Abstracts Book

XI Latin American Symposium of chronobiology

Cholula, Puebla, México
May 4-5 2011
Sponsors
Welcome

It is an honor to receive in Mexico scientists and students in the field of Chronobiology, visiting us from different Latin American countries, as well as from other parts of the world. In this meeting, as has occurred in the past, we expect a fruitful interchange of ideas, the beginning and consolidation of collaborations and to continue promoting the interest of the students in this field.

Many topics of Chronobiology will be discussed in this meeting. The genetic basis of the circadian rhythms, the physiological foundations of rhythms, the mechanisms of synchronization, the rhythms of behavior and the consequences of disorders of the temporal organization of the organism on health. Topics vary from basic science to applications in the social environment.

Sincerely,

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Universidad de Guanajuato
Scientific Program
XI Latin American Symposium of Chronobiology

May 4-5, Cholula, Puebla, Mexico

Scientific Program

May 4, Wednesday

09:00-09:50  Conference 1. Time in the brain
             Diego Golombek (Universidad Nacional de Quilmes, Argentina)

10:00-12:00 Thematic session 1. From genes to society
              Chair: Claudia Moreno

             Adaptation to environmental light/dark cycle and clock genes
             Mario Pedrazzoli (Universidade de São Paulo, Brazil)

             Internal and external temporal organization
             Luiz Menna-Barreto (Universidade de São Paulo, Brazil)

             Not all adolescents are sleep deprived: environmental influences on
             sleep-wake cycle
             Fernando Louzada (Universidade Federal do Paraná, Brazil)

             Effects of sleep deprivation on cognitive performance at different times of
             day
             Pablo Valdez (Universidad Autónoma de Nuevo León, Mexico)

             Job satisfaction and discrepancies between social and biological timing
             Claudia Moreno (Universidade de São Paulo, Brazil)

12:00-13:30  Student projects session

13:30-15:30  Lunch
15:30-16:20  **Conference 2. From the sense of time to genes and back**  
*Urs Albrecht (University of Fribourg, Switzerland)*

16:30-18:30  **Thematic session 2. Ionic Mechanisms underlying circadian oscillations**  
Chair: *Raúl Aguilar-Roblero*  

- Excitatory synaptic transmission increases the intracellular calcium concentration in suprachiasmatic nucleus neurons.  
  *Charles N. Allen (Oregon Health & Science University, USA)*  

- Fast delayed rectifier potassium current: critical for input and output of the circadian system.  
  *Christopher S. Colwell (UCLA Medical School, USA)*  

- Participation of intracellular calcium-release channels and calcium pumps in the circadian rhythmicity of the suprachiasmatic nucleus.  
  *Adrián Báez-Ruiz (Instituto de Neurobiología, UNAM, Mexico)*  

- As time goes by: how aging affects clock cell physiology  
  *Stephan Michel (Leiden University Medical Center, Netherlands)*  

- From the nucleus to the membrane: Ca\(^{2+}\) release from RyR signals time in SCN neurons.  
  *Raúl Aguilar-Roblero (Instituto de Fisiología Celular, UNAM, Mexico)*

18:30-20:00  **Poster session 1**

20:00-21:30  Dinner

21:30-22:30  **Late night discussion**

**May 5, Thursday**

09:00-09:50  **Conference 3. Clock gene expression in peripheral clocks of mice and humans**  
*Nicolas Cermakian (McGill University, Canada)*
10:00-12:00  **Thematic session 3. Mechanisms of synchronization**  
Chair: Carolina Escobar  

Distinct molecular mechanisms in the suprachiasmatic nucleus underlie circadian clock photoentrainment by advances or delays  
*William J. Schwartz (University of Massachusetts Medical School, USA)*  

The Oscillatory Carnivale: synchronization, desynchronization, and resynchronization of the circadian clock  
*Juan J. Chiesa (Universidad Nacional de Quilmes/CONICET, Argentina)*  

Mechanisms for food entrainment: the contribution of extra-SCN pacemakers  
*Carolina Escobar (Universidad Nacional Autónoma de México, Mexico)*  

Social synchronization in non-human primates  
*Carolina Azevedo (Universidade Federal do Rio Grande do Norte, Brazil)*  

Entrainment in a subterranean rodent: field work meets mathematical modeling in South America  
*Gisele Akemi Oda (Universidade de São Paulo, Brazil)*

12:00-13:30  **Poster session 2**

13:30-15:30  Lunch

15:30-17:30  **Thematic session 4. Generating rhythms in invertebrates: where do they come from?**  
Chairs: Maria Luisa Fanjul, Mirian D. Marques  

Role of the Drosophila Clock gene in circadian and non-circadian responses to light  
*Amita Sehgal (University of Pennsylvania, USA)*  

Molecular dissection of cricket’s clock systems  
*Kenji Tomioka (Okayama University, Japan)*
Stingless bees and their clocks  
Mirian D. Marques (Universidade de São Paulo, Brazil)

Crustacean β-PDH I but not β-PDH II and their putative roles in pigment dispersion and circadian regulation  
Horacio de la Iglesia (University of Washington, USA)

Sixth abdominal ganglion modulates the circadian rhythms of locomotor activity and abdominal posture in crayfish  
Leonardo Rodríguez Sosa (Universidad Nacional Autónoma de México, Mexico)

The crayfish circadian clock: a biochemical and molecular approach  
Julio Prieto-Sagredo (Universidad Nacional Autónoma de México, Mexico)

17:30-18:20  Conference 4. Chrono-nutrition and chrono-dietetics  
Shigenobu Shibata (Waseda University, Tokyo, Japan)
Student Projects Session

May 4, Wednesday
12:00-13:30

PR.1 Participation of the anterior Paraventricular Thalamus (PVT) in the entrainment of the biological clock to light.
Aura Becerril Parra, Raúl Aguilar Roblero.

PR.2 Sexual behavior and sexual function as a potential "Zeitgeber". Alterations in the sexuality of nurses who work shift-work.
Andres Camargo-Sanchez, Martín Alejandro Piloneta-Rueda, Carmen Lucia Niño-Cardozo.

PR.3 Study of variability circadian body temperature and sleep-wake cycle of the student night shift workers.
Ferreira Luciane Carmona, De Martino Milva Figueiredo.

PR.4 Characterization of social factors associated with diurnal sleepiness in kindergarten children.
Aline Silva Belísio, Fernando Mazzilli Louzada, Pablo Valdez, Carolina Macêdo Azevedo.

PR.5 Effects of advance and delay of the sleep phase on the components of attention.
Diana Juarez, Pablo Valdez.

PR.6 Effects of coffee ingestion on the components of attention after a 24 h sleep deprivation.
Mariana Reyna, Pablo Valdez.

PR.7 Effects of a 15 minutes nap on the components of attention
Juventino Cortez, Pablo Valdez.
## Poster Session 1

**May 4, Wednesday**  
**18:30-20:00**

| P1.01 | Stress and chronotype analysis in day and night shift students.  
 | Carla Parada Pazinatto Andreoli, Milva Maria Figueiredo De Martino. |
| P1.02 | Chronotype assessment: validation of the MCTQ in a sample of university students from Brazil.  
 | Marilene Farias Alam, Karla V. Allebrandt, Mabel Mascarenhas Wiegand, Giovana Dantas, Rosa Maria Levandovski, Rosana Mendonça De Souza, Maria Paz Hidalgo. |
| P1.03 | Retirement, wrist temperature rhythm and time estimation.  
 | Mario Miguel, Luiz Menna-Barreto. |
| P1.04 | Synchrony in circadian activity rhythm socially adjusted in juveniles common marmosets (Callithrix jacchus).  
 | Paula Rocha De Melo, Camila Moura Bessa, Fernanda Fernandes Kolodiuk, Henrique James, Bruno Silva, Alexandre Augusto Lara Menezes, Carolina Macêdo Azevedo. |
| P1.05 | Irregularity of sleep schedules, daytime sleepiness and sleep quality of students and teachers in the morning shift.  
| P1.06 | Flying mice: Internal forced desynchronization in a murine model of chronic jet-lag.  
 | Leandro Pablo Casiraghi, Gisele Akemi Oda, Juan José Chiesa, Wolfgang Otto Friesen, Diego Andrés Golombek. |
| P1.07 | Effects of a 30-minutes advance in school schedule on sustained attention in children.  
 | Juventino Cortez, Diana Juarez, Jorge Borrani, Aida Garcia, Candelaria Ramirez, Pablo Valdez. |
Effects of 24-h sleep deprivation on attention.  
Juventino Cortez, Diana Juárez, Aida García, Candelaria Ramírez, Pablo Valdez.

Relationship between sleep patterns and cognitive performance in elderly humans.  
Aparecida Caroline Araujo, Luiz Menna-Barreto.

Comparative analysis of biological rhythms in fullterm and preterm babies.  
Clarissa Bueno, Luiz Menna-Barreto.

Circadian variations in alertness.  
Javier Talamantes, Aida García, Candelaria Ramírez, Pablo Valdez.

Circadian variations in cognitive inhibition and flexibility.  
Benito Martínez, Aida García, Javier Talamantes, Candelaria Ramírez, Pablo Valdez.

Effects of sleep reduction on the components of attention.  
Diana Juárez, Martha Guerrero, Layla Arroyo, Juventino Cortez, Aida García, Candelaria Ramírez, Pablo Valdez.

Effects of sleep deprivation on working memory.  
Jacqueline Del Angel, Gabriela Iracheta, Diana Juárez, Juventino Cortez, Aida García, Candelaria Ramírez, Pablo Valdez.

Sleep-wake cycle and activity-rest cycle in elderly people.  
Anahi Flores, Xochitl Ortiz, Aida García, Candelaria Ramírez, Pablo Valdez.

Sleep pattern, learning and memory, in nursing personnel working in a Bogotá hospital, Colombia, 2011.  
Carmen Niño-Cardozo, Andres Carmargo-Sanchez.

Motor activity of manatee (Trichechus manatus manatus) in semi-captivity conditions: preliminary results.  
Victoria Eugenia Holguín, Victor Manuel Alcaraz, Jairo Ignacio Muñoz.
P1.18  Sleep habits, chronotypes and obesity/overweight in medical students at UNAM.
Eduardo González-Guerra, Donaji Heredia-Garcia, Carolina Escobar-Briones.

P1.19  Mapping chrono pre-metabolic syndrome in Mexico City.

P1.20  Experimental chrono-homeopathic study with Aconitum napellus in female mice.

P1.21  Discussion about the interaction between medicine and human body biological clock.
Feng Xiu Jie, Zhuang Hong Yan.

P1.22  The association among sleep quality and mid-sleep phase with use of antihypertensive drugs.
Rosa Maria Levandovski, Till Roenneberg, Karla Viviane Allebrandt, Maria Paz Loayza Hidalgo.

P1.23  Type 1 Diabetes mellitus (T1DM) itself seems to have no impact on the rest/activity rhythm (RAR).
Mark Thomas Ugliara Barone, Denise Reis Franco, Mario Kehdi Carra, Fabiola Schorr, Geraldo Lorenzi, Luiz Menna-Barreto.

P1.24  Differences in sleep and daily metabolic profiles between overweighted and normal mice Neotomodon alstoni.
Citlalli Fuentes Granados, Pilar Durán, Manuel Miranda Anaya.

P1.25  Chrono homeopathic actions of Aconitum napellus upon human heart rate.

P1.26  Alterations in the characteristics of circadian variation of BP in non-dippers.
Razia Sultana, Atanu Kumar Pati.
P1.27  Emerging Leaders in the Americas Program (ELAP).
       Martin Roland Ralph

P1.28  Sleep patterns and risk factors for metabolic syndrome among professional public transport drivers.
       Ma. Elena Durán Lizarraga, Juan Manuel Ponce, Miguel Angel Palomino O Garibay.

P1.29  Custom-made software for digital signal analysis on chronobiology.
       Arturo Vega-Gonzalez, Raul Aguilar-Roblero.

P1.30  Chronobiologic Viscum album study in breast cancer.
       Alfredo A. Abuín Landín.

**Poster Session 2**

May 5, Thursday
12:00-13:30

P2.01  Rat liver gabaergic system characterization during food entrainable oscillator expression.
       Olivia Vazquez Martinez, Monica Villalobos Leal, Isabel Mendez, Diego Hernandez Saavedra, Mauricio Díaz-Muñoz.

P2.02  Regulation of hepatic mitochondrial beta-oxidation during the expression of the Food Entrained Oscillator.
       Julieta Berenice Rivera, Mauricio Diaz-Muñoz.

P2.03  Sub-cellular localization and release of the mitochondrial enzyme Ornithine Transcarbamylase, in rat liver during the expression of the Food Entrained Oscillator.

P2.04  Role of the intracellular calcium signaling during hepatic food-entrainment.
       Adrián Báez-Ruiz, Mauricio Diaz-Muñoz.

P2.05  The hepatic zonation of the Phosphoenolpyruvate Carboxikinase changed in a daytime restricted food schedule.
       Moises Perez-Mendoza, Mauricio Diaz-Muñoz.
P2.06  Hepatoprotective effects of food restriction in the induction and development of Hepatocellular Carcinoma in rats.  
*Christian Molina-Aguilar, Mauricio Díaz-Muñoz.*

P2.07  Circadian rhythm of energy expenditure and consumption of oxygen by indirect calorimetry in enteral nutrition therapy.  
*Marlene Pooch Leuck, Rosa Maria Levandovski, Claudia Will Machado, Maria Paz Hidalgo.*

P2.08  Behavioral and temperature changes that precede food entrainment.  
*Estefa Espitia, Dulce María Palomares-Vazquez, Rodrigo Ivan Osnaya, Carolina Escobar.*

P2.09  Differential involvement of Orexin cells in food entrainment.  
*Angeles Jiménez, Mario Caba, Carolina Escobar.*

P2.10  Scheduled feeding does not reentrain the estrous cycle in female Wistar rats maintained in constant light.  
*Donají Heredia García, Mariana Pliego Caballero, Isabel García-Peláez, Carolina Escobar Briones.*

P2.11  Aftereffects of entrainment in the field provide new insights into the switch from diurnality to nocturnality in the subterranean rodent Tuco-tuco (Ctenomys cf. knight).  
*Barbara Mizumo Tomotani, Danilo Eugênio De França Laurindo Flôres, Patricia Tachinardi, Jose Demetrio Paliza, Gisele Akemi Oda, Veronica Sandra Valentinuzzi.*

P2.12  Effect of overweight on entrainment of locomotor activity in the volcano mouse Neotomodon alstoni: gender differences.  
*Vania Patricia Carmona Alcocer, G. Ivette Aguilar González, Manuel Miranda Anaya.*

P2.13  Overweight in females reduces the entrainment of circadian locomotor activity in the volcano mouse Neotomodon alstoni.  
*Ivette Aguilar González, Vania Patricia Carmona Alcocer, Manuel Miranda Anaya.*
P2.14 Comparative analysis of circadian rhythms of the mosquitoes Aedes aegypti and Culex quinquefasciatus under temperature cycles. 
Gustavo Bueno Rivas, Carla Gentile, Alexandre Afranio Peixoto.

P2.15 Modulation of the circadian rhythm of body temperature by intense activity in a subterranean rodent (Ctenomys cf. knighti). 
Patricia Tachinardi, Barbara Mizumo Tomotani, Danilo Eugênio De França Laurindo Flôres, Jose Eduardo Pereira Wilken Bicudo, Veronica Sandra Valentinuzzi, Gisele Akemi Oda.

P2.16 A subterranean rodent’s “natural entrainment”. 
Danilo Eugênio De França Laurindo Flôres, Barbara Mizumo Tomotani, Patricia Tachinardi, Gisele Akemi Oda, Verónica Sandra Valentinuzzi.

P2.17 Locomotor activity and sleep rhythms in the Mongolian gerbil Meriones unguiculatus are differentially affected by short and long photoperiod. 
Cinthia Rocio Juárez-Tapia, Pilar Durán, Manuel Miranda-Anaya.

P2.18 Effect of different photoperiods on the rhythmic induction of Hypoxia-inducible Factor-1 and Heat Shock Pathway. 
Rosa Maria Velazquez-Amado, Elsa G. Escamilla-Chimal, Ana Gabriela Garcia-Tecpan, Maria Luisa Fanjul-Moles.

P2.19 Low temperature pulses produce circadian effects on the locomotor activity rhythm of the crayfish P. Clarkii. 
Irving Palma-Anzures, Julio Prieto-Sagredo, Maria Luisa Fanjul-Moles.

P2.20 Inter-individual variability of Nephrops norvegicus biological rhythms and its implications in stock assessment. 
Valerio Sbragaglia, Jacopo Aguzzi, Juan Antonio García, Juan José Chiesa, David Sarrí, Antoni Manuel, Francesc Sardà.

P2.21 Crustacean Hyperglycemic Hormone MRNA oscillation in the crayfish Procambarus clarkii eyestalk. 
Janikua Nelson-Mora, Julio Prieto-Sagredo, Maria Luisa Fanjul-Moles.

P2.22 Determination of intron movement in per gene. 
Gabina Calderon Rosete, Francisco Martinez Perez, Juan A. Gonzalez Barrios, Luis Kameyama, Jorge L. Fuentes Lorenzo, Leonardo Rodríguez Sosa.
P2.23 Daily fluctuations in the biosynthesis of Phosphatidylcholine and activity of its key enzyme CTP: Phosphocholine Cytidylyltranferase in synchronized cultures of fibroblasts. 
*Victoria América Acosta Rodríguez, Sebastian Marquez, Mario Eduardo Guido.*

P2.24 The effect of gating Ryanodine receptor son the intracellular calcium concentration in SCN neurons in mice. 
*Daniel Quinto-Muñoz, Stephan Michel, Raul Aguilar- Roblero.*

P2.25 Circadian regulation of cytoplasmic MRNA-granules. 
*Juan Ignacio Lescano, Mario Eduardo Guido, Eduardo Garbarino Pico.*

P2.26 Opsin expression and light responses in RGC-5 cells . 
*Paula Sofia Nieto, Diego Javier Valdez, Victoria América Acosta-Rodríguez, Mario Eduardo Guido.*

P2.27 SCN driven changes in the activation of Arcuate Nucleus α-945-MSH neurons. 

P2.28 Circadian rhythms of PER2 in mouse primary hepatocyte cultures . 
*Martha Luitje, Penny Molyneux, Mary Harrington.*

P2.29 Differential development of the diurnal clock gene expression in the olfactory bulb and Suprachiasmatic nucleus of European Rabbit. 
*Oscar Hernández-Campos, Rodrigo Montúfar-Chaveznav, Ivette Caldelas*
Abstracts

Conferences
Chronobiologists are obsessed with time and timing, in particular those dealing with periodic occurrences of physiological phenomena. However, it is worth to consider that timing mechanisms in the brain largely exceed biological rhythmicity and can be considered from multiple points of view. In this lecture we shall discuss time from different perspectives, including a putative sense of time, learning of time and timing, suggestions for neuroanatomy and neuropharmacology of timing, time as related to learning and memory, time in the cultural domain and, of course, time as a cycle (we are chronobiologists after all, right?). Indeed, neural timing mechanisms range from the millisecond to diurnal, and possibly annual, frequencies. Although some of these frequencies appear to be interrelated, the underlying timing mechanisms are usually considered independently. The precise knowledge of the origin and regulation of neural oscillations, in different periodicities and levels of organization, offers a unique perspective with which to study how the brain deals with time and timing, both in physiological and pathological conditions. Time waits for nobody.

