026±243ms, 6345±341ms, 6613±323ms, p=0,015); and 6s during the evening (5741±169ms, 6059±224ms, 6466±369ms, 6081±171ms, 5882±173ms, p=0,030). Discussion: While the short-interval productions during the morning increased progressively through the year, the medium-interval productions during morning and evening sessions increased during the winter polar night. The variations found in the short-interval productions may be related to isolation conditions, whereas the prolonged exposure to artificial light conditions during winter possibly modulates the seasonal differences observed in medium-interval productions.

(Sponsored by ANPCyT PICT Start-up 2013-0710, PIDDEF 2014/17n°06)

Chronopharmacological approach for glioblastoma treatment: effects of the novel drug 1A.

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Glioblastoma, the primary brain tumor with highest incidence in the adult population, has a 90% mortality rate (five-year), a 14 month average survival time and had no therapeutical improvements in the last 30 years. Research for novel drugs and treatment strategies becomes critical. It was reported that the efficacy of several drugs is modulated by the circadian system leading us to hypothesize that a chronopharmacological approach would improve the efficacy of glioma treatment. We studied the effects of 1A (a Rac1 inhibitor), a novel candidate drug to glioblastoma treatments and Temozolomide (current treatment of choice) when applied at different circadian times to LN229 glioma cells.

Our results show that 1A extends the survival time in mice. Because two of the main roles of Rac1 are related to cell proliferation and migration, we studied the effects of 1A and TMZ over these processes when applied at different circadian times. We found that the effectivity of 1A is rhythmic and depends on the administration time showing a minimum of 15% inhibition of proliferation when applied 28 hs after a serum shock and a maximum of 60% inhibition when applied at 43 hs. Migration assays were performed at 28 hs and 43 hs with no significant effects at 28hs and over 50% inhibition of migration observed at 43 hs. Toxicity showed a similar result than proliferation. In primary murine astrocytes, 1A was not toxic in neither of the circadian times. Our results suggest that effects of this drugs are modulated by the circadian system.

Oscillators in a regular lattice display phase sub-oscillations that carry zeitgeber frequency information.

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In mammals, the suprachiasmatic nuclei (SCN) are hypothalamic structures comprised of glial cells and neurons which are functionally heterogeneous circadian oscillators. Because SCN cells function coordinately in order to drive physiological and behavioral circadian rhythms, the SCN can be considered as a network of oscillators which works in synchrony. A subset of SCN cells receive direct environmental light-dark information through the retinohypothalamic tract and this information is communicated to the rest of the network through coupling between SCN cells, ultimately allowing the entrainment of the SCN to the light-dark cycle. Our aim is to understand how phase and period information from the light-dark cycle is transmitted into the SCN oscillator network. To achieve this we study a Kuramoto model on a square lattice on which an external oscillating force with amplitude B and frequency wf perturbs a subset of oscillators. We find that for high coupling strength, the system exhibits phase traveling waves while the effective frequency of each node in the network is set to the external frequency (wf), a phenomenon known as frequency locking. Interestingly, for low coupling strength, the system also displays traveling waves but in this case those oscillators which do not receive direct external stimulus not longer exhibit frequency locking.Nevertheless another phenomena emerge in those nodes: their phases sub-oscillate with a frequency determined by the external stimulus. We numerically show that phase sub-oscillations are a robust phenomenon which carry zeitgeber frequency information.

Sex differences in the homeostatic accumulation of sleep pressure.