In the last decade research on circadian rhythms has been complemented with a molecular understanding of basic cellular mechanisms that make up a circadian rhythm in a cell. This molecular mechanism has lead to a large enthusiasm bearing the hope that a complex behavior can be explained at the cellular and molecular levels. However, nowadays the good old systemic view on circadian rhythms swings back. Understanding a cell and its rhythms does not mean that we understand circadian rhythms at the physiological level. The exciting challenge is to understand how the multiple clocks in our
organism interact with each other to produce a coherent systemic circadian rhythm that is truly useful for the organism. The mechanisms important to understand in this context will include neuroendocrinological signaling mechanisms, electrophysiological signal transduction and metabolic signaling mechanisms. In this lecture I will delineate some of the important steps in the identification of molecular circadian rhythms and go on to illustrate how systems properties contribute in an important manner to the circadian rhythms that we can observe.

C3

CLOCK GENE EXPRESSION IN PERIPHERAL CLOCKS OF MICE AND HUMANS

Nicolas Cermakian
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Most tissues and organs harbour circadian clocks, which regulate cell function within the tissues and their physiological functions. These circadian clocks rely on a set of clock genes and proteins, organized in transcriptional-translational feedback loops. Tissue-specificity was noted in the genes regulated in a daily fashion across tissues, in the response of the different tissue clocks to resetting cues, but also to a certain extent in the composition of the molecular clockwork itself. Our aim is to understand the differential regulation of clocks across the circadian system, both in mice and in humans. In a first series of experiments, we identified new nuclear receptors of the REV-ERB and ROR families as transcriptional regulators of the Bmal1 gene. Analysis of the expression of the genes encoding ROR factors showed that their expression and circadian rhythmicity are tissue-specific. Even for a single factor, RORgamma, two isoforms display strikingly different circadian expression patterns, which can be explained by the differential regulation of alternative promoters by clock transcription factors. In another project, we have studied the expression of human clock genes in peripheral blood mononuclear cells (PBMCs) from subjects under different lighting conditions. We have applied this approach to an experiment simulating night shift work conditions, showing that resetting of a human peripheral clock (in PBMCs) follows different kinetics compared to the central suprachiasmatic nucleus (SCN) clock (as measured by melatonin and cortisol rhythms). Finally, we have analysed clock gene expression in post-mortem brain samples from Alzheimer’s disease (AD) patients and control subjects. We found that different extra-SCN brain structures show rhythmic clock gene expression in humans, analogously to the rodent brain. Notable differences in the phase of clock gene rhythms and phase
relationships between genes and regions were observed in the brains of AD compared to those of controls, indicating altered synchronization among brain oscillators in the brain of AD patients. Supported by the Canadian Institutes of Health Research and the Natural Science and Engineering Research Council.

CHRONO-NUTRITION AND CHRONO-DIETETICS

Shigenobu Shibata
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Circadian rhythm system controls the drug action through ADME (absorption, distribution, metabolism and excretion) of drug. For example statin compounds, anti-hypercholesterolemia drugs are preferred to take at night, because HMG-CoA reductase which is target enzyme for drug shows circadian rhythm of its enzyme activity and gene expression with peak at night. The subjects examined how the drug action is dependent on clock time are so called as “Chrono-pharmacology”. As same as such drug, ADME of nutrients/foods may be controlled under circadian clock system. Actually, SGLT1, GLUT2 and GLUT5, all are transporter for sugar uptake at small intestine show circadian rhythm of gene and protein expression. Expression time of these genes is well corresponded to the time for absorption of sugar when animals take food. Therefore, the subjects examined how nutrients/foods are dependent on clock-time are called as “Chrono-nutrition” and “Chrono-dietetics”. Thus, circadian clock controls the drug effect and also foods/nutrition effect. In this lecture, I will mention the importance of idea of these new subjects to understand the interaction between circadian clocks and food/nutrition. (1) When total amount of food per day is constant, increase of mouse body weight was higher in one mealtime group than two meal-times group, and change of breakfast/dinner affected the body weight and leptin gene expression. (2) When casein in the diet was substituted by amino acids or polypeptides, and casein percentage (14%) in diets was decreased to 8% or 6%, and mealtime was set at one or two per day, fat contents of liver was dependent on such various factors. (3) When compared daytime and nighttime administration of food (digestible or indigestible), increase of blood glucose is stronger in the daytime than in the nighttime in both corn starch- and potato-treated mice, suggesting that insulin secretion response is faster and stronger at night.

Acknowledgments: This research is partially supported by Bio-oriented Technology Research Advancement Institution (2010).
Abstracts

Thematic Sessions
The light/dark cycle given by the rotation of earth around its own axis and around the sun has been shown to be the strongest zeitgeber that entrain circadian rhythms, inclusive to humans. Due to tilt in earth north/south axis in relation to the sun plan, the sunlight reaches earth differentially depending mainly on the latitude, resulting in an enormous variability of isolation levels and daylength along the latitudinal cline. Practically all physiological processes are regulated by the signal coming from light/dark. The suprachiasmatic nuclei, in the hypothalamus, is the first step in temporal processing of light/dark signaling and this processed signal is widespread through a net of nuclei inside hypothalamus giving rise to circadian control of endocrine, autonomic and muscular systems and consequently behavior. Part of the signal processing is related to gene expression, a group of genes, called clock genes, are directly involved in the regulation and maintenance of circadian rhythms in the body. Variations in these genes, as polymorphisms and mutations, are associated with aberrant or differential expression of circadian behavior. The strong link between these clock genes and environmental conditions derived from the position of the earth in the solar system make them evident candidates to natural selection associated to light/dark cycle. The study of these genes and their interaction with environment results in a better comprehension of the human evolution related to the adaptation to annual variations of light/dark cycle through the latitudinal cline and its consequences to human health and social organization.
INTERNAL AND EXTERNAL TEMPORAL ORGANIZATION

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Distinct circadian rhythms tend to establish relatively stable phase relationships and this fact has been read as an index of health status by several authors. The concept of Internal Temporal Organization (or Order) has been proposed to identify that property of organisms, and based on that concept we detect internal desynchronization. External desynchronization, on the other hand, has not been discussed as a phenomenon comprising phase changes involving specific circadian rhythms and singular external time cues. In order to gauge these phase relationships we propose the concept of External Temporal Organization. Assessments of variability in the phase relationships between external time cues and internal markers may inform more about both the health status and adaptation to temporal challenges such as shiftwork and jet-lag.

NOT ALL ADOLESCENTS ARE SLEEP DEPRIVED: ENVIRONMENTAL INFLUENCES ON SLEEP-WAKE CYCLE

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The objective of this study was to investigate the role of environmental factors in sleep duration among adolescents living in rural areas. A total of 1140 students (569 males), aged 10-19 years, and attending two schools in rural regions in southern Brazil, completed a questionnaire about their sleep habits. Demographic data were also obtained. Prevalence ratios (PR) were estimated for the cases of more than 9 h of sleep on weekdays. Sleep duration in adolescents with and without electric lighting at home was analyzed. Average sleep duration at night was 9.63 (1.64) h on school-going days and 10.14 (2.42) h on weekends. The prevalence of adolescents sleeping for more than 9 h at night on school-going days was 58.3%. Older adolescents showed a tendency to
delay their sleep onset times, which is associated with a reduction of sleep duration. Adolescents without electric lighting at home slept longer on school-going days (P < 0.001) and on weekends (P = 0.013) when compared to those with electric lighting at home. From multivariate analysis, age (P < 0.001), school schedule (P = 0.007) and work (0.042) were factors affecting sleep duration. In contrast to the data previously reported for urban populations, we found a high prevalence of adolescents sleeping for more than 9 h on school nights. Data on populations living in less industrialized regions reinforce the idea that technological advances are associated with the negative impact of sleep phase delay in adolescents.

S1.4

EFFECTS OF SLEEP DEPRIVATION ON COGNITIVE PERFORMANCE AT DIFFERENT TIMES OF DAY

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Sleep deprivation produces an increase in reaction time and errors in the execution of many tasks, such as motor, verbal comprehension, arithmetic calculation, time perception, and impairs performance of truck drivers, airline pilots, shift workers and medical residents. These effects are modulated by time of day, so that worse performance is observed during the night and early in the morning. A decrease in attention can be responsible for the impairment in the execution of many tasks. Attention has four components: tonic alertness, phasic alertness, selective attention and sustained attention. The objective of this work was to analyze the effects of sleep deprivation on the components of attention at different times of day. Participants were undergraduate students attending school at a morning shift. They responded a Continuous Performance Task designed to assess the components of attention. There were three groups: a group without sleep deprivation, a group with 24-h of sleep deprivation and a group with partial sleep deprivation, which slept 5 hours the night before the recording session. They were recorded at 06:00, 08:00, 10:00 and 12:00 h. 24-h sleep deprivation produced lower levels of all components of attention during the morning, whereas partial sleep deprivation reduced tonic alertness and increased reaction time. These effects on the components of attention can reduce the efficiency at work and school, and increase the risk of accidents during a morning shift.
The discrepancies between social and biological timing are reflected on shift worker's well-being. The agreement between work schedules and chronotypes could be one possible solution to increase job satisfaction and well-being among shiftworkers. The aim of this study was to verify the association between job satisfaction and chronotypes among nursing personnel, day and night workers. We also explored other variables which could be associated to job satisfaction as seniority at the hospital and at the same shift, sleep duration, quality of sleep, sleepiness (Epworth scale), willing to change and sleep timing. We have studied 514 nurses (registered nurses; licensed practical nurses and nurses assistants), mean age 34.9 years old (SD 9.5), who worked at a public university hospital. Chronotype was calculated by using the morningness-eveningness questionnaire (MEQ). The correlation between job satisfaction and independent variables was checked by Spearman test or, in case of normal distribution, by Pearson’s correlation test. Significant variables were tested in a linear regression analyses separated for day and night shift. The analysis was adjusted by job category, age and sex. The final model showed that for the day workers sleep quality and MEQ score were variables associated with job satisfaction, meaning that the higher the morningness the higher satisfaction was found among day workers. In contrast, for those who work at night, the associated variables with job satisfaction were sleep quality and seniority, but not the chronotype. Our results suggest that working at night is not a solution for reducing discrepancies between social and biological timing even for evening types, since it is not reflected in job satisfaction.
Neurons of the hypothalamic suprachiasmatic nucleus (SCN) contain a molecular circadian clock that is entrained to the environmental light-dark cycle by a axons projecting from light-sensitive retinal ganglion cells via the retinohypothalamic tract (RHT). In individual SCN neurons the intracellular calcium concentration is a key component of the neural signaling pathways generating and entraining circadian rhythms. Whole cell patch clamp electrophysiological and calcium imaging techniques were used to study excitatory RHT neurotransmission to the SCN and changes of the intracellular calcium concentration in SCN neurons. Stimulating the optic chiasm at frequencies that simulated the firing frequency of light-sensitive retinal ganglion cells evoked excitatory postsynaptic currents (eEPSCs). The mean eEPSCs amplitude was stable at 0.08 Hz and was used as control. Stimulation at 0.5 - 50 Hz evoked short-term synaptic depression observed as a frequency dependent reduction of the steady-state eEPSCs amplitude. In spite of the synaptic depression, the total charge transfer into SCN neurons during first second of stimulation increased in a frequency-dependent manner and saturated at 20 Hz. Similar stimulation of the RHT evoked a variety of Ca2+ responses in SCN neurons. The amplitude of calcium response was dependent on the stimulation frequency and duration but independent of the time of day. RHT stimulation induced excitatory postsynaptic potentials (EPSPs), which generated action potentials and increased calcium in both the soma and dendrites in SCN neurons, while EPSPs alone produced no detectable change in calcium. The rise in calcium induced by RHT stimulation was directly dependent on the number of action potentials fired by the SCN neuron.

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The ability to generate intrinsic circadian rhythms in electrical activity appears to be a key property of central pacemaker neurons and one essential to the function of the circadian timing system. Previous work has demonstrated that suprachiasmatic nucleus (SCN) neurons express the fast delayed rectifier (FDR) potassium current and raise questions about the function of this current. Here, we report that mice lacking both Kcnc1 and Kcnc2 genes (double knockout, dKO) fail to express the Kv3.1 and 3.2 channels in the SCN as well as exhibit a greatly reduced FDR current. SCN neurons from these dKO mice exhibit reduced spontaneous activity during the day as well as reduced NMDA-evoked excitatory responses during the night. Interestingly, the daily rhythm in PER2 expression in the SCN was not altered in the dKO mice though the photic induction of c-FOS was attenuated. Behaviorally, the dKO mice exhibited extremely disrupted daily rhythms in wheel running behavior. In a light/dark cycle, some of the dKO mice were arrhythmic while others expressed a diurnal rhythm with low amplitude and significant activity during the day. When placed in constant darkness, the dKO mice exhibited low amplitude, fragmented rhythms and attenuated light-responses. Together, this data is consistent with the hypothesis that the FDR current is critical for the generation of robust circadian rhythms in behavior as well as the synchronization of the circadian system to the photic environment.
The role played by intracellular calcium in the physiology of the principal mammalian pacemaker, the suprachiasmatic nucleus (SCN), is not completely understood. The aim of this project is to study the participation of intracellular calcium-handling proteins such as the Ryanodine Receptor (RyR), Inositol-trisphosphate Receptor (IP3R) and calcium-dependent ATPase pump (SERCA) in the circadian rhythmicity of SCN. Results from Dr. Gabriella Lundkvist’s laboratory using unilateral SCN cultures from transgenic mice carrying luciferase as reporter gene for PERIOD2, showed a severe damping (period and phase were undetectable after this treatment) after treatment with the SERCA inhibitor cyclopiazonic acid (CPA: 10, 20, 50 and 100 µM during 3 days). Short term CPA treatment (50 µM for 2 h) showed a phase delay of ~2 h on PER2, without change in the period. This means that SERCA inhibition by CPA is washable but with an apparent PER2 phase shift. SCN explants were treated for 2 h with 80 µM (inhibitory) and 100 nM (activatory) of ryanodine at 2 different times: peak and trough of PER2 (early morning and early evening, respectively). No effect on phase and period was detected in these experiments. These results suggest: 1) SERCA seems to participate in the molecular clock machinery; 2) RyR is not affecting PER2 rhythmicity. Further experiments using IP3R inhibitors (i.e. 2-APB or xestospongin) are necessary in order to characterize the possible participation of this intracellular calcium release channel in the circadian rhythms of PER2 in SCN cultures.

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The daily control of behavior and physiology in mammals is subject to age-related changes like fragmented sleep-wake rhythms and low amplitude as well as mistimed rhythms in many bodily functions. One of the factors contributing to a loss in temporal order with age seems to be a weakening of the neuronal pacemaker residing in the suprachiasmatic nucleus (SCN). The degree and level of age-related deficiencies in SCN function is largely unknown although it has been suggested that aged SCN neurons are less synchronized within the neuronal network generating a lower amplitude timing signal.

The aim of our study was to identify cellular components of pacemaker neurons affected by aging in mice. Using patch clamp technology, we recorded membrane properties and ion currents of SCN neurons in brain slices of young (3-6 month) and old mice (> 20 month). Old mice exhibited striking deficits in neuronal properties with no significant circadian rhythms in resting membrane potential or spontaneous firing frequency. Recordings of fast delayed rectifier (FRD) and transient A-type potassium current (IA) did reveal age-dependent deficiencies in both potassium currents, but not in the slow delayed rectifier. Especially the lack of circadian modulation in IA in old SCN neurons offers a possible mechanistic explanation for the lack of circadian frequency modulation.

To evaluate the effect of cellular deficit on the network level, we recorded multiunit electrical activity (MUA) rhythms in SCN slices. Surprisingly, we found significant, but rather small age-related changes in amplitude and shape of the MUA rhythms. We suggest that cells in MUA recordings were more imbedded in the neuronal network of the SCN. Although we found that coupling and synchronization within SCN network was also affected by age the network still seems to be able to compensate for more drastic and earlier age-dependent deficits in cellular physiology.
FROM THE NUCLEUS TO THE MEMBRANE: Ca$^{2+}$ RELEASE FROM RyR SIGNALS TIME IN SCN NEURONS

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Cytoplasmic Ca$^{2+}$ mobilized from intracellular Ca$^{2+}$ storages may be one of the first transmission elements linking the molecular oscillator to the circadian modulation of firing rate in SCN neurons. Intracellular Ca$^{2+}$ homeostasis in the SCN is under circadian control and its manipulation affects expression of overt circadian rhythms. In SCN neurons recorded from brain slices by perforated patch, it has been shown that pharmacological opening of the intracellular RyR-Ca$^{2+}$ channel increases its firing frequency, whereas the closure of the channel decreased firing rate. Chronic administration of different doses of ryanodine directly into the SCN affects behavioral circadian rhythms in rats. Activation of RyRs by ryanodine 100 nM leads to shortening of endogenous period, whereas inhibition of the RyRs by ryanodine 100 µM disrupts circadian rhythmicity. Since period and phase of the rhythm returned to basal values of period and phase after the treatment, the changes in overt rhythm seem to involve the output rather than the clock mechanism itself. In the present work we studied the effect of 100 nM ryanodine on BK currents in SCN neurons recorded by perforated patch. We studied the tail currents induced by 50 msec voltage pulses (-60 to 60 mV, 20 mV steps). Basal recordings were made in Krebs solution added with TTX (1mM) and bicuculine (10 µM); experimental recorded were made adding ryanodine (100 nM) to the previous solution (n=8). Ryanodine administration increase the BK current in about 12.5% of recorded neurons, decreased in 62.5% and has no effect in the remaining 25%. This suggests that the effects of ryanodine on SCN firing frequency involve modulation of BK currents. These findings support the role of intracellular RyR-Ca$^{2+}$ channels in the output pathway from the molecular circadian oscillator in the SCN.