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There are differences between men and women regarding their sleep/wake cycle and sleep structure. Male population present a more pronounced eveningness preference than female. Additionally, when sleep deprived, women have greater rebound of slow wave activity in comparison with men, indicating the existence of a potential difference concerning the homeostatic process of sleep. Thus, this study aimed to evaluate the differences in sleep structure between men and women, as well as the rate of increase in the homeostatic sleep pressure for men and women with regular sleep/wake schedule. Forty-six healthy young adults took part in the study, all monitored by actigraphy throughout the week preceding the experiment day, when they had the opportunity to take a nap for up to 90min. We accessed the sleep macrostructure through the standart EEG sleep stage scoring and a power spectrum analysis was applyed. Spectral analysis detected significantly higher power densities over a wide frequency range (F3/F4 for theta and spindles ($p<0.05$); C3/C4 for delta, theta and spindles ($p<0.05$)) in the female versus male subjects, as accessed by t-test between groups. Also, according with ANCOVA analysis, women have greater increase rate in homeostatic sleep pressure than men (difference in β-value between groups: F4 (Delta: $p<0.05$; Slow Oscilations: $p<0.05$), C3 (Delta: $p<0.05$; Slow Oscilations: $p<0.05$) and C4 (Delta: $p<0.05$; Slow Oscilations: $p=0.180$)), supporting the existence of differences between sexes regardind the homeostatic process of sleep.

Circadian control of lipid and redox metabolisms in proliferative glioblastoma cancer cells.

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Immortalized cell lines contain circadian clocks conducting transcriptional/translational rhythms in gene expression whereas metabolic rhythms can persist without transcription. Circadian rhythm disruption by modern life may cause higher cancer risk; however, little is known about clock functioning in tumor cells. Here we evaluated glycerophospholipid (GPL) and redox metabolisms in cultures of glioblastoma T98G cells under proliferation (P) or partial arrest (A), synchronized with dexamethasone (100 nM) (time 0) and collected at different times. In arrested cultures, mRNAs for clock- (*Bmal1, Per1, Rev-erbα)* and GPL enzyme genes, and ^{32}P-GPL labeling exhibited circadian rhythmicity; oscillations were also found in the redox state/ peroxiredoxin oxidation cycles. In proliferating cells, circadian rhythms of gene expression were lost or their periodicity shortened whereas the metabolic rhythms persist with a similar or longer period to that observed under A Also, cell viability significantly changed over time after bortezomib (500 nM) treatment.Nevertheless, cell viability and redox state rhythms were altered when *Bmal1* expression was knocked down by CRISPR/ Cas 9 genomic editing technology. Results support that a metabolic clock operates in proliferative tumor cells regardless the molecular clock; property that may confer tumor susceptibility for a time-dependent chemotherapy.

(Sponsored by FONCyT; PICT 2013 nro 021 and SeCyT-UNC; CONICET).

Behavioral rhythms in the lab and acute effect of light and darkness in tuco-tuco (*Ctenomys aff. knighti*), a subterranean rodent.

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The running wheel is commonly used in rhythm studies assuming that the wheel-running time corresponds to the activity time in nature. However, the tuco-tuco (*Ctenomys aff. knighti*) is a subterranean rodent which is diurnal in the field and nocturnal in the lab. Because of that peculiarity, our first goal is to verify which are their behaviors in the lab, their distribution throughout the 24 hours, and how much the running wheel activity contributes to the total activity. The second goal is to verify what are the acute effects of light and darkness on their rhythms. 18 adult males and females (150-250g) had their behaviors recorded by cameras. Their running wheel activity, general activity and body temperature were also monitored. They were kept in a LD 12:12 (L = 100 lux) regimen and received light and darkness pulses to verify possible masking effects in their rhythms, going through the same protocol three times: 1) without wheel, 2) with wheel, and 3) with a blocked wheel. Most of animals demonstrated little to no rhythmicity in specific lab behaviors, except for running wheel activity. The wheel activity is also the main component of the general activity that is inhibited by the light pulse, but we found some masking on body temperature rhythm as well. Interestingly, there was a single individual that switched from nocturnal to diurnal when kept without a running wheel, and presented the masking patterns expected for diurnal animals.

(Sponsored by CNPq (130130/2016-8), FAPESP (2013/50482-1; 2014/20671-0), CONICET (PIP 11220120100415), ANPCyT (PICT2013-2753)).

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