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Thematic Session: 3

S3.1 DISTINCT MOLECULAR MECHANISMS IN THE SUPRACHIASMATIC NUCLEUS UNDERLIE CIRCADIAN CLOCK PHOTOENTRAINMENT BY ADVANCES OR DELAYS

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Photic entrainment of the circadian clock’s oscillation insures a stable phase relationship between the clock and the environmental light-dark cycle. Possible molecular substrates in the suprachiasmatic nucleus (SCN) were investigated by entraining hamsters to T cycles (non-24-h light-dark cycles) consisting of a single 1-h light pulse repeated as either a short (23.33 h) or a long (24.67 h) cycle; under these conditions, the light pulse of the short cycle acted as "dawn," whereas that of the long cycle acted as "dusk." Analyses of the rhythmic, photo-inducible clock genes Per1 and Per2 revealed fundamental differences in their expression under these two entrainment modes. Light at dawn accelerated the clock, advancing the onset of the Per1 mRNA rhythm and acutely increasing mRNA transcription, whereas light at dusk decelerated the clock, delaying the offset of the Per2 mRNA rhythm and tonically increasing mRNA stability. The results provide insights on the nature of SCN “morning” and “evening” oscillators and the possible roles of non-parametric and parametric entrainment mechanisms.
THE OSCILLATORY CARNIVALE: SYNCHRONIZATION, DESYNCHRONIZATION, AND RESYNCHRONIZATION OF THE CIRCADIAN CLOCK

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The non-parametric synchronization to light-dark (LD) cycles of the suprachiasmatic nucleus (SCN) is determined by differential phase-shifting established by circadian-dependent transduction pathways, which is predicted by phase-response curve (PRC). Light-induced phase advances during the late subjective night occur after Glutamate/Ca2+ activation of neuronal nitric oxide synthase, through phosphorylation by its Ca2+-dependent kinase II, increasing nitric oxide (NO) levels. Intracellular NO enhance the activity of guanylyl cyclase, increasing cyclic guanosine-monophosphate (cGMP) levels, whose dependent kinase activity is a putative transcriptional regulator of the period 1 gene, necessary for phase changes. A regulatory step in this pathway is the degradation of cGMP by phosphodiesterases (PDEs). We have found that extracellular NO levels are implicated in the SCN communication in the non-parametric synchronization to advancing 23-h LD cycles (T23 cycles, but not to delaying T25) wheel-running rhythms in golden hamsters. Critical synchronizing conditions were also studied, under different chronic LD-shifts (i.e., chronic jet-lag CJL) schedules. A particular advancing schedule (6h advances every 2 days) induced the desynchronization of the general activity rhythm of C57/BL6 mice into two stable circadian components: one with a period of ≈21h, was driven by the light schedule, and the other with a >24h period. We developed a mathematical model of two coupled circadian oscillators that was able to explain the desynchronized patterns, and predicted the experimental results later found under different CJL protocols. Finally, pharmacological tools were employed for the resynchronization of the circadian clock. Even above the saturation curve, increasing cGMP levels by inhibition of PDE produced proportional increases in light-pulse behavioral advances in hamsters. This manipulation of light effects accelerated the resynchronization to a single LD-advance in a jet-lag protocol. These experiments provide a framework for studying the link between circadian synchronization and physiological alterations, as well as putative pharmacological tools for its prevention.
Overweight (OW) and obesity are increasing problems in industrialized countries; recent studies have related obesity with changes in the circadian regulation. Females of volcano mouse *Neotomodon alstoni*. Previous studies in males showed that OW mice are less efficient entraining to the jet lag protocol and they have shorter phase shifts to light pulse than controls. The aim of the present work was to study if the OW and control adult females of *N. alstoni*, either have effect in the entraining of circadian locomotor activity to a jet lag protocol and in phase shifts when exposed to a 1 h light pulse (200 lux). Locomotor activity was recorded by means of infrared light beams in a no-running wheel environment. Two experiments were set, first, controls (51.15±1.3 g) and obese (70.47±2.1g) mice were exposed to LD 12:12 photoperiod and 6 h advances and then delays were set every 2 weeks respectively. In this experiment no differences in transient cycles were found, and that is different to the observed in males. A second experiment consisted on animals exposed to LD (12:12) for at least 10 days, thereafter, mice were maintained DD. Ten days later, a light pulse was given at different circadian times (CT 02, 06, 14, 22) and phase shifts were estimated. In the OW animals phase delays are significantly reduced compared with controls, phase advances have not differences. Our results indicate that phase shift induce by light in females of *N alstoni* seems to be affected by the overweight conditions in delay zone, and that this species could be of particular interest to study the effect of obesity upon circadian physiology. Supported by PAPIIT IN225311
Feeding schedules have shown to be powerful timing signals driving behavioral, metabolic and hormonal systems. To the present time the location of the food entrained pacemaker is not known. A series of brain structures regulating metabolic homeostasis, alertness, movement and food reward are entrained to feeding schedules and their disruption results and specific alterations of food entrained rhythms including the reduction of intensity in food anticipatory activity. The clock gene machinery as we know it today is not sufficient to explain food entrainment, because mice or rats lacking one of the canonical genes can still anticipate a scheduled meal and exhibit entrainment of peripheral rhythms by feeding schedules. Present evidence suggests that food entrainment results from a system, build up by different oscillatory processes and is constituted by a yet unidentified network of central and peripheral structures. Food entrainment is initiated by a repeated metabolic state of scarcity that drives an oscillating network of brain nuclei in interaction with our organs, the amount of starvation as well as the quality of food may determine the intensity of food anticipation and cellular entrainment in different organs. Oscillations driven by energy availability in the cell and/or by temperature may be the signals that restart and entrain cellular processes in peripheral oscillators. In this presentation possible mechanisms that constitute the food entrained system will be discussed as well as the contribution of food entrainment to the SCN driven circadian system. Acknowledgements: The experimental work to be presented in this symposium is funded by CONACyT 82462 and by DGAPA PAPIIT IN-203907.
The role of social cues in circadian synchronization has been investigated in social mammals. In natural conditions, the social cues have a secondary role in relation to the light-dark cycle. In the absence of other cues, there is an increase in the circadian system response to social cues, such as observed in bats in caves or in beavers and voles over wintering under snow. In primates, social cues have become dominant over other stimuli in regulating behavior over the course of evolution. However, the effect of these cues on circadian activity rhythm was evaluated in few species. In initial studies conducted in common marmosets, a diurnal primate that shows strong social bonds within the group, the social cues were suggested as a weak zeitgeber. In these studies, the mutual acoustic social contact induced only pseudo-splitting and relative coordination between isolated males. Also, social entrainment was observed between pairs that were in auditory contact only among highly familiar individuals. Although in recent studies, we observed stable synchronization by entrainment between isolated males under LL conditions and the rest of the marmosets’ colony maintained outdoor, in natural LD cycle. The role of social cues on photic synchronization was also observed. After phase advances of LD cycle, the presence of a previously synchronized conspecific accelerates the synchronization of the circadian activity rhythm of isolated males. The mechanisms of social entrainment in marmosets are unknown; some authors suggest the action of non-specific arousal elicited by conspecific vocalizations. To evaluate this, we applied pulses of 1-h of conspecific vocalization in animals maintained in LL-conditions. We suggest that the duration and the composition of vocalization of the pulse applied were not sufficient to promote entrainment. Thus, additional evaluation of pulses of higher duration and different composition are necessary to clarify the characteristics of the social cues and their effect on the circadian system in marmosets. 

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Daily entrainment mechanisms of circadian oscillators have long been investigated in model species, under systematic manipulations of light/dark cycle parameters in the laboratory settings. In particular, it has been shown that even daily minute light pulses are able to entrain circadian oscillators, linking biological clock studies with physical theories of periodically pulsed oscillators and synchronization. These are gross models of what occurs in nature, but have yielded considerable understanding of entrainment mechanisms, motivating the construction of experimental Phase Response Curves (PRCs). A parallel line of investigation, launched by P.de Coursey (1986) have considered how organisms are daily exposed to the light/dark cycle in nature, questioning the representativeness of artificially imposed light/dark conditions of the lab and mathematical models based on PRCs. Here we present our joint field, laboratory and modeling work investigating daily entrainment and light exposure patterns of subterranean rodent species known as “tuco-tucos” (Ctenomys cf. knighti, Rodentia: Ctenomyidae), which are native of South America. Our studies have revealed one more rodent species that switches from diurnality to nocturnality when transferred from field to lab conditions and we use both the laboratory measured PRC and mathematical modeling for approaching this intriguing phenomenon.

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Thematic Session: 4

S4.1 LASC102

ROLE OF THE Drosophila CLOCK GENE IN CIRCADIAN AND NON-CIRCADIAN RESPONSES TO LIGHT

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The circadian gene, Clock (Clk), encodes a major transcriptional activator of the molecular clock in Drosophila and mammals. Despite its importance for generating a rhythm, little is known about the role of Drosophila CLK in the determination of circadian period and in behavioral responses to light. We have recently identified a novel hypomorphic allele of Clk that lengthens circadian period. The availability of this mutant allowed us to also assay the role of CLK in circadian responses to light. Previously, CLK was shown to regulate non-circadian effects of light on behavior. We now find that circadian responses are also affected in Clk mutants. Cellular and molecular mechanisms underlying these phenotypes will be discussed.

S4.2 LASC107

MOLECULAR DISSECTION OF CRICKET’S CLOCK SYSTEMS

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Most insects show daily rhythms in their physiology, including general activity. The rhythm is driven by an endogenous timing mechanism, so-called circadian clock. Although the molecular oscillatory mechanism has been extensively studied in the fruit fly, Drosophila melanogaster, recent molecular technology allows us to use crickets as a new model insect. We have investigated the molecular clock work and its role in photoperiodic time-measurement in the cricket, Gryllus bimaculatus and Modicogryllus siamensis. We first made molecular cloning of clock and clock-related genes such as period (per), timeless (tim), cryptochrome2 (cry2), clock (clk), and cycle (cyc), and found that per, tim, cry2 showed clear rhythms under light dark cycle (LD) and constant
darkness (DD). Experiments with RNA interference of these genes revealed that per and clk play an important role in rhythm generation since their knock-down resulted in a loss of rhythms both at molecular and behavioral levels, while tim is not essential for the rhythm generation. We also found that light-induced resetting of the clock was associated with transcriptional changes of clock genes. These properties are different from those of Drosophila but rather resemble those of mammalian clock. We also found that disruption of the circadian oscillation prevented the photoperiodic time-measurement in M. siamensis. Nymphal development of this species normally completes within two months in long-day conditions but takes nearly 6 months in short-day conditions. Maternal RNAi of the per gene disrupted the photoperiodic responses in nymphal development as well as the circadian rhythm in the progenies, suggesting the photoperiodic time-measurement requires the functional circadian clock.

The organization of activities inside a stingless bees colony depends upon several rhythmic components. Essential activities as egg-laying by the queen and foraging obey well defined temporal patterns. Among these patterns, a large diversity of frequencies is found going from ultradian present in the activity of the queen and that of young nurses until the infradian pattern of the seasonal diapause. Circadian rhythms can be detected as secondary components in several rhythmic expressions of nurses’ activity but its importance grows with age of the worker, becoming the major expression in foragers’ activity rhythm. As it is the case in other insect groups, circadian rhythms of M. quadrifasciata workers seem to be generated in the central nervous system, more precisely in the protocerebral region. Using classical histological and immune techniques it has been possible to detect probable components of the circadian system that apparently comprises a web of multiple oscillators - neurons and glial cells - which do not assemble together to establish a discrete structure. The region receive inputs from the visual system and the optic lobes neuropils: lamina, medulla and lobula are distinct and evident. In the protocerebral ganglion, two large neuropilar groups are present: corpora pedunculata, the large, bilateral “mushroom bodies”; and the central complex in the
median line. Under the calyces of the *corpora pedunculata*, neurosecretory cells have been detected. They present spherical nucleus and granular soma and they cluster together at the *pars intercerebralis* and *pars lateralis*. The use of markers for corazonin and pigment dispersing factor allowed the detection of putative output pathways leading to *corpora cardiaca* and *corpora allata*, in the retrocerebral complex.

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S4.4 LASC105

CRUSTACEAN β-PDH I BUT NOT β-PDH II AND THEIR PUTATIVE ROLES IN PIGMENT DISPERSION AND CIRCADIAN REGULATION

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Species that live in the intertidal zone are exposed to the 24-h light-dark cycle and to cyclic ebb and flow of tidal waters. Accordingly, both circadian clocks and biological clocks that oscillate in synchrony with the tide, namely circatidal clocks, have been described in several intertidal organisms. Whether these two biological timing systems share common neural and molecular mechanisms remains unknown. In an effort to identify biological clock components in an intertidal decapod crustacean, the crab *Cancer productus*, we recently cloned homologs of *Drosophila* genes whose expression is essential to sustain normal circadian rhythmicity. One of them is the pigment dispersing factor (PDF) gene, expressed by eight pacemaking ventral lateral neurons in each of the fly’s brain hemispheres. We have previously identified two members of the pdf family in *C. productus*, β-pigment-dispersing hormone I (β-PDH-I) and β-PDH II. The distribution of β-pdh I-expressing neurons in the crab’s brain is similar to that of PDF in *Drosophila*. Furthermore, β-PDH I shares closer sequence homology with PDF than PDH II does, and it is expressed in neurons that also show CYCLE-like immunoreactivity in the crab’s brain. Taken together, these results suggest that β-PDH I in *C. productus* may represent a functional homolog of PDF, which in *Drosophila* is a critical peptidergic signal to drive circadian outputs and to couple the circadian network. To further investigate this possibility we transformed *Drosophila* pdf-null mutants by overexpressing either β-PDH I
or β-PDH II neuropeptides in the PDF circuit. Both transformants expressed the corresponding mRNA and peptides in the PDF circuitry. Whereas the circadian locomotor activity of β-pdh I-expressing flies was virtually undistinguishable from pdf-transformed null flies, β-pdf II could only accomplish a modest rescue of some of the phenotypes associated with the loss of PDF function in the pdf01 mutant. Finally, PDH-I, but not PDH-II, was as efficient in stimulating the PDF receptor as the native Drosophila PDF in an in vitro assay.

S4.5 LASC14
SIXTH ABDOMINAL GANGLION MODULATES THE CIRCADIAN RHYTHMS OF LOCOMOTOR ACTIVITY AND ABDOMINAL POSTURE IN CRAYFISH

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Our previous observations on the electrical activity of the caudal photoreceptor (CPR), in the sixth abdominal ganglion (6th AG), in crayfish have been driven in the context of a distributed circadian-pacemaker system. We think that CPR also modulates the motor behavior in crayfish. The objective of this study is to analyze the effects of injury in the 6th AG on two functions: 1) spontaneous locomotor activity (SLA), and 2) abdominal postures (AP), both exhibited by crayfish in an aquarium. Experiments were made at regulated conditions in adult intermolt Procambarus clarkii crayfish with random gender. We formed three groups: 1) control, 2) injured by sectioning connective between fifth and 6th AG, and 3) injured by ablation of the 6th AG. Initially, all the crayfish were collocated in LD condition (12:12) by two weeks. Then, both experimental groups were taken back to LD condition by one or more weeks. Crayfish were then collocated for observation in LL conditions. Data were analyzed along 24-h cycle. The main results are the following. 1) For the SLA, the tree groups displayed circadian rhythmicity. Both experimental groups have shown changes of phase, with respect to control group. 2) AP were observed and classified into four categorical abdominal positions. We noticed circadian rhythmicity in the AP behavior in all groups. Both experimental groups showed changes on three of the four abdominal postures, out of phase compared with control group. In some cases ultradian rhythms were observed for both, SLA and AP. Taken together, these data suggest that the 6th AG modulates the locomotion activity and abdominal posture in crayfish, probably through the CPR or some possible neuromodulators.

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The crayfish Procambarus clarkii shows a variety of rhythmic physiological and behavioral processes controlled by the circadian timekeeping system. The multioscillatory and distributed nature of the circadian system of the animal has been proposed. Several authors have demonstrated the presence of circadian oscillators in retina, the eyestalk, particularly the X-organ and the brain, and recently 6th caudal ganglion but more experiments are required to fully explain the mechanism by which these putative pacemakers may interact and generate the precisely timed phases of daily rhythms. One important issue is to understand which are the biochemical and molecular mechanisms regulating the interaction between positive and negative transcription factors forming the crayfish circadian loop. Then here we investigated whether four proteins that interact in the feedback loop of the molecular clock described for *Drosophila* are expressed in the putative circadian pacemakers of crayfish: retina, eyestalk and brain and whether their abundance cycle in a circadian and daily manner consistent with elements described for the circadian clock of insects. Using immunochemical and biochemical methods we localized the presence of some of the clock proteins proposed for insects in the retina, eyestalk and brain of adult crayfish. By means of Western blot and statistical methods we analyzed their daily and circadian relationship. The cyclic changes in clock protein levels are associated to cyclic changes in their mRNA levels, so a different set of experiments were designed to sequence the per and clock mRNA and measure their daily expression pattern. Our results showed that CRY, PER, TIM and CLK are localized either in retina, eyestalk or brain neurons and neuropils. Their significant cyclic expression pattern in the eyestalk suggest that they interact as has been described for the homologous proteins in the *Drosophila* model. The molecular preliminary results on *per* and *clk* mRNA sequence, as well as their cyclic behavior suggest a certain identity with the same genes in other insects.
Abstracts

Posters
Considering that studying and working conditions of modern times have a strong tendency to improve, and that the night shift is a reality in favor of people who seek qualifications and have a desire to occupy a better position in the labor market, which is undoubtedly becoming more demanding, we investigated stress and its relation with diurnal preference in 116 students with a mean age of 24.11. This study analyzed the presence of stress and chronotype among college students in the University Center Herminio Ometto, Uniararas, by using two questionnaires: the Identification of Chronotype and the Lip Stress Symptom Inventory (LSSI). Chronotypes were classified as follows: 17 morning ones (14.7%), 31 evening ones (26.7%) and 66 (56.9%) indifferent ones. The results showed statistically significant data (Fisher’s exact test) for the variables “work” and “estages of stress” with high frequency alert (7.04%, n = 5) and “nearly exhaust / exhaust” (16.9%, n = 12) among students that worked and high frequency nonstress (32.56%, n = 14) and “resistance” (62.79%, n = 27) among those students that did not work. It was concluded that 66 (56.9%) students were at the stage of resistance and 53.4% (n = 62) presented more exacerbating psychological symptoms. The significant psychological symptoms of stress in different chronotypes and consequent stages of alert and near-exhaustion and depletion presented by students who study and work lead us to suggest that the set of information collected is used for some thoughts on training and favorable conditions for students who work and study in different shifts.
In the last years, the interest in the study of the individual difference known as circadian typology or chronotype is increasing. Several instruments have been developed for identification of circadian typology with proven reliability and validity. Objectives: The validation of the Munich ChronoType Questionnaire (MCTQ) in a sample of undergraduate students from Brazil. Methods: This cross-sectional study analyzed data from 246 subjects, university students from Brazil, aged 17-35 years who filled the MCTQ and MEQ in a Portuguese version. Preferences in sleep schedules were assessed taking into account workdays (study hours) and free days and the amount of exposure to ambient light. The mid-sleep on free days was corrected for sleep debt and the validation was made using the ROC curve. Statistical analyses (SPSS 18.0) included the Pearson’s correlation coefficient (r). Also discriminant analyses was used to define which variables of MCTQ presented the highest discriminant coefficient between evening-type and the other chronotypes. Results: The mid-sleep phase distribution in this sample was around 5:00 to 6:00 am (local time). MCTQ and MEQ showed normal distribution. The ROC curve showed AUC = 0.76 (CI95%: 0.70; 0.83), Sensitivity of 74% and Specificity of 68% for a cut-off = 5.5. MEQ presented negative correlation with mid-sleep phase corrected to sleep debt (Pearson correlation: r = - 0.48; p<0.001). The variables that presented the highest discriminant coefficient were MSF (0.89) and sleep onset in free days (0.86). The total discriminant coefficient was 70%. Conclusion: This study showed a good sensitivity and specificity. Also a good correlation between the MEQ and MCTQ questions related to
sleep times on free days was observed. But in discriminat analysis only MSF and sleep onset presented higher levels of discrimination between evening-type and the other chronotypes. This means that MCTQ and MEQ measure different dimensions of human behavior. This study is part of a project which is intended to continue evaluating an extended number of chronotypes, in Brazil.

P1.03

RETIREMENT, WRIST TEMPERATURE RHYTHM AND TIME ESTIMATION

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Introduction: The present study is intended to describe the relationship between healthy aging, and cognitive functions, particularly focusing on the time estimation capacity and circadian system parameters. Methods: Actimetry, wrist temperature, sleep log, Horne and Ostberg questionnaire, Pittsburg Sleep questionnaire (PSQI), quality of life questionnaire (WHOQOL) data were collected from young adults (n=15, 20-30 years) and older adults (n=16, 50-70 years). Following their usual daily routines, the subjects were instructed to perform a time estimation procedure that comprises the production of 10 seconds, in 5 different phases of day for 10 consecutive days. Results and Discussion: A circadian phase-dependent modulation on time estimation was found for both groups (morning vs. afternoon vs. evening sessions), and the magnitude of deviation or accuracy from the proposed 10 seconds was similar between groups, showing no age dependent modulation. The age-related differences found in the circadian timekeeping organization were only related to phase and not to amplitude or spectral power density and seem to be insufficient to impair the capacity of estimation of short intervals. Retirement is often seen, regardless of culture and nation, as the end of productivity and as a yellow sign to the lifespan. If one considers the relationship between retirement and biological ageing processes, however, there is apparently no direct correlation. Obviously, several aspects of the circadian timekeeping system change with age, including alterations in phase relationship of rhythms to the environmental zeitgebers, reduced sensitivity of the circadian pacemaker to time cues, decreased amplitude and stability of the circadian rhythms. Nonetheless, our group and others have described the importance of the exposition to strong signals, as the social zeitgebers and physical activity, on the preservation of a “healthy” circadian timekeeping system, culminating in the lack of differences between young and aged people.
SYNCHRONY IN CIRCADIAN ACTIVITY RHYTHM SOCIALLY ADJUSTED IN JUVENILES COMMON MARMOSETS (Callithrix jacchus)

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In the circadian activity rhythm in marmosets, characterized as diurnal, the onset of activity occurs just before the light phase and end up two hours before dark. Although the light-dark cycle is the main Zeitgeber in this species, social cues also act as a weak Zeitgeber. The social cues are important for this species living in groups of 15 individuals, in which a breeding pair exerts dominance over the others in the group. The offspring are usually twins, and the twin brother is an important social partner, enhancing opportunities for social interactions. However, in the same-sex twins cortisol levels are highest during the juvenile stage in the future dominant animals. Moreover, in males, these changes are accompanied by higher levels of agonism and androgens. To evaluate the synchronization between the individuals in groups of marmosets, we calculated the correlations between the profiles of the motor activity of juveniles and other family members over the months. The motor activity of each family member was monitored by actiwatches (MiniMitter) from 5th to 12th months of life of juveniles. Families consisting of a dyad of twins (four male / female and one male / male) and their parents were kept under controlled lighting (LD 12:12h), temperature and humidity. The correlation of motor activity profile of each animal with other members was calculated for 15 alternately selected days at each age. To compare the correlations in each age according to the dyads (juvenile 1/juvenile 2; juvenile 1/mother, juvenile 1/father; juvenile 2/mother; juvenile 2/father, mother/father) in the three families studied, the repeated measures ANOVA was used. The correlation between twins was stronger than the correlations between the twins and their parents or between the breeding pair (Tukey, p<0.05) and remained high over the months (ANOVA, p<0.05). As was noted that the family consisting of a dyad of males showed the lowest correlations (Tukey, p<0.05), we performed a second analysis in which the correlations between twins of the five families were compared throughout the ages. From this analysis it was observed that the dyad of males showed the lowest correlation across all months (ANOVA, p<0.05). Considering that the twin brother is an important social partner is likely that it is a social cue stronger than the other family members adjusting the circadian rhythm of activity. However, this
correlation is weaker in the dyad of males than in dyads of different sexes, possibly due to competition between individuals of the same sex in this species. Despite these results, it is necessary to expand the collection of data with same-sex dyads to confirm this hypothesis.

The school schedule influences the sleep-wake cycle and can promote partial sleep deprivation and irregularity on sleep schedules. Therefore, the aim of this study is to compare the irregularity of the sleep schedules, daytime sleepiness and sleep quality of adolescents studying in the morning and adults working as teachers in the morning shift. The sample was 45 adults (38±9 years) and 150 adolescents (15±0.5 years) of both sexes. The time in bed and the irregularity were assessed from the sleep log by 7 days and the irregularity was obtained by the standard deviation of the sleep and wake up hours. The times of highest daytime sleepiness and sleep quality during the week and weekend were obtained from the questionnaire “Health and Sleep”. The comparison between groups was performed by ANOVA and $X^2$ (p<0.05). The bedtime (BI) and wake-up time irregularity (WI) were higher among adolescents (BI= 90±71min; WI= 117±52min) than adults (BI= 52±24min; WI= 57±30min). Time in bed increased on weekends compared with weekdays in adolescents (1h) and adults (51min-ANOVA; p<0.05). The time of higher sleepiness was between 8h-10h am for adolescents and 12h-14h pm for adults, during the week. Over the weekend, there was a decrease in the frequency of adolescents (50%) and adults (29%) who felt sleepiness ($X^2$, p<0.05). Both groups showed worse levels of sleep quality during the week compared with weekend. Therefore, teachers also have difficulties to fulfill morning classes, as has been observed in adolescents. Although this difficulty be higher among adolescents. On weekend, there was a significant improvement in the parameters related to sleep for both groups. Thus, it’s necessary to discuss temporal organization of the school schedule for adolescents and teachers, who can show losses due a partial sleep deprivation in the school days.
FLYING MICE: INTERNAL FORCED DESYNCHRONIZATION IN A MURINE MODEL OF CHRONIC JET-LAG

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We studied locomotor activity rhythms of C57/bl6 mice under a chronic jet-lag protocol (6 h phase advances of the light-dark schedule (LD) every two days (ChrA)). Periodogram analysis indicated two components of the activity rhythm: a short-period component (21.01±0.04h) that followed the LD schedule, and an independent long-period component (24.68±0.26h). Onsets of free-running rhythms after release in constant darkness (DD) were significantly predicted by both components. Our mathematical model of two coupled circadian oscillators subjected to different jet-lag protocols states that the system processes chronic jet-lag schedules as new zeitgebers with periods equal to 24 + Step (the ratio of shift size, in h, to the intershift interval, in days; i.e. the Step Components), that induce a higher level of desynchronization as Step Components increase. The model predicted a lesser degree of desynchronization under a 21h period zeitgeber (T21; step=+3/1=+3) than under ChrA (step=+6/2=+3), which was confirmed by experimental data. The model also predicted less desynchronization under phase delaying than under phase advancing protocols. Indeed, most mice subjected to a chronic delay of the LD cycle through 6h shifts every two days (Step= -6/2= -3) displayed synchronous entrainment, showing an activity rhythm with a period of 26.92±0.11h driven by the predicted 27h zeitgeber. All together, our results indicate that the increase of the Step Components emulates the effect of a decrease in the amplitude of the arising zeitgeber.

In this work we present a new model of circadian forced desynchronization through a chronic jet-lag protocol, and a mathematical model that not only explains the behavior found but also provides framework for understanding health issues associated with chronic phase shifting of the circadian system.

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EFFECTS OF A 30-MINUTES ADVANCE IN SCHOOL SCHEDULE ON SUSTAINED ATTENTION IN CHILDREN

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People are exposed to several types of changes in schedule, such as shiftwork and daylight saving time. An advance in the schedule produces changes in the sleep-wake cycle and sleep deprivation. These disturbances may affect sustained attention, which is the capacity to respond efficiently to the environment during prolonged periods. There are three indices of sustained attention: general stability of efficiency, time on task stability and short-term stability. The objective of this study was to determine the effects of a 30-minutes schedule advance on the indices of sustained attention in children. Participants were 21 elementary school students (13 males and 8 females; age: 10.94±0.65 years); attending school in a morning shift, from Monday to Friday. Recordings were made at school settings in February, when some elementary schools of the State of Nuevo León advance their start time, from 08:00 (winter schedule) to 07:30 (spring schedule); the other schools did not change their start time (08:00 h). Nine participants attended a school that did not change its schedule and 12 participants attended a school that advanced 30 minutes its start time. All participants kept a sleep diary during three days before and one day after changing schedule. All participants responded a continuous performance task (CPT) at 08:00 h, one Monday before and one Monday after changing schedule. Participants attending the school that change the schedule advanced their waking time by 32:24±9:45 minutes, and showed a lower level of short term stability as measured by the median longest hit runs (before change = 24±16.47 correct responses, after change 16.33±6.86 correct responses, T=3, p<0.01). After the change in schedule, this measure was also lower compared to participants that did not change schedule (median longest hit runs = 28.22±14.77 correct responses, U=26.50, p<0.05). In conclusion, a 30-minutes advance in schedule impairs short-term stability, an index of sustained attention of children, early in the morning.
Sleep deprivation produces sleepiness and tiredness, as well as impairment in the performance of many activities. These effects on performance could be due to a decrease in attention, which is the capacity to respond efficiently to the environment. This cognitive process has four different components: tonic alertness, phasic alertness, selective attention and sustained attention. The objective of this work was to assess the effects of 24-h sleep deprivation on the components of attention. Participants were 7 undergraduate students (3 male, 4 female), mean age=18.12±1.24 (16-20) years. All participants kept a sleep diary during eleven days. They were registered in the laboratory the day before sleep deprivation at noon (12:00 h); spent the night without sleeping in the laboratory, and were registered the day after sleep deprivation at 06:00 h, 08:00 h, 10:00 h and 12:00. At all these intervals they responded a continuous performance task (CPT), designed to assess the components of attention. Participants slept 5:52±1:33 h the night before sleep deprivation. After sleep deprivation there were not significant differences among measures taken from 06:00 h to 12:00 h, so an average of these measures was used for the statistical analysis. Participants showed less efficiency after sleep deprivation in tonic alertness (before=98.78±0.94 correct responses, after=85.28±8.78 correct responses; T=0, p<0.05), selective attention (before=67.59±14.43 correct responses; T=0, p<0.05), phasic alertness (before=94.70±5.06 correct responses, after=74.11±15.71 correct responses; T=0, p<0.05) and sustained attention (before=0.23±0.14 linear regression of correct responses, after=-0.44±0.13 linear regression of correct responses; T=1, p<0.05). There were not differences in the reaction time of any of the components. In conclusion, 24-h sleep deprivation impairs all components of attention. These effects could explain the reduction in efficiency of many activities observed in sleep deprived persons.
In the elderly, sleep disorders show positive correlations with memory impairment. This may be attributed to an imbalance in the regulation of internal timing mechanisms or to an insufficient exposure to environmental cues capable of entraining circadian rhythms. Thus, the objective of this project was to investigate possible relationships between the voluntary change of routine in the elderly with changes in their sleep/wake cycle, as well as possible links with memory. We studied a group of five volunteer elderly individuals attending an outreach program at our university. Data were collected in two moments: nine days before start the program and again nine days, three months later. Activity and wrist temperature data were automatically collected with Tempatilumi, the Horne & Östberg's chronotype questionnaire and the Pittsburgh Sleep Quality Index and a Word Memory Test. Data were arranged in series and analyzed using Statistic®. Overall, no significant changes were found in characteristics of nocturnal sleep and the test of memorizing words, when compared before and after participation in activities. However, the number of nighttime awakenings (p=0.009) and duration of naps (p=0.01) decreased, on average, after participation. This lack of significant results may be related to the suspension of the activities due to a strike at the university or because of the low number of participants. Since the observed number of naps and nocturnal awakenings may reflect both the sleep hygiene recommendations given during the research, as well as participation in social activities.
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Introduction: Rhythmicity can be seen in some variables in newborns, evolving from an ultradian to a circadian pattern. However there are doubts about the functional status of this system in the first months of life and how environment factors can influence the rhythmic expression in neonates. In this study we compare the evolution of rhythmicity in sleep/wake, rest/activity, body temperature and feeding behavior in fullterm and preterm babies in a neonatal care unit. Material and Methods: Nineteen preterm and seven fullterm babies were followed during their stay in the neonatal care unit since the first week of life until discharge. We recorded motor activity with an actigraph and wrist temperature with a skin thermistor. Sleep/wake, feeding and activity diaries were also filled by the neonatal care unit staff. Subjects were divided in five groups according to gestational age (GA). The resulting individual time series were used to build actograms and were divided in 7 days series according to GA. The Lomb Scargle periodogram and the Cosinor algorithms were applied to the resulting time series. Results and Discussion: A non-24-hour circadian rhythm in wrist temperature, with an unstable circadian period, is present for all groups since the second week of life, even for the most preterm babies. Otherwise, we found an ultradian pattern for motor activity for most babies until the 34th week of gestational age, when a circadian rhythm appears. Babies born with more than 34 weeks of GA and the fullterm ones show a dominant circadian rhythm in motor activity. Feeding behavior has exclusively a 3 hours rhythm for all groups. Sleep/wake and staff/mother procedures also have a 3 hours rhythm in preterm newborns while more than half of the fullterm babies also exhibit a circadian rhythm for both variables.
Alertness is a basic cognitive process that enables the person to produce general responses. Alertness supports other cognitive processes such as attention and memory. The objective of this study was to analyze circadian variations in alertness. 10 females and 2 male students, age: 18.31 years (± 2.39 SD), participated in this study. They were recorded in a constant routine protocol during 30 h. Rectal temperature was recorded each minute, and performance was assessed every 100 minutes using a continuous performance task (CPT) and a concurrent alarm task, with a duration of 12 min. CPT required using the right hand to press key 1 to any number (except “9”) appearing at the screen, key 2 to a “9”, and key 3 to a “4” after the “9”. The concurrent alarm task required to hold a key pressed with the left hand; if the key was released, an alarm sound indicated the participant to press the key again. Alertness was measured as sequences of omissions and sequences of commission errors in the CPT and frequency of alarms and latency to cancel the alarms in the alarm task. Rectal temperature and performance in the CPT showed circadian variations. There were circadian variations in frequency of alarms, latency to cancel the alarms and sequences of omissions. There were higher frequency of alarms (Friedman = 133.51, p< 0.001), longer latency to cancel the alarms (Friedman = 122.95, p< 0.001) and higher sequences of omissions (Friedman = 145.71, p< 0.001) during the night and early in the morning. Sequences of commission errors did not showed circadian variations (Friedman = 22.44, p = NS). In conclusion, there were circadian variations in frequency of alarms, in the latency to cancel the alarms as well as in the sequence of omissions, but not in the sequences of commission errors. These findings contribute to explain the time of day variations on daily activities such as driving a car as well as on the efficiency on many working activities.
Errors and accidents at work increase during the night; this could be due to circadian variations in inhibition and flexibility, two components of executive functions. Inhibition is the capacity to restrain inappropriate responses, and flexibility is the capacity to change responses according to environmental demands. The objective of this study was to identify circadian variations in inhibition and flexibility, using a shifting criteria task. Participants were 8 undergraduate students, age: 17.75 ± 0.46 years (17-18 years), 1 male and 7 female. They were kept in a constant routine protocol for 28 h. Rectal temperature was recorded each minute and responses to a shifting criteria task were recorded every 100 min. In this task, numbers “1” or “2” in color blue or red were displayed on a computer screen. Participants had to press keys “1” or “2” according to the following conditions. First, they had to press a key that matched the number displayed. Second, they had to press the non-matching key to the number shown; these responses were taken as indices of inhibition. Third, they had to shift the criteria (match, non-match) every 4 to 6 numbers, depending on the color of the number; these responses were taken as indices of flexibility. Rectal temperature showed circadian rhythms (mean acrophase = 15:45 ± 1:23; cosinor fit %R = 80.25 ± 11.83, p < 0.001). During the night and early morning, there was a decrease of efficiency in inhibition (Friedman = 42.03, p < 0.001), an increase of latency in inhibition (Friedman = 44.61, p < 0.001) and a decrease of efficiency in flexibility (Friedman = 66.07, p < 0.001). In conclusion, there were circadian variations in inhibition and flexibility. The decrease in these functions during night and early morning could be a risk factor for night shift workers.
Most of the people living in cities reduce their sleep during workdays, compared to weekends. Sleep reduction produces sleepiness, tiredness and a decrease in school and working performance. The decrement in performance could be due to a lower level in a basic cognitive process, such as attention. This cognitive process has four components: tonic alertness, phasic alertness, selective attention and sustained attention. The objective of this study is to determine the effects of sleep reduction on the components of attention. Participants were 32 undergraduate students, (age = 18.03±1.23 years, mean ± standard deviation). They were divided in two groups: an Unrestricted Sleep Group (USG, N=21) and a Restricted Sleep Group (RSG, N=11). The USG slept free and was registered in the laboratory at noon (12:00). The Restricted Sleep Group arrived to the laboratory at 06:00 h and was registered at noon (12:00 h). The night before the laboratory session, the USG slept 10:15±1:15 h, while the RSG slept 5:04±0:19 h. The sleep reduction group showed lower efficiency in tonic alertness (correct responses USG=99.03±0.82%, RSG=96.03±4.08%, U=50, p<.01), and longer reaction times in tonic alertness (USG=366.87±77.43 ms, RSG=431.00±70.16 ms, U=60, p<.05), selective attention (USG=455.16±66.87 ms, RSG=551.26±67.70 ms, U=27, p<0.001) and phasic alertness (USG=374.24±68.95 ms, RSG=453.48±50.08 ms, U=20, p<0.001) compared to the unrestricted sleep group. There were no significant differences in sustained attention. In conclusion, sleep reduction produces a lower level of tonic alertness and an increase in reaction time. These effects could explain the decrease in school and working performance.
EFFECTS OF SLEEP DEPRIVATION ON WORKING MEMORY

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Sleep deprivation affects performance of many activities. Working memory is crucial for performance; this cognitive process is the capacity to maintain information for brief periods (seconds). It has two storage components: phonological, essential for verbal information processing, and visuospatial, essential for visual information processing. The objective of this study was to analyze the effects of sleep deprivation on the components of working memory. Participants were 10 undergraduate students, age 17-18 years (mean = 17.5 ± 0.53 y). They responded phonological and visuospatial tasks at 20:00 h and 06:00 h, during this period they stayed awake all night in the laboratory. The phonological storage component showed lower percentage of correct responses at 06:00 h (20:00 h = 86.64 ± 1.99 %, 06:00 h = 47.76 ± 9.46 %; T = 0.00, p < 0.01). Also, the visuospatial storage component showed at 06:00 h, lower percentage of correct responses (20:00 h = 83.66 ± 3.03 %, 06:00 h = 69.08 ± 5.43 %, T = 7 p < 0.05) and higher reaction times (20:00 h = 901.88 ± 47.49 ms, 06:00 h = 1042.85 ± 42.55 ms, T = 8 p< 0.05). Sleep deprivation affects the phonological and visuospatial components of working memory, crucial for verbal processing required to reading, and visual information processing required for driving a car or solving arithmetic problems.

SLEEP-WAKE CYCLE AND ACTIVITY-REST CYCLE IN ELDERLY PEOPLE

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In elderly people the nocturnal sleep period shortens, with an associated increase in diurnal sleepiness and frequency of naps. These modifications in sleep can be due to changes in the period and the phase of the sleep-wake and the activity-rest cycle as age
advances. The objective of this study was to analyze the sleep-wake and activity-rest cycle in elderly people. It is expected that elderly people will present more awakenings, more naps and a fragmentation of the activity-rest cycle. Participants were 6 seniors (4 male, 2 female) mean age 70.33±7.87 (65-86) years, without neurological or degenerative diseases and without sleep disorders. All participants answered the morningness-eveningness self-assessment questionnaire, then answered a sleep diary and used a wrist activity monitoring device in the non-dominant hand, for 18 consecutive days. One participant was definitely morning chronotype (age: 70 years), two were moderately morning type (ages: 65, 67 years) and three were intermediate type (ages: 66, 68, 86 years). The participants slept from 23:10±1:37 h to 07:37±1:27 h (midsleep: 03:23±1:37 h; total sleep time: 8:25±0:26 h). They woke up an average of 1.26±1.34 times per night, higher frequency of awakenings occurred in the oldest participant (3.81±0.81 times per night). Only two participants took naps during the day (ages: 66, 86 years). Using a spectral analysis, an activity cycle with a 24:16±0:00 h period was found in all participants. Five participants also showed a 12:00±0:00 h, only the youngest of the sample did not show this cycle (age: 65 years). In conclusion, it was found that in elderly people, nocturnal sleep period shortens, awakenings increase and the activity cycle is fragmented into two cycles: 24 h and 12 h.

P1.16 LASC68
SLEEP PATTERN, LEARNING AND MEMORY, IN NURSING PERSONNEL WORKING IN A BOGOTÁ HOSPITAL, COLOMBIA, 2011

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Objective: to determine the relationship of: work time, sleep pattern, day time sleepiness, learning and memory, in nursing personnel that work in a hospital in Bogotá, Colombia in 2011. Background: A great number of studies consider night shift work as responsible for alterations in the sleep pattern. Also considerable evidence supports the role of sleep in learning and memory processes. Methods: a descriptive, exploratory and cross-sectional study was carried out based on a sample of 57 nurses: 28 nurses working in daytime shiftworks (6 work hours/day) and 29 working in night shiftworks (12 hours/day each other day) in a general hospital in Bogotá, Colombia. Social and demographic variables such as: age, years of work, years in shiftwork and sleep hours were included. An Epworth Sleepiness Scale (ESS-VS) - Colombian Version: "Prueba de Aprendizaje Auditivo-Verbal de Rey (PAAVR)" - was used in order to evaluate memory and learning.
To evaluate the nursing personnel chronotype, a Composite Morningness Scale (CSM) was applied. The instruments were administered by the researchers at the end of the shiftwork. Findings: This study showed that the participant’s slept hours/day mean was 7.11 hours (± sd 2.1 hours). Morning chronotype was the most registered with 59.6% and evening chronotype registered just 5.3%. Overall participants showed a normal day time sleepiness 42.1%, followed by excessive day time sleepiness high severity 31.6%. Significant differences between day and night shiftwork were not found in our study. Results using Prueba de Aprendizaje Auditivo-Verbal de Rey showed no significant differences between shiftworks. Conclusion: results reported here may be influenced by hour and date work time distribution at night shiftwork. In this hospital nurse personnel are allowed to rest three hours during the shiftwork. This rest may be beneficial to sleep debt and allows synchronization with activities they must develop after night shiftwork.

P1.17

Motor activity is being registered of 7 manatees in exterior captivity at the Aquarium of Veracruz, Mexico, with AW4 actiwatch actimeters for a year. The actimeters are programmed to collect data continuously, with a recording interval of 5 minutes, 24 hours a day during 12 months. Activity data has been collected from all individuals, obtaining Actograms and Periodogram through the day, along with behavioral data records during the photoperiod for posterior correlation analyses. This observation records are divided in morning and evening periods. Based on data from the U.S. Navy website (http://aa.usno.navy.mil/data/docs/RS_OneDay.php) with the location coordinates of Veracruz Aquarium (19º 11' 14.8'' N, 96º 07' 19.8'' W), average photoperiod data, sunrise and sunset (hr), as well as average of morning and evening twilight start (hr) was obtained. As preliminary data, the average intensity record of each individual was obtained; plus the feeding schedules of observation days for each individual were recorded. Our preliminary results show 4 important activity peaks, possibly related to the feeding schedules, suggesting an anticipatory behavior at feeding hours. However, more records are needed to corroborate this finding. This study is contributing in the chronoeological aspects that could be relevant for the species adaptation to diverse temporal
cycles, which are determined by geophysics phenomena and are very difficult to study under natural conditions. Actimetry can be a robust technique to obtain long term records of activity-rest rhythm, allowing the investigation of relationships of environmental factors that could affect the manatee activity cycle.

SLEEP HABITS, CHRONOTYPES AND OBESITY/OVERWEIGHT IN MEDICAL STUDENTS AT UNAM

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Adolescents and young adults have the capacity and the preference of staying up late at night and reducing the sleep hours. Recent studies indicate that short sleep leads to metabolic disturbance and propensity to overweight and obesity. Poor sleep is a common feature among medical students and a high incidence of overweight and obesity is reported among medical professionals. The aim of this study was to examine the chronotypes, sleep habits (quantity and quality) of medical students inscribed in the first two years in the Medical Faculty and to explore the association between obesity/overweight and sleep habits. We used a cross sectional design at the Faculty of Medicine UNAM, Mexico City. We assessed sleep quality with the Pittsburgh Sleep Quality Index (PSQI) and chronotypes with the Horne and Östberg questionnaire. We calculated the Body Mass Index (BMI) by auto-report of weight and height. We applied 1006 questionnaires and obtained 966 complete questionnaires for sleep quality, 984 for chronotypes and 986 for height and weight auto-report. The median of the subjective quantity of sleep hours during the week days was 5 hour for students in the morning groups and of 6 h for the afternoon schedules. With the PSQI we obtained a proportion of 73.9% bad sleepers. Men had lesser “bad sleepers” than women, 67.6% vs. 76.8% respectively (p<0.05) and the afternoon schedule have lesser bad sleepers than the morning students, 61.3% vs. 80.7% respectively (p<0.05). The more prevalent chronotype was the intermedium (76.8%), then the morning (16.2%) and finally the evening one (7.1%). There was a difference by sex, the proportion of women with early chronotype was 17.9% and that of men was 12.4% (p<0.05). The mean BMI was 23.25 kg/m² (+ 3.33 S.D.) Only 3.8% of the students had a BMI below normal, 71.9% was normal, 20.4% was overweight and 4% indicated obesity. We observed that early schedule students had lesser proportion of overweight/obesity than those of late
There was an association between chronotypes and obesity/overweight ($p = 0.003$). Conclusion: medical students have poor sleep quality and it’s poorest in women and early schedule. The chronotype was associated with overweight/obesity. Supported by CONACyT 82462 and PAPIIT IN224911.

**P1.19**

**MAPPING CHRONO PRE-METABOLIC SYNDROME IN MEXICO CITY**

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Introduction. Among attempts to reduce the prevalence of hypertension and diabetes have been developed preventive sequences: pre-diabetes and pre-hypertension. Since such chronic pathologies conducted to renal and cardiovascular irreversible damages and death. Under a chronomic perspective the study of temporal structure or chronome of blood pressure (BP) or glucose metabolism is possible to detect circadian patterns, variable vascular disorders (VVD) or syndromes (VVS). We had found in normal healthy persons circadian hyper amplitude tension (CHAT) and intolerance to glucose (hyperglycemia). Both factors constituted a pre-metabolic syndrome. Now after confirmed in different age and gender groups, it has been defined as chrono-pre-metabolic syndrome (CPMS), to differentiate from classical health lineal approaches related to the study of the metabolic syndrome itself. Methodology. At the Chronic Research Center of Escuela Nacional de Medicina y Homeopatía-IPN several medical graduated students studied in different Mexico City locations the BP chronome complemented with glucose tolerance test (GTT) in a total of 200 apparently healthy subjects of both genders from 18 to 65 years of age, by monitoring BP with ambulatory automatic instruments (A&D Co.) programmed at 30 and 60 min intervals during day-night, respectively during 2 to 7 consecutive days. The GTT test was performed with digital glucometer. Data was analyzed by the least squares approach of single cosinor method. Results. It was observed circadian rhythmic systolic (S) BP over 74% of studied populations. In young men was detected a circaseptan SBP. The incidence of Chat varied from 10 to 17%. Incidence of CSPM ranged from 4 to over 12.0%. Discussion. Detection of Chat and
CPMS seems to be useful chronomic predictive tools that must be wide extended in preventive and general medicine, in such manner that might reduced the progress to irreversible cardio and metabolic syndromes ending in cardio-renal vascular damages. Acknowledgment: ICyT-DF Grant: PICDS08-82.

EXPERIMENTAL CHRONO-HOMEOPATIC STUDY WITH *Aconitum Napellus* IN FEMALE MICE

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Introduction. *Aconitum napellus* (Acn) as a homeopathic remedy is used as a topical agent to relieve pain, itching and inflammation, and as an internal agent to reduce febrile states, among others. Objective. To explore the chronobiological effects of Acn upon the body temperature of mice treated at 6 different times over 24h.

Methods. BALB/c female mice were synchronized in six chambers each with 12h Light (L) - 12h Dark (D), but with L-onset staggered by 4h between chambers. Rectal temperature (RT) of all mice was measured in °C at baseline (B) or 1h after oral treatment with placebo (P) or two different doses of Acn (6C and 30C) in six studies over an 8d span: 1) B; 2) P; 3) Acn 30C; 4) Acn 6C; 5) Acn 30C; 6) Acn 6C. The difference in RT for each mouse from the respective B+P timepoint mean RT was computed following each Acn treatment, and data from each of the 6 studies were analyzed by ANOVA and for circadian rhythm by the fit of a 24h cosine.

Results. A significant rhythm in RT was found at B and after P, with no significant differences in rhythm parameters (mean (M) = 35.58 vs. 35.69 °C, acrophase (Ø) = 15:31h vs. 15:40h); B & P were combined. A significant rhythm was found in original RT after all Acn doses in studies 3-6 and overall for 30C and 6C. Acn induced hyperthermia overall for each dosage. The overall change in BT was also rhythmically significant for each Acn dosage (M = 30C: +1.95°C, 6C: +1.70°C), with greatest hyperthermia observed during the L-span for each dose (Ø = 30C: 08:56h, 6C: 05:17h). Conclusion. Acn administered to mice induced hyperthermia overall and in a time-dependent manner, with greatest effects during the resting (L) span.
DISCUSSION ABOUT THE INTERACTION BETWEEN MEDICINE AND HUMAN BODY BIOLOGICAL CLOCK

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Through literature retrieval, this paper discusses the interaction between the medicine and human body biological clock. Medicine is the important factor to affect the clockwork. Modern research also found that many medicines in the human body process will show their circadian rhythms change. That is to say, the different time of administration shows different clinical curative effect and different adverse reactions, this relates greatly to human body biological clock. This paper discusses the two aspects respectively, the influence of the medicine on human body biological clock and human body biological clock on medicine.

THE ASSOCIATION AMONG SLEEP QUALITY AND MID-SLEEP PHASE WITH USE OF ANTIHYPERTENSIVE DRUGS

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Objective: The objective of this study was to investigate the association of sleep quality and mid-sleep phase with the use of antihypertensive drugs. Design: Cross-sectional study. Participants: A total of 1047 subjects (351 men and 696 women) with an average age of 44 ± 12 years, living in an essentially rural area in south of Brazil. The population is a community of German descendants, significantly homogeneous in terms of culture, socio-economic level, biological factors, and daily exposure to environmental light. Main outcome measures: Sleep quality was evaluated using the Pittsburgh Scale (PSQI), and mid-sleep phase using the Munich Chronotype Questionnaire (MCTQ). An antihypertensive drug was measured by self-reported information through the use of standard drugs according to pharmacological ATC codes (adrenergic receptor agonists
and antagonists, calcium channel blockers, beta blocking agents and diuretics). Results: In this study, 20.4% (N = 214) of the participants reported use of antihypertensive drugs. Subjects using antihypertensive drugs showed significantly lower mid-sleep phase (t test = -4.82: p< 0.0001), poor sleep quality (Man Whitney test; p< 0.0001), and higher BMI (t test 10.82; p<0.0001), and age (t test 14.69; p<0.0001) than those not using antihypertensive drugs. There were no difference between genders (chi-square; p=0.224), smoking status (chi-square; p=0.487), and sleep duration on free (t test -1.36: p=0.174) and work days (t test 0.35: p=0.582). A multilinear regression model was used to control colinearity and potential confounding factors with self related antihypertensive drugs as a dependent variable. BMI, PSQI and mid-sleep phase were independent factors predicting the use of hypertension drugs (r2 = 0.25, F = 57.60, p<0.0001). Conclusion: In spite of the fact that this was a cross-section study, there was a clear association of poor sleep quality, and an advanced mid-sleep phase with the use of antihypertensive drugs.

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P1.23 LASC88

**TYPE 1 Diabetes mellitus (T1DM) ITSELF SEEMS TO HAVE NO IMPACT ON THE REST/ACTIVITY RHYTHM (RAR)**

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It is widely known that poor glycemis control in individuals with diabetes mellitus (DM) may impact sleep, decreasing sleep quality and leading to sleep fragmentation. At the same time, there are strong evidences that poor sleep quality, sleep disorders, and short sleep length favor insulin resistance, making it more difficult to maintain a good glycemic control in individuals with DM. In the present study, data collected during 10 consecutive days, with the Brazilian actimeter Tempatilumi, were analyzed in order to verify if there were differences between the RAR of people with T1DM and controls. The T1DM group
had 12 people of both genders, without chronic complication, and age mean of 25.4 ± 2.8. In the control group there were 7 people of both genders, and age mean of 29.7 ± 5.5. Although no difference between both groups RAR, in terms of period, amplitude, rhythmic potency, total variance percentage, and acrophases location was observed, the mean of total rest time was shown to be positively associated with the mean sleepiness including the individuals of both groups (r=0.64; p=0.003). Specifically in the T1DM group the night rest was positively associated with the mean sleepiness (r=0.59; p=0.04), which is not verified when the control individuals are included in the analyses (p=0.14). On the other hand, in the T1DM group, there is a tendency of association between the glycemic variability (glycemic SD) and the mean glycemia and the mean night rest duration (r=0.57; p=0.05 and r=0.54; p=0.7, respectively). Therefore, we understand that although there is no difference between the RAR of individual with and without diabetes, the glycemia level or variability may affect the phases of this rhythm.

P1.24

DIFFERENCES IN SLEEP AND DAILY METABOLIC PROFILES BETWEEN OVERWEIGHED AND NORMAL MICE Neotomodon alstoni

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Obesity is a public health problem of great importance in emerging countries, related to other disorders such as metabolic syndrome (MS), which leads to cardiovascular problems and type II diabetes. The causes of obesity are complex and recent research has found a link between the circadian clock, sleep patterns and energy balance. The volcano mouse (Neotomodon alstoni) is an endemic rodent from Mexican Neovolcanic Transversal belt. This species, in captivity and fed regular laboratory rodents diet, develops obesity in a high percentage of mice, as well as some signs characteristic of MS. Since previous studies on sleep and circadian rhythms in this species, makes it a suitable model where to study the affections of overweight upon circadian physiology. The aim of the present work is to analyze daily profiles of some metabolic-related blood parameters (leptin, insulin, triglycerides, corticosterone and glucose), and the main electrocorticographic characteristics of sleep-wake cycle, between normal (CTL) and overweighted (OW) volcano mice. In CTL mice, the results of this study showed that there are differences were found between the highest and lowest concentration in the daily profiles, of all the blood parameters (except the leptin). OW mice however did not show daily differences, although the average concentration in almost all the hours tested were
higher in OW than in CTL mice (except the corticosterone). The analysis of the sleep-wake cycle evaluating the temporal distribution of the vigilance states, indicate a polyphasic architecture. A trend was observed in OW animals to increase the percentage of Slow Wave Sleep and decreasing time Wakefulness compared to CTL. The percentage of Rapid Eye Movements Sleep was similar between groups. The differences observed in this species are consistent with other reports in which leptin deficient or obesity induced rodents, shows alteration in the sleep-wake pattern. From this and other related studies, we propose the volcano mice as a good model for studying the daily rhythms and metabolic disorders related to obesity, mainly from the cyclical patterns of metabolites associated to energy balance.

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**P1.25 CHRONO HOMEOPATHIC ACTIONS OF Aconitum napellus UPON HUMAN HEART RATE**

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Introduction. Aconitum napellus (Acon) as a homeopathic remedy is used as a topical agent to relieve pain, itching, inflammation, and as an internal agent to reduce febrile states. Acon, one of the most poisonous known steroidal alkaloids, is safe only in extremely minute oral doses and has a short duration of action. Any circadian time-related consequences of Acon administration of this effect are unknown. Objective. To explore the effects of Acon at very low dose upon human heart rate (HR) circadian rhythm. Methods. It was invited to participate 20 post graduate students (10 female and 10 men) of Escuela Nacional de Medicina y Homeopatía-IPN, from 18 to 50 years of age under bioethical consent form. All of them monitored HR and blood pressure (not presented). Once basal HR profiles were obtained, two groups of students were randomized in such manner to received either globules with Acon 30H (Similia Co.) or placebo (ethanol 83%=Pl) at diurnal or evening times, during 2 to 7 days. Data was analyzed for circadian rhythm by the least-squares fit of a 24h. Rhythm detection was considered significant if p<0.05 that allowed detection of Amplitude (A) MESOR (M) and Achrophase (ø) Results. It was detected circadian rhythm of HR in 37 circadian profiles (92.5%). Pl induced increment of M-HR and Acon.30CH did not showed apparent effect
on M-HR. However, showed an apparent increment of A-HR. Individual observations manifested A-HR increment in 6 subjects (60%). Diurnal Acon induced ø advancement from 4.5 and 6 h. Nocturnal Acon induced M increment in two subjects (6,14). Conclusion This is the first chronopilot clinical assay where homeopathic compound was explored on the HR temporal structure or chronome of healthy subjects. Acon 30H did not show adverse toxic adverse effects, but chronomodulated actions on the timing and Amplitude of HR.

P1.26
ALTERATIONS IN THE CHARACTERISTICS OF CIRCADIAN VARIATION OF BP IN NON-DIPPERS

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The present study was planned to examine BP variability in the normotensive human population drawn from southern-central region of India. Further, the relative role of the predictors, such as age, BSA and BMI was also studied. One hundred seventeen (117) subjects, aged between 6 to 80 years (median = 24 years), participated in the study, voluntarily. The Systolic blood pressure (SBP), diastolic blood pressure (DBP), and pulse rate (PUL) in each subject were monitored at least for two consecutive days, using ABPM (TM2430-13). The measurements were recorded every 15-minutes during day time and every 30 minutes during night time. Derived variables, such as mean arterial pressure (MAP) and double product (DP) were computed. Data were analyzed using A&D analysis software (TM2430-13), Cosinor rhythmometry and SPSS (10.0). The subjects were classified as dippers (10-20% dip, clinically normal), extreme dippers (>20% dip), non-dippers (<10% dip) and risers (daytime BP less than that in nights). Out of 117, 52 subjects displayed deviated nocturnal dipping in blood pressure. There were only three risers; therefore the data on them were excluded from group analysis. Subjects were also grouped as function of age. Non-dipping was more prominent among elderly subjects (> 45 y). Further, BSA predicted SBP, wake time SBP, DBP and MAP significantly, whereas BMI was found to be a significant predictor of wake time DBP and sleep time MAP. Independent of age, BSA and BMI, the acrophases of DBP, PUL, MAP and DP occurred in late afternoon between 14:00 to 16:00. However, the occurrence of acrophase of SBP significantly delayed in non-dippers as compared to dippers and extreme dippers. The amplitudes of DBP and MAP had an inverse relationship with the age. It is concluded that non-dipping is more frequent in elder subjects (>45 years) in southern-central Indian
population and that the non-dippers display decreased amplitude and later acrophase as compared to dippers and extreme dippers.

P1.27

EMERGING LEADERS IN THE AMERICAS PROGRAM (ELAP)

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The Government of Canada, through the Department of International Affairs and Trade, offers scholarships to Canadian publicly funded universities or colleges as part of the Canadian Commonwealth Scholarship Program (CCSP) and Government of Canada Awards (GCA). The goal of the Emerging Leaders in the Americas Program (ELAP) is to support the development of human capital and a next generation of Leaders in the Americas while strengthening the linkages between Canadian post-secondary institutions and the ones in Latin America and the Caribbean. ELAP scholarships allow talented students to engage in international research projects that: a) provide tangible added value to each student’s educational program and career development; and b) introduce students (and their Canadian hosts) to new ideas, techniques, and research linkages. We are pleased to announce that this year 400 scholarships were awarded under this program in diverse areas of inquiry including commerce, technology, health and social sciences, and the arts. Significantly, 4 awards were given to Chronobiology students who will study at the University of Toronto Centre for Biological Timing and Cognition (CBTC), Toronto, Ontario, in collaboration with the CBTC at the Lakehead University in Thunder Bay, Ontario, and the CBTC at Trent University in Peterborough, Ontario.
Sleepiness and sleep complaints are common among professional drivers. Sleepiness is a problem that affects the drivers well-being, and has consequences in their performance and safety. Assessment of prevalence and research into the risk factors are an important health issue and as public transport drivers have a high prevalence of sedentary habits, diabetes and obesity. Our aim is to describe the distribution of chronotypes and explore the association between sleep patterns and risk factors for metabolic syndrome (MS) in this population with the collaboration of the Centro para el Fomento de la Educación y la Salud de los Operarios del Transporte Público de la Ciudad de México (CENFES, AC).

The Horne-Östberg questionnaire, the Sleep Hygiene Index (SHI), the fatigue severity scale (FSS), the obstructive sleep apnea syndrome (OSAS) questionnaire and a medical examination and laboratory analyses, (both of these were made in the medical unit of CENFES, AC) were completed by 200 professional public transport drivers during september and august 2010. The mean (SD) age of the population was 39.4 (11) yrs. The 83% of them have a matutine chronotype, 45% report a sleep onset latency >30 minutes. The mean (SD) SHI score was 21.9 (4.5). The 38% had a score >10 for the the FSS, the mean (SD) score was 21.8 (11.73). The prevalence of OSAS was 38%. Out of the drivers analyzed, 35.5% had obesity, 41% were overweight, 25% had glycemia >110 mg/dl and 15% had hypertension. These results show that the prevalence of risk factors for MS and sleep disorders in this study was higher that the observed in other studies for general population. As different health problems were identified, we are working in the design of a systematic education about these disorders as well as health promotion interventions.
Research in the field of biological rhythms requires precise quantitative and qualitative tools for the signal analysis of time series. The methodology for analysing a biological rhythm has been the subject of much investigation. Nonetheless, many issues still have no definitive answer, for example, in the search for periods and phase markers there are various methods and the researcher must be able to determine those that are the most appropriate and reliable in a particular case. Furthermore, it happens that all those methods are not included in a single software package. The present work is aimed to describe a system for the signal analysis of chronobiological data. This custom-made software, named DiSPAC, had several options for analysis and display. It includes the following analysis: spectral density, periodogram, cosinor, auto-correlation, and phase plane. The graphics and display features include: graphic estimation of phase and period, rhythm architecture, stability analysis and four types of actogram. DiSPAC was tested with real and artificial data sets to determine the reliability of the algorithms used to calculate the outcome measures reported. This allowed the building of a solid and reliable software application. The DiSPAC software was written in C/C++ for MS-Windows operating system. At the highest level of operation the DiSPAC software has a graphical user-friendly interface and it was able to provide a range of outcome measures from chronobiological data. Supported by grant CONACyT 49740 and FONCICYT 91984 funded by the European Union and CONACyT, PAPIT IN204811.
In Cuba breast cancer incidence rate grows alarmingly. In the year 1978 it was 24.6 being presented 1169 new cases of this illness. Already in the year 2002 were diagnosed around 1800 new cases, with an incidence rate of 35.1 for each 100,000 women, occupying today in our country the first place in incidence in all cancers that affect the feminine sex and the third place in both sexes. To face this challenge of the public health in Cuba new therapeutic resources they are developed that enlarge the conventional therapy. Inside them they are the coming ones of the treatments optimization with chrononcology, the immunotherapy and the natural medicine. In this context it is very diffused the therapy in the entire world with pharmacological natural phytocomplex with *Viscum album*, a hemiparasit plant with substances that have been demonstrated goods actions as immunemodulater effects on the linf cells implied in the detection, recognition, attack and clearing of transformed cells, cytotoxic and proapoptotic effect on the tumoral cell that opens a road toward a holistic vision that has in it counts the what, how much and how it is administered, but also to who, when and where. One of the most excellent properties in *V. album* phytocomplex is the presence of biologically active substances with a vectorial polarity that transcends the pharmacological, morphological and ecological dimensions. This dynamics could be represented at molecular grade and in function of the time, for the presence of three macro-molecules groups that act as dynamic attractors and that they explain as much its therapeutic effect as resinchronizator of biological rhythms (chronobiotic effect). To characterize and evaluate the clinical effectiveness and temporary optimization of the *Viscum album* preparations, we mensure in the present work, with the use individual tempactilumi sensors, capable to register the corporal temperature, the movement (actimetry) and intensity light external exposition that enlarge and supplement the clinical possibilities of mensuration of variable as quotient frequency cardiac/respiratory rate in the exhaustive study of human rhythms and metabonomic profile in patient with breast cancer.
It is few known about the action of GABA outside of the nervous system, firstly in the liver with circadian rhythms implications. This organ has all the GABAergic elements: the molecule, the sintetics and catabolic enzymes, transports, as well the hyperpolarizant action of GABA in hepatic slides and cells. In a previous microarrays experiment, we found many changes in the hepatic transcriptional level of classical neurotransmitors systems, like GABA, glutamate and serotonine, follow an feeding restriction protocol which promote the expression of a biological clock sincronizaced by the food access (FEO). In this proyect we propose that the food restriction will provoke changes in the expression and activity in GABA receptors, as well in the catabolics and sintetics enzyme of GABAergic system in the liver. The general goal is characterized if there are changes in the GABA A (subunit a5) and GABA B (subunit 2) by Western bolt and immunohistochemistry experiments; we want to quantifyied the succinate semialdehyde deshydrogenase activity, GABA transaminase (catabolic) and glutamate descarboxilase (anabolic) In our protocol we use Wistar male rats in a food restricted schedule by three weeks in which we present the food from 12:00 to 14:00. The controls are rats with: 1) Ad libitum food, 2) Fasting rats of 24 and 48 h, and 3) Re-feeding rats with a 24 and 48 h of fasting but two hours of food before de sacrifies.
Restricted feeding schedules (RFS) promote numerous metabolic, physiological, and chronobiological adaptations to optimize the handling of nutrients. This condition activates responses in hypothalamic and midbrain areas, as well as in peripheral organs involved in nutrient and energy metabolism that form part of a circadian clock known as the food entrained oscillator (FEO). RFS is associated with marked behavioral arousal previous to food access known as food anticipatory activity (FAA), and an extreme hyperphagia after the 2-h mealtime. Food restriction induces adaptations in the metabolic handling of energetic substrates by the liver and the adipose tissue during the FAA. For example, lipolytic release of free fatty acids (FFA) and production of ketone bodies are largely increased, whereas in the liver, the levels of triacylglycerols are reduced, but glycogen is only partially decreased. Fatty acids act as endogenous ligands of peroxisome proliferators-activate receptors (PPARalpha, beta, and gamma), which are transcriptional factors that influence peroxisomal and mitochondrial activities. PGC1-alpha coactivates PPARalpha starting the transcription of genes that encode for enzymes participating in the mitochondrial beta-oxidation of fatty acid, such as carnitine palmitoyltransferase 1 alpha (CPT-1 alpha). This enzyme controls the transfer of long-chain fatty acid-CoA molecules from the cytosol into the mitochondrial matrix. The aim of this project was to determine metabolic parameters associated to beta-oxidation of fatty acids in liver mitochondria in rats entrained by circadian food availability. Control group: animals fed ad-libitum. Experimental group with restricted food access (from 12:00 to 14:00 h) during 3 weeks. At the end of the restricted food protocol, different subgroups of animals were sacrificed at 3 h intervals, starting at 08:00 h, to complete a 24 h cycle. The results showed by ad-libitum group: PPAR alpha and PPAR gamma showed a robust diurnal rhythmicity with a peak at 17:00 and 23:00 h respectively. PPAR beta displayed a nocturnal valley at the middle of the light period (17:00 h). FR group: PPAR alpha and PPAR gamma showed similar rhythmicity with a peak at 11:00 h (during FAA) and a valley at 23:00 and 02:00 h respectively. PPAR beta also exhibited a valley, but during the dark period (05:00 h). Perspectives: Explore: 1) complete mitochondrial beta-oxidation, 2) the expression of PGC-1 alpha and CPT-1 alpha and 3) activity of CPT-1 alpha.
It is well known that the master clock in mammals is the suprachiasmatic nucleus in the hypothalamus which is entrained by the light/dark cycle. Nevertheless, it has been proposed the existence of another oscillator that is elicited when animals are subjected to restricted feeding schedules. This biological clock is known as the food entrained oscillator (FEO). It is believed that complex and dynamic interactions among peripheral oscillators are crucial for its emergence. However, information about the mechanisms of these interactions is still missing in order to achieve a more complete understanding of the FEO. Recently, it has been proposed that the enzyme Ornithine Transcarbamylase (OTC) can act as a signaling molecule during the proliferative phase of liver regeneration. Exploring this novel statement, we have hypothesized that this enzyme could have the same role during the expression of the FEO, and hence, the aim of this study was to analyze the effects of the FEO on the subcellular localization and the release of this enzyme out of the liver. The qualitative presence and the activity were measured by means of western blot and a colorimetric method, respectively. Samples of serum, total liver homogenate, isolated mitochondrion and cytosol were collected along 24 h (in 3 h intervals) from rats under restricted feeding schedule (from 12:00 to 14:00 h daily), from a control group fed ad-libitum, and from a fasting-refeeding groups (feeding condition control). Liver fractions were used to detect OTC by immunofluorescence. The results suggest that the FEO has a clear effect in the localization and rhythmicity in the presence and activity of the OTC, and that there is a distinct pattern of the activity and presence within the different cellular compartments. It was also observed that FEO expression caused striking difference in comparison to control groups. Altogether, our results have led us to think that, during the FEO; this enzyme could have a novel function besides its classic catalytic role in urea cycle.
The important role played by intracellular calcium concentration [Ca2+] in the hepatic physiology and metabolism is widely accepted. For example, calcium signaling in the liver varies according to nutritional states (fasting or feeding). The main proteins responsible for this regulation are calcium-releasing channels, as the ryanodine receptor (RyR) and the Inositol 1,4,5-trisphosphate receptor (IP3R), and the sarco-endoplasmic reticulum calcium ATPase (SERCA), which returns the calcium to the endoplasmic reticulum lumen. However, it has not been described if this calcium-handling proteins show a circadian rhythmicity in this organ, neither if this cation participates in the modulation of the liver circadian clock. In order to gain understanding in these points, we implemented the next experimental protocols: 1) we characterized by biochemical approaches (specific ligand-binding experiments and enzymatic essays), protein levels (immunoblotting) and zonational distribution (immunohistochemistry) how the IP3R, RyR and SERCA respond to fasting, feeding, and time-shifted food restriction; 2) at Dr. Michael Menaker's laboratory (University of Virginia), liver explants cultured from transgenic rats carrying a Per1 clock gene associated to luciferase gene reporter were tested chronically with drugs that inhibit RyR (ryanodine), IP3R (2-APB) and SERCA (thapsigargin). Data indicated that a combined influence of circadian rhythmicity and nutritional conditions, modified the intracellular calcium regulating machinery. Feeding conditions as well as the expression of the Food-entrainable Oscillator (FEO) modulate the rhythmicity of the main liver calcium-handling proteins. These results suggest that intracellular calcium dynamics plays a regulatory role in the circadian system of the liver. Also, the period of Per1-luc expression, measured during and after drugs administration, showed different lengthening patterns according to the drug tested (thapsigargin produced the most severe period lengthening). These results suggest that Ca2+ from internal deposits could regulate the circadian molecular clock in the liver of rats entrained by light or by restricted meal access. PAPIT IN204811
Animals in a restricted food schedule (RFS) expressing the food entrained oscillator (FEO) showed a temporal profile of 24 h characterized by hypoglycemia and increased phosphoenolpiruvate carboxykinase (PEPCK) activity with a period close to 12 h. It has been reported that in RFS before food access high levels of circulating FFA and ketone bodies, increased ATP and an oxidized redox state, partial reduction of hepatic glycogen and a high level of glucose. It has been suggested that the liver metabolism adopt a rheostatic adaptation during the RFS. As a consequence, our hypothesis is that RFS promotes an upregulated response of hepatic gluconeogenesis (even as a higher glycemia, or an increased glycogen production in the periportal hepatocytes). Hence, we explored the process responsible of maintaining the glycemia and the regulation of the hepatic gluconeogenesis during the expression of FEO. We analyzed the gluconeogenic response and the distribution of PEPCK a key enzyme of the gluconeogenesis in the liver by alanina tolerance test and immunohistochemical localization. We found significant differences in the circulating glucose formed from intraperitoneally injected alanine. RFS rats showed a similar response in comparison to the animals fed AL, whereas the fasting group increased the level of glucose 20 min postinjection. By other way, the hepatic zonation changed in the animals under RFS, increased the presence of PEPCK in the periportal hepatocyte versus pericentral hepatocite before food access. These data suggest that PEPCK could be active synthesizing glycogen.in this type of hepatocytes. Therefore our results suggest that gluconeogenesis is upregulated in the RFS condition than in AL. Hence, the metabolic changes detected in the liver also strengthen the notion that RFS promotes a rheostatic adjustment in liver physiology during FEO expression.

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Chronobiology has been focused in recent years to find the strategy to eliminate or controlling cancerous cells in mammals, the suprachiasmatic nucleus (SCN) of the hypothalamus is considered the master clock, capable of conferring a temporary order to the internal processes and the behavior of the organism. Other circadian oscillators have been identified in different organs and tissues such as lung and liver. Food restricted protocol to a few hours a day have shown that alternative pacemakers are expressed even when the SCN is injured. This new circadian oscillator is known as food entrainable oscillator (FEO). Under these conditions, the food becomes relevant as a major synchronizer of biological rhythms, even with an intact SNC. The FEO is an emerging phenomenon sustained by daily alternating episodes of daytime restricted. The exact anatomical location of the FEO is unknown. At the molecular level, genes involved in generating and maintaining circadian rhythms in mammals are known as clock genes. These genes are able to regulate the rates of cellular proliferation and apoptosis. Among the genes that are influenced by the circadian clock and control cellular division and apoptosis are the oncogene cMyc, cyclins, caspases and P53 just to name a few. Previous reports have shown that restricted access to food reduced the inset and development of neoplastic growths. The aim of this project is to investigate the possible hepatoprotective effect of food restriction during the induction of hepatocellular carcinoma (HCC) with diethylnitrosamine (DEN).
CIRCADIAN RHYTHM OF ENERGY EXPENDITURE AND CONSUMPTION OF OXYGEN BY INDIRECT CALORIMETRY IN ENTERAL NUTRITION THERAPY

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Introduction: The importance of enteral nutrition has grown in recognition resulting in new methods of administration. That leads to many questions such as: what are the chronobiologic effects of continuous or intermittent nutrition therapy? Objectives: The aim of this study was to evaluate the use of enteral nutrition as a Zeitgeber of biological rhythm. Energy expenditure and oxygen consumption were measured by indirect calorimetry in continuous or intermittent nutrition patterns. Methods: A randomized controlled clinical trial was conducted from December 2009 to November 2010. Thirty four neurological patients received through the same kind of calibrated nasogastric tube the standard protein and energy intakes calculated for each subject. The continuous group (15 patients) received continuous feeding throughout 24 hours; the intermittent group (19 patients) received as follows: 8, 12, 16 and 20 hours during 2 hours infusion. Nutritional support was identical for both groups. The measures were performed during 30 min, with 12 measurements for each patient during 3 days, Measure A: 7:30 am (fasting group intermittent), B: 10:30 C: 14:30; and D: 21:30 hours. Results: The mean age was 69.5±8, 50% were male; BMI 22±3.9kg/m² (men), 25±5.6 kg/m² (women). The total energy expenditure showed no significant difference between groups. Oxygen consumption showed a significant difference between continuous and intermittent groups (212±117 ml/min; 257±125 ml/min (p=0.048), respectively.) The variables energy expenditure and oxygen consumption were analysed by ANOVA-one way in different times during the day and some statistically difference was found among all times (A, B, C and D) in both nutritional groups. Comparing energy expenditure and oxygen consumption between the groups (continuous and intermittent) by Mann-Whitney, there was a statistically significant difference in time B and C (p=<0.01). In the intermittent group, the average of the test (fasting) was 1798 kcal/24h, in subsequent tests there was a decrease of 1% and 6% on thermogenesis in test B and C, and an increase of 4% in test D. In the continuous group the average of the test during 24 hours was 1532 kcal/24h. We observed a decrease of 11.8% and 13.87% in tests B and C, and 11.4% of increase in test D. Conclusion: We observed in this study a circadian variation of energy expenditure and oxygen consumption in continuous and intermittent method of enteral
nutrition, suggesting that only one measurement per day may not necessarily show the patient energy consumption. Moreover, the energy expenditure and oxygen consumption was higher in intermittent groups in all times.

P2.08

BEHAVIORAL AND TEMPERATURE CHANGES THAT PRECEDE FOOD ENTRAINMENT

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Behavioral and temperature changes that precede food entrainment Estefia Espitia Bautista, Dulce Palomares Vázquez, Rodrigo Iván Osnaya Ramirez, Carolina Escobar. Departamento de Anatomía, Facultad de Medicina, Universidad Nacional Autónoma de México, México DF 04360 Keywords: Food anticipatory activity, circadian rhythms, food entrainment. Introduction Feeding schedules entrain behavior and physiological circadian rhythms changing their oscillations and organizing their peaks of maximum expression around feeding hours. Food entrainment is achieved when food is restricted to a few hours and is given in a regular and predictable way. Food anticipatory activity (FAA) develops after 4-5 days and is strongly expressed after 3 weeks in such protocol. In peripheral clock this process also seems to develop after several cycles although in our group we have reported that with unpredictable feeding schedules modifications in behavior and metabolism can be observed after 24 hours of the last meal. The process and mechanisms underlying the process to develop FAA may lead to uncover pacemakers underlying food entrainment. Purpose: The aim of this study is to characterize the process in the circadian pattern of behavior and temperature in response to the first and second events of food access in a paradigm of food entrainment. Method: Adult male Wistar rats weighting 250-300 g at the beginning of the experiment were maintained in a 12:12h LD cycle, controlled temperature and free access to food and water. General activity was monitored with movement sensors under each cage, and temperature loggers (i-buttons) were inserted under the abdominal wall of the rats. Rats were divided in three groups; 1. Ad libitum (AL) control group had free access to food and water, 2. Restricted feeding group for one event (RF-1p) was fasted on day 6 and 7 and on day 8 the food was supplied for 2h from 12:00 hrs. to 14:00 hrs. 3. Restricted feeding for 2 events (RF-2p) was fasted on day 6 and 7 on day 8 and 9 food was delivered for 2h. For both RF groups, the feeding events were followed by 2 days of total food deprivation.
to evaluate behavior and temperature food-entrainment. Results: The following day after the food event the RF-1p group showed behavioral activation and an increase of temperature at the same phase as food was given. The RF-2p group showed behavioral activation that lasted for two days after the pulses, they also showed a change in temperature corresponding to the phase of the feeding event. On the first day of food deprivation a low amplitude FAA was observed. Conclusion: Data here provided confirm that development of FAA is a gradual process and that it starts developing as early as day 2 of RF.

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Rats under a restricted feeding schedule develop food anticipatory activity (FAA) 2 - 3 h prior food access, characterized by increased arousal, foraging and exploratory behavior. This FAA disappears when rodents are allowed ad libitum food access and reappears for several cycles when animals are fasted. Previously we reported in rats under a restricted feeding schedule (RFS) an increase in the expression of c-Fos protein in the dorsomedial (DMH), in the perifornical area (PeF) and in the lateral hypothalamic area (LH) anticipating and reacting to food intake. Considering that these hypothalamic structures contain abundant orexin (Orx) producing neurons and promote arousal, reward and metabolic balance, we explored the participation of the orexinergic system in rats under RFS and analyzed the expression of c-Fos in Orx cells by double label immunocytochemistry during and after food access, as well as in rats exhibiting persistent activation in fasting after RFS. RFS induced a daily rhythm of activity on Orx cells with highest levels at the time of FAA in the DMH, LH and PeF. In previously food-entrained rats, after 48 h of fasting the activation of Orx cells persisted in the PeF and LH with a similar temporal pattern as observed during RFS, however the DMH showed increased activation during fasting in all time points. We conclude that Orx cells in PeF and LH are related to a self sustained oscillator involved in FAA, whereas the DMH is related to the
arousal mechanisms during FAA possibly associated with the metabolic condition resulting from the feeding-fasting alternation.

P2.10

SCHEDULED FEEDING DOES NOT REENTRAIN THE ESTROUS CYCLE IN FEMALE WISTAR RATS MAINTAINED IN CONSTANT LIGHT

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Introduction: A large number of women are exposed to night-work that alters circadian rhythms, disturbs menstruation, pregnancy and leads to early menopause and polycystic ovary (PCO) syndrome, characterized by menstrual irregularity, hirsutism, anovulation, and infertility. The rat estrous cycle is tightly regulated by the circadian system, under constant bright light (LL), rodents become arrhythmic and the estrous cycle stops, follicles develop to the preovulatory stage and secret estrogen, the ovary contains no corpora lutea nor ova. Constant secretion of estrogen results in continuous vaginal cornification, high uterine weight, and continuous sexual receptivity, constituting a state of persistent estrus (PE) that leads to PCO. Restricted feeding schedules (RFS) are strong entraining stimuli for locomotor activity and peripheral oscillators in intact and SCN lesioned animals. Thus, it is possible that RFS can be used to reestablish circadian rhythms, resynchronize the estrous cycle and prevent and/or treat PCO. Methods: 12 female Wistar rats (weighing 150-250g) divided in 2 groups. Group A: n=4, experiment length 12 weeks and group B: n=8, experiment length 15 weeks. All rats were housed in individual acrilic transparent cages placed over movement sensors, under controlled LD cycle (lights on at 07:00–19:00 h), temperature between 20-22°C, water and food ad libitum. During the experimental phase rats were exposed to LL, and restricted food access. The estrous cycle was determined with daily vaginal smears in baseline and in each experimental condition for 3 weeks. Group A. The effects of 6 weeks in LL and the recovery in a 12-h LD cycle were tested. Group B. After 8 weeks in LL, rats were exposed to a 12 h cycle of scheduled food access (SF) for 4 weeks (food from 20:00-8:00), vaginal smears were taken during the last 10 days. Results: In an LD cycle all rats were cycling (4-5 days cycle). Actograms and activity analysis indicated a synchronized rhythm with a period of 24 h. LL induced loss of rhythmicity in general activity, confirmed by no-peak on the periodogram and an equal activity percentage during subjective day and night, vaginal cytology indicated loss of rhythmicity and PE. In group A, when LD was
reestablished, estrous cycle reappeared after 4 days in 50% of the rats while 50% remained in diestrus. In group B, general activity entrained slowly to SF, after 4 weeks exhibited a significant period of 24 h with a low amplitude, and rats remained in PE. Conclusion: The estrous cycle depends on the light-dark alternation. In LL when circadian rhythms are altered, persistent estrus appears. SF for 12-h synchronizes circadian rhythms of behavior, but not the estrous cycle.

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P2.11

AFTEREFFECTS OF ENTRAINMENT IN THE FIELD PROVIDE NEW INSIGHTS INTO THE SWITCH FROM DIURNALITY TO NOCTURNALITY IN THE SUBTERRANEAN RODENT TUCO-TUCO (Ctenomys cf. knight)

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Ctenomys cf. knighti, a subterranean rodent from La Rioja, Argentina, is clearly nocturnal when kept in the laboratory under LD 12:12 (L: 200 lux) in cages with running-wheels. However, in a semi-natural enclosure (10x5 meters) continuous observation during the light phase of the day of one individual during summer and another in winter surprisingly revealed intense aboveground activity throughout the day, outside their burrows. This indicates that C. cf. knighti could be another species that switches from diurnality to nocturnality when transferred from the field to the laboratory, as has been reported in some other wild diurnal rodent species (Smale et al., 2003). The next step was to verify whether the observed diurnal aboveground activity is a component of their total daily diurnal activity (above and underground) or, alternatively, tuco-tucos are not entrained in the field and thus the observed aboveground activity is just part of randomly timed activity bouts during the 24 hours of the day. In order to approach this question, and based on the fact that circadian oscillators often display aftereffects of entrainment, after the finalization of each observation experiment we immediately transferred the animals from the field enclosures to constant laboratory conditions. In this way, we could register the aftereffects of the previous field synchronization and access the phase of the synchronized oscillator in the field. The result was surprising: the activity phase of both animals coincided with the external night. In order to verify the generality of these results,
we brought 11 more animals now directly from the field to the laboratory under constant conditions: five in running wheels and six with infrared motion sensors. Independently of the measuring device, aftereffects indicate nocturnality in the field. We conclude that the activity phase of tuco-tuco is nocturnal, as dictated by the LD entrained oscillator but its expression is diurnal in the field, due to masking by some environmental cycle other than the light-dark. Some hypothesis about the masking factors are temperature, oxygen content cycles in the underground environment or even predation risk in the surface.

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Recent studies have related metabolic diseases with changes in circadian regulation. Overweight (OW) and obesity have received particular interest because their increasing in industrialized countries. The volcano mouse Neotomodon alstoni, endemic of central Mexico, develop symptoms related with the metabolic syndrome in most organisms in captivity, when fed with standard rodent diet, which makes this species interesting to study circadian behavior and obesity. The aim of the present study is to elucidate if overweight differentially affects the entraining of locomotor activity between genders in volcano mouse, through a phase response curve (200 lux, 1hr) and traditional jet lag protocols. Controls (49.57± 0.81 g), OW (75.81± 2.06g) males and controls (51.15±1.3 g) and OW (70.47± 2.1g) females were used. Locomotor activity was recorded by means of infrared light beams. Two experiments were set: First, mice were exposed to LD 12:12 and 6 h advances and then delays, were set every 2 weeks respectively. A second experiment consisted on animals exposed to LD (12:12) for at least 10 days, thereafter, mice were maintained in DD. Ten days later a light pulse was given at different circadian times (CT 02, 06, 14, 22) and phase shifts were estimated. In the present study, no differences were noted between control females and males in the PRC. When OW animals were compared, a phase delayed zone was significantly reduced in both genders, however, OW males did not show evident phase advance zone, while OW females still present the phase advance like in controls. In a jet lag protocol not significant differences were found in females but OW males require larger number of transient days to fully entrain. Our results indicate that the photic entrainment in overweight mice N
alstoni is differentially affected between genders, and we propose this species could be of particular interest to study the effect of gender overweight and obesity upon circadian physiology. Supported by PAPIIT IN225311

P2.14 LASC26
COMPARATIVE ANALYSIS OF CIRCADIAN RHYTHMS OF THE MOSQUITOES *Aedes aegypti* AND *Culex quinquefasciatus* UNDER TEMPERATURE CYCLES

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Many species of mosquitoes are vectors of important diseases. Their activity and blood feeding rhythms, which are important for pathogen transmission, are under circadian control. However, relatively little is known about the molecular genetics of the endogenous clock controlling these rhythms. We are studying the locomotor activity rhythms and circadian expression of the main clock genes in *Aedes aegypti*, a diurnal vector of Dengue and Yellow fever and *Culex quinquefasciatus*, a nocturnal vector of Filariasis and West-Nile fever. Our previous work revealed conserved circadian expression patterns between the two species in most genes, except cryptochrome2, under light-dark and constant darkness conditions and we are currently examining the effects of temperature cycles. We observed that both species are entrained by temperature cycles in constant light and showed differences in their activity behavior that resemble their patterns in light-dark conditions. *Ae. aegypti* is more restricted to the thermophase while *Cx. quinquefasciatus* has its activity more restricted to the cryophase. At the molecular level, we observed mRNA cycling in temperature cycles, albeit in lower amplitude than in light-dark cycles, and some species-specific differences in their clock gene expression patterns. Our results suggest that both species use temperature, as well as light, to concentrate their activity in a specific phase of the day.
MODULATION OF THE CIRCADIAN RHYTHM OF BODY TEMPERATURE BY INTENSE ACTIVITY IN A SUBTERRANEAN RODENT (*Ctenomys cf. knighti*)

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Most mammals show a daily body temperature (Tb) rhythm which is endogenous and entrained by light/dark (LD) cycles. Usually, the highest temperatures occur during the same phase of motor activity, which often may mislead to the conclusion that the Tb rhythm is a mere consequence of the activity/rest rhythm. Nevertheless, several studies have verified that both rhythms persist independently of each other. Intense activity may, however, modulate some parameters of the Tb rhythm. Quantifying this modulation helps dissecting the exogenous and endogenous components of the Tb rhythm when the animal is under different experimental conditions or in its natural habitat. We investigated this issue in a subterranean rodent, the tuco-tuco *Ctenomys cf. knighti*, found in northwest Argentina. First we studied their Tb under a LD cycle and verified a daily Tb rhythm with the highest temperatures occurring during the dark, synchronously to running-wheel activity. Furthermore, Tb rhythm persisted under constant darkness (DD), an evidence of its endogenous nature. Interestingly, we observed a much smaller variability in rhythmic patterns (period, phase and amplitude) compared to other subterranean rodents. In order to study the exogenous components of the Tb rhythm due to intense activity, its parameters in the presence and absence of a running-wheel were compared. To continuously measure temperature and gross motor activity, telemetric transmitters were implanted intraperitoneally. Three tuco-tucos were housed individually in cages equipped with running-wheels and exposed to the following lighting conditions: 1) DD (44 days); 2) LD (12:12, 14 days); 3) reestablishment of DD (16 days). The wheels were then removed and the exposure to the lighting conditions repeated. After running-wheel removal, mean gross motor activity diminished 54-62% in two animals. Free-running period (ranging from 23.6 to 24.4 hours) and phase relationship with activity rhythm did not change significantly. The amplitude, in turn, decreased about 0.5°C in 2 animals. These results will be important for further analysis of experiments using running-wheels as well as studies of Tb rhythmicity of tuco-tucos in their natural habitat, where they show frequent bouts of intense activity while digging.

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A SUBTERRANEAN RODENT’S "NATURAL ENTRAINMENT"

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Subterranean rodents are interesting organisms for the study of "natural entrainment" (Hut et al, 1999) for they spend most of the day inside underground tunnels, where there is little daily variation in environmental variables. Therefore, it has been proposed that entrainment depends mostly on aboveground excursions, e.g., during foraging, when there might be contact with more robust cycles. Our field data on the tuco-tuco (Ctenomys cf. knighti) indicates that these animals perceive the aboveground light-dark cycle by several random bouts of light-exposure during the photophase of the day. Summer observations through 11 consecutive days revealed an average peak of exposure bouts between 9 and 10 a.m. In order to access whether such light-dark pattern acts as an entraining agent, we first constructed in laboratory the Phase Response Curve (PRC) for 1h light-pulses (1000lux). Its shape is qualitatively similar to other curves reported in the literature. How the tuco-tuco's clock, with its associated PRC, responds to irregular light-exposure patterns is being investigated by means of computer simulations. Using the software Circadiandynamix (Friesen & Friesen, 2009), two selected oscillator-configurations, A (type-1 PRC) and B (type-0 PRC), were submitted to potentially entraining cycles, consisting of "light pulses" uniformly distributed throughout a fixed phase range of the day. Several simulations were performed by changing this phase range from 2 to 12 hours. Unexpectedly, oscillator A maintained an apparent stable entrainment even under the most irregular cycle (12-hours phase range). Oscillator B, in turn, presented a pattern resembling relative coordination under this same regimen. Future studies will be performed using qui-square (instead of uniform) distribution of pulses within the phase range, to simulate more precisely our field data. Simulation results are consistent with a nocturnally phased oscillator, the field records described above indicate that tuco-tucos do express aboveground activity during the photophase.

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Changes in the photo-phase length in a 24-hour cycle, influences the circadian system in several organisms, including mammals. This effect may be reflected in different physiological functions and behavior such as locomotor activity, hormones release and the sleep-wake cycle. Mongolian gerbil Meriones unguiculatus, presents changes in the activity profile regarding photoperiod and have been considered as a semidiurnal or crepuscular mammal. It also has been established that seasonal photoperiod induce physiological changes in the reproductive system of this species. The aim of this study was to evaluate the effects of three different photoperiods (LD 12:12, long photoperiod 16:08-LP- and short photoperiod 08:16-SP-) on the architecture of locomotor activity and sleep-wake cycle in adult male Mongolian gerbils. Our results indicate that the locomotor activity profile is organized according to the photoperiod tested. In LD 12:12, animals showed a bimodal pattern with main components associated to lights on and off respectively. When exposed to LP, gerbils increased their total activity and maintain their bimodal profile associated with light transitions. When exposed to SP, total activity gets reduced and the component related to lights on disappears. By other hand, results of temporal distribution of the vigilance states indicate that, in LD 12:12, gerbils spent about 65% of the recording time in wakefulness, mainly diurnal; and near 5% of REMs. When exposed either to SP or LP, wakefulness increases in both light and darkness, while sleep gets reduced. Also, during SP the main wake state becomes nocturnal. We proposed that the changes observed in both locomotor activity and sleep-wake patterns, could be analyzed considering the morning and evening oscillators theory, and become Mongolian gerbils as a good animal model for studying the effects of seasonal photoperiod changes in both circadian and homeostatic processes. Suported by CONACyT fellowship to CRJT.
EFFECT OF DIFFERENT PHOTOPERIODS ON THE RHYTHMIC INDUCTION OF HYPOXIA-INDUCIBLE FACTOR-1 AND HEAT SHOCK PATHWAY

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Both irradiance and photoperiod length of the environmental daily cycles are stress factors that strain Procambarus clarkii circadian rhythms, and as a consequence changes in this species metabolic, oxidative, and behavioral rhythms occur. Extreme luminous situations may drive this species to a state of behavioral and metabolic depression that allows this crustacean to cope with the adverse luminous environment. This adaptive response should involve signaling pathways able to translate the environmental changes into metabolic and behavioral response by means of genes expression mechanisms related with the generation of ROS. Many of the ROS-mediated responses, as GSH system protect the cells against oxidative stress and reestablish “redox homeostasis”. Then here we propose HIF-1 as a transcription factor related to ROS, to heat shock pathway activity and participating in the daily adaptive response to extreme light cycles in crayfish. In this work we investigated two structures that have been proposed as circadian pacemakers of crayfish P. clarkii: the retina and the brain-optic lobe complex (BOL), determining whether equatorial (LD 12:12) and extreme (20:4) daily cycles, activate the expression of HIF-1, HSP 70 and 4-hydroxy-2-nonenal (HNE), a marker of lipid oxidation, and whether these markers expression levels depend on the LD cycle and the time of day. Retinas and BOL complex of three groups of crayfish submitted to LD:12:12, LD 20:4 and constant darkness were dissected at two times of day and processed by western blotting (WB) using polyclonal antibodies. The immunoreactivity was visualized by chemiluminescence and the blots were digitized and analyzed. The results revealed an effect of time on HIF-1 and HSP-70 relative abundance both in retina and BOL. Although we did not find any effect of time on lipid oxidation levels. This study suggests a rhythmic HIF-1-dependent increase in HSP expression that should be highly up-regulated during extreme photoperiodic conditions. Supported by PAPIIT IN218811.
LOW TEMPERATURE PULSES PRODUCE CIRCADIAN EFFECTS ON THE LOCOMOTOR ACTIVITY RHYTHM OF THE CRAYFISH _P. Clarkii_

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The unrestrained crayfish maintained under darkness exhibits a circadian unimodal activity rhythm able to entrain to light-dark cycles showing two activity peaks one at lights on and other just after lights off, displaying a maximal activity by night. This circadian rhythm free runs with a period values about 24 h and is synchronize by parametric and non parametric light stimulus. Although it has been proven the activity rhythm’s ability to compensate temperature its ability to temperature synchronization is unknown. In this work we analyzed the effect of cold water pulses on different groups of unrestrained crayfish. All organisms were placed individually in double compartment aquaria as reported elsewhere. Movements were detected by an array of photocell couplers placed in each of the two compartments. Interference of the light beam caused by movements of the animal were electronically transduced and transferred to a data collected computerized system. The crayfish were maintained under light-dark (LD) 12:12 at least 7 days, at 8th day at ZT 10, ZT13 and ZT23 the aquarium water was changed and cold (6.5°C) water was introduced in the aquarium for 30 min and after changing water again the animals were kept under darkness at least for 10 days. In a control group animals were subjected to a similar protocol but stimulated with white light pulses at same ZTs than the experimental group. Activity of 24 h period was conventionally doubled plotted as actograms and analyzed with Spad 9 (Omnialva, Mex) and TAU (Minimiter, USA) programs. When applied at the beginning of the subjective night cold water pulses produced phase advances meanwhile at the end of the subjective night the pulses produced phase delays. The phase change magnitude were similar to those produced by light pulses but turned back, these findings suggest different temperature and light synchronizing pathways.

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The temporal niche of most marine species is likely determined by different light intensity experienced with depth. The Norway Lobster (Nephrops norvegicus) shows niche switching of daily behavioral activity, being nocturnal on the shallow and lower shelf (50-100 m), while diurnal at the slope (200-400 m). Biodiversity conservation and stock assessment studies should not only consider diurnality/nocturnality in relation to depth, but also the ethological features of behavioural patterns in sampling strategies, since populations cyclically disappear from collection windows. We investigated daily (24-h based) behaviour in order to determine activity phenotypes at different depths: shelf (100 m) and slope (400 m). We used 113 animals held in individual aquaria, endowed with three infrared detection barriers (i.e. at burrow mouth for Door-Keeping, at intermediate and opposite locations for Proximal- and Distal-Emergence, respectively). We applied a monochromatic blue (480 nm) light intensity cycle protocol of different intensity (simulating the depth). We recognized by both periodogram and waveform analyses three different rhythmic phenotypes: Burrow-focused behavior (Door-Keeping, 66.6%), burrow-oriented behavior (Proximal-Emergence, 16.6%) and “poorly” burrow-oriented activity (Distal-Emergence, 4.5%), as well as arrhythmicity (12.3%). Furthermore, we identified a “sleeping period” (< 20 min) in 23.4% of animals. The results suggest that Nephrops posses a plastic activity rhythm as required by its wide depth range of distribution. Present data were extrapolated at the population level, being laboratory results on a large sample of animals of importance for a more precise stock assessment.
The crayfish’s eyestalk X organ-Sinus gland system (XO-SG) is part of this animal’s neuroendocrine system. It synthesizes and secretes peptidic hormones important for the metabolic regulation. One of them is the crustacean hyperglycemic hormone (CHH), involved in the glycemic regulation and controlled by the circadian timekeeping system. Previous works using immunochemical and hemolytic essays had demonstrated the CHH’s secretion in the P. clarkii’s in the XO-GS complex and the retina, both organs proposed as circadian pacemakers of P. clarkii, however up to now daily and circadian variation of the synthesis of CHH either in eyestalk or retina have not been proven. The objective of this work was to find out whether in the eyestalk a CHH’s synthesis daily rhythm exists and whether chh mRNA is synthesized in the retina of crayfish. To characterize RNA in both organs, total RNA was extracted from the eyestalk at different times of day: 0300, 0700, 1100, 1300, 1500, 1900 and 2300 hours, and at 0700 h for the retina. Tripure (Roche) reactive was used following manufacturer’s instructions. Oligonucleotides based in the mRNA CHH sequence (GeneBank accession no. AB027291) were designed for the reverse transcriptase-PCR reactions (RT-PCR). For internal control, B-actin oligonucleotides were used. All the amplicons obtained were purified and sequenced. Once the amplicons were identified as being part of the B-actin and CHH mRNAs, Semi-quantitative RT-PCR reactions were carried out with the total RNA extracted from the eyestalk at different hours. Temporal variation was analyzed for the mRNA expression by band quantification in a Gel Logic 200 system (Kodak) and the Molecular Imaging software (Kodak). The results show eyestalk chh mRNA daily variations with a peak at 0700 h and the lowest value at 1500 h. For the retina it was possible to identify chh mRNA.
This work was supported by PAPIIT IN 218811.
The first described clock gene, per (period), was encountered in Drosophila melanogaster and is composed by 9 exons and 8 introns. In mammalian, Per proteins may be transcribed by 3 genes. The difference in the number of introns, the position and the phase among the members from a gene family has been used to propose the origin of intron. An early model for this origin suggests that the introns were present in the ancestral genome and they were eliminated in the prokaryotic cells while in eukaryotic they continue. There is another model which claims that the presence of the introns in eukaryotic cells is recently, because the introns were absent in prokaryotic cells. To per gene, the intron analysis among invertebrates and vertebrates was determined between D. melanogaster and Homo sapiens suggesting that the origin in vertebrate corresponds to the early model. However, the putative intron relation among invertebrates and vertebrates to explain the gene evolution with other invertebrate no has been established yet. In this work, the intron analysis from arthropods and vertebrates were revised to determine the putative intron evolution. The results showed that the intron position and phase suggest that new introns were obtained in vertebrate, probably were eliminated from the ancestral gene. Some common introns had phase 2 or 3, suggesting the presence of alternative splicing in this regions in arthropods. In addition, we investigated the possible exon skipping in per mRNA RT/PCR from the crayfish Cherax quadricarinatus with cDNA RACE library from abdominal ganglia obtained of animals adapted to cycles light:dark (12:12 h) shows 3 amplicons. Our experimental results in crayfish C. quadricarinatus are analyzed and discussed in relation to the hypothesis that some introns are ancient in the per gene while others were included in the genetic evolution to participate in alternative splicing.

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An open question in biology is how the lipid components of the biological membranes are synthesized and assembled to maintain membrane homeostasis and diverse cellular processes. Circadian clocks regulate diverse biochemical functions, whereas cell cultures make up a model of peripheral oscillators to investigate metabolic oscillations. We previously reported that phospholipid biosynthesis oscillates in synchronized fibroblast cultures; however, the way phosphatidylcholine (PC) synthesis is daily regulated in cell cultures, is unknown. PC is mainly synthesized through Kennedy’s pathway with CTP: phosphocholine cytidylyltransferase (CCT) as key enzyme. In mammals, there are two genes encoding for CCT: Pcyt1a that encodes the CCTa protein from alternative transcripts CCTa1 and CCTa2 and Pcyt1b gene encodes the CCTb2 and CCTb3 proteins from differentially alternative spliced mRNA CCTb2 and CCTb3. Here, we investigated possible temporal changes in PC biosynthesis and CCT activity and expression in quiescent NIH 3T3 fibroblasts after a 2 h-serum synchronization. We found that PC labelled with [3H]glycerol or [32P]phosphate in the cultures exhibits a daily variation with levels peaking at 4-6 h after serum stimulation and decreasing by 29-32 h. Significant daily variations were also seen in CCT activity with higher levels at 6 and 35 h. Moreover, CCT expression across time for the most abundant isoforms (CCTa1 and CCTb2) at mRNA and protein levels displayed complex pattern expression. Both CCTa1 transcript and protein peaked at 3 h post-stimulation, while CCTb2 mRNA was higher at 9-12 h. We also detected increased levels of CCTb isoforms during 9-18 h using an antibody that recognized both CCTb2 and CCTb3 proteins. Results demonstrated that synchronized fibroblasts exhibit temporal variations in PC biosynthesis which could be due, at least in part, to changes in CCT activity and/or to a concerted and differential expression of different CCT isoforms.

Supported by ANPCyT-FONCyT (PICT967 – PICT898), CONICET, SeCyT-UNC, MinCyT-Cba.
The Suprachiasmatic Nucleus (SCN) is responsible for circadian rhythms of physiological and behavioral processes in mammals. The intracellular calcium concentration ([Ca\textsuperscript{2+}]) in this neurons shows a circadian oscillation, which is taught to be regulated by the ryanodine receptors (RyRs) widely expressed in neurons in the SCN. In a previous study we found that the spontaneous firing rate (SFR) of these cells increases when the RyRs are activated and decreases when they are blocked pharmacologically. In this study we used calcium imaging with Fura-2 AM to see the effect of gating the RyRs on the [Ca\textsuperscript{2+}] in SCN slices from mice. Recordings were conducted during day and during night. We activated the RyRs applying 100nM of Ryanodine and blocked them with 10\textmu M of Dantrolene. We hypothesized that activating or blocking the RyRs will either increase or decrease the [Ca\textsuperscript{2+}], respectively. We found that the basal [Ca\textsuperscript{2+}] was higher in the day than in the night, as previously showed. As expected, we also found that activating the RyRs increases the [Ca\textsuperscript{2+}] while blocking them diminishes it. This effect was higher at night than during day. On the other hand, we saw that not all of the cells responded equally to the drugs. Most of them acted as we expected, but in some neurons the [Ca\textsuperscript{2+}] decreased when activated the RyRs or increased when they were blocked. In some cases they did not change. In conclusion, the RyRs can directly affect the [Ca\textsuperscript{2+}] in SCN neurons and thus probably regulate the membrane excitability throughout the day, for example, modifying the SFR as we described before.

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Gene expression is modulated by clocks, 5-20% of the mRNAs expressed in a given tissue show daily oscillations. Presumably, this is largely generated by changes in the transcription of those genes; however, the relative importance of mRNA post-transcriptional processing has not been established. Stress granules (SG) and P-bodies are cytoplasmic subdomains involved in the regulation of translation, mRNA decay and storage. They are formed by RNA and a number of factors involved in mRNA cytoplasmic processing. Considering that some of the SG and P-body components are expressed under circadian basis, we have analyzed whether these foci show rhythmic changes. NIH3T3 cells were synchronized by serum shock and fixed at different times. SG and P-bodies were detected by ICC. P-body per cell (number) showed fluctuations peaking 28 h after synchronization, and reaching highest areas 14 h after the shock. We applied arsenite (oxidative stress) for inducing SG. The number of SG/cell did not change through time; however the area and perimeter of them showed daily variations with peak 14 h after serum shock. Further studies will be carried out with other markers to confirm these results; however they suggest that these foci, or a subpopulation of them, are regulated by a circadian clock.

Supported by: IBRO, CONICET, FONCyT, SECyT-UNC y Ministerio de Ciencia y Tecnología de Córdoba.
The vertebrate retina contains at least three classes of photoreceptor cells, cones and rods responsible for vision, and intrinsically photoresponsive retinal ganglion cells (RGCs) involved in diverse non-visual functions such as photic entrainment of daily rhythms and pupillary light responses. In this work we investigated the potential capability of the rat RGC line (RGC-5) as intrinsic photosensitive cells by assessing the presence of visual and non-visual opsins as well as the responses to brief light pulses on the expression of the immediate-early gene protein c-Fos, and intracellular Ca\(^{2+}\) mobilization. We observed that cultured RGC-5 cells express opsin mRNAs such as retinal G protein coupled receptor (RGR), encephalopsin/panopsin (Opn3), neuropsin (Opn5) and cone opsin but not melanopsin (Opn4) or rhodopsin. Furthermore, the expression of the OPN5 in RGC-5 cells was confirmed by immunocitochemistry. In addition, we report for the first time the localization of this opsin within the mammalian (rat) retina: OPN5 is expressed in some neurons of the inner nuclear layer and in the ganglion cell layer. Strikingly, functional assays in RGC-5 cells showed significant changes in intracellular Ca\(^{2+}\) levels in subsets of RGC-5 cells after bright white light pulses of 30 to 60 sec of duration while cells exhibit the induction of the c-Fos protein by a 30 min-light pulse. In summary, our results indicate that RGC-5 cells express diverse putative functional photopigments and display the intrinsic photic induction of c-Fos protein and changes in intracellular Ca\(^{2+}\) mobilization. Supported by: ANPCyT-FONCyT, (PICT 04 967/PICT 06 898), SeCyT-UNC and MinCyT-Cba.
The Arcuate Nucleus (ARC) is known to respond to metabolic cues as Glucose, Free Fatty Acids, Leptin, Ghrelin and Insulin, these signals are known to inform neurons within the ARC about metabolic state of the organism. Previous studies, have demonstrated anatomical connections between the ARC and the Suprachiasmatic Nucleus (SCN), suggesting an involvement of the SCN in ARC sensorial function. Therefore, the aim of the present study was to investigate the influence of the SCN on ARC activity, especially with respect to the anorexigenic neurons $\alpha$-Melanocortin Stimulating Hormone ($\alpha$-MSH). This neuronal population is known to be activated after food intake. In order to determine whether the SCN may influence ARCs neuronal activity, c-Fos IR was determined at six time points along the LD cycle on two groups, fed Ad libitum and 48h fasted animals. The results show that $\alpha$-MSH neurons from the ARC present a peak in their activity at the end of the dark phase; this activation persists despite fasting. To determine the involvement of the SCN on ARC neuronal activity, bilateral SCN lesion were made and animals sacrificed at the acrofase $\alpha$-MSH neuronal activity. An almost complete loss of the activation within the ARC was observed. To determine how the SCN is driving the activation of $\alpha$-MSH neurons, SCN unilateral lesions were executed and the neuronal activation at the lesioned and non-lesioned side of the ARC were quantified. The loss of $\alpha$-MSH neuronal activity at the lesioned site demonstrates that the SCN activates $\alpha$-MSH neurons via neuronal connections, suggesting that temporal information is transmitted to the ARC may affect its function.

Acknowledgement: This study was supported by DGAPA project IN215308 and Conacyt 79797
The hypothalamic suprachiasmatic nucleus (SCN) coordinates circadian rhythms from peripheral tissues in vivo, serving as the main circadian pacemaker. Here we studied a target tissue, the liver, to compare its cellular properties as a circadian oscillator. Isolated suprachiasmatic nucleus (SCN) cells are capable of sustaining molecular and electrical rhythms in vitro, and intercellular communication is necessary to generate a coordinated, stable output from the SCN. Consistent with this model, SCN explants maintain rhythms for up to several weeks in vitro, while treatments that disrupt intercellular communication cause the whole-explant rhythms to damp as single-cell oscillators become desynchronized. Whether rhythms in peripheral cell types are also synchronized within other target tissues remains controversial. To begin to address this question, we measured luminescence rhythms in explants of various tissues from PER2::LUCIFERASE knock-in mice. Consistent with previous reports, we observed that SCN and liver explants damped at similar rates, while esophagus, spleen, lung and thymus damped more quickly; rhythmicity in all tissues could be reinstated by a media change. The ability of liver explants to sustain stable rhythms suggests that there may be a mechanism for synchronizing liver clock cells in the absence of systemic cues. To further address this possibility, we cultured primary mouse hepatocytes in the collagen gel sandwich (CGS) configuration, which has been reported to maintain cellular health and hepatocyte-specific function for several weeks. Rhythms in PER2::LUC bioluminescence from CGS hepatocyte cultures were maintained for up to 54 days, with four media changes, and were dependent on seeding density. Rhythms damped at rates intermediate between those from SCN and liver explants. Hepatocytes plated on a single layer of collagen did not maintain rhythms for more than a few days, consistent with previous reports. These results suggest that individual liver clock cells could be functionally coupled in vitro if culture conditions are optimized. We are investigating the role of gap junctions in hepatocyte coupling, using a gap junction blocker as well as immunocytochemical localization of gap junction proteins, in order to understand how these proteins that connect hepatocytes may function in generation of circadian rhythmicity in this organ.

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Differential Development of the Diurnal Clock Gene Expression in the Olfactory Bulb and Suprachiasmatic Nucleus of European Rabbit

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In altricial mammals, during pre-visual stages of development the olfactory system plays an important role. In the case of the European rabbit (Oryctolagus cuniculus), the newborn were usually maintained in a dark nursery burrow and the lactating female only visit their young for a few minutes once approximately every 24 hr; under this conditions the newborn rabbits depend entirely on pheromonal cues on the mother’s ventrum to locate nipples and suckle efficiently. In addition, the daily exposition to maternal olfactory cues to newborn rabbits, such as the maternal pheromone 2MB-2 is possible to synchronize the locomotor activity and core body temperature rhythms. Despite the undoubted importance of the suprachiasmatic nucleus (SCN) as a circadian pacemaker, there is considerable evidence of other circadian oscillators in the mammalian olfactory bulb (OB) with properties similar to and largely independent of the SCN. In order to understand the development of the rabbit’s circadian system, we characterized the 24-h pattern of expression of the clock genes in the OB and SCN of pre-visual week-old rabbits and compare these with the pattern of expression of visual juvenile rabbits several weeks after weaning. In order to characterize the diurnal pattern of clock gene expression in the OB and SCN of pre-visual rabbits, 43 newborn were used. The rabbits were maintained in constant darkness and fed by a lactating female every 24-h, from the postnatal day 1 to 7 (P1- P7). After nursing on P7, pups were killed in the dark at 3 hours intervals, in order to obtain eight groups (n=5-6 pups/group) distributed evenly across the 24 hours cycle. The visual rabbits (n=46) were weaned at P25 and maintained in an L:D cycle, at the P45 the juvenile rabbits were sacrificed at the same time points across the cycle. The expression of the clock genes Per1, Cry1 and Bmal1 were determined using in situ hybridization, using oligodeoxynucleotides labelled with S35, in sagital sections containing the OB and coronal sections containing the SCN. The autoradiography films were digitalized and quantified the relative optical density of at least three sections of each subject; the data were analyzed by COSINOR and ANOVA. We report for the first
time: 1) that Per1, Cry1 and Bmal1 are expressed in the OB and in the SCN of the newborn and juvenile rabbits, 2) the expression in the OB takes place mainly in the mitral cell layer, and 3) the diurnal pattern of clock genes expression develops earlier in the OB, than in the SCN of newborn rabbits. Our findings suggest that the OB is a potential element of the newborn pre-visual circadian system, since the molecular machinery develops earlier than in the SCN.

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Abstracts

Student Projects
The PVT is a structure that is part of the circadian system, there are mutual connections between the PVT and the suprachiasmatic nucleus (SNC), suggesting that PVT is involved in the modulation of the circadian rhythms. Salazar-Juarez et al (2002), stimulated the anterior PVT in Wistar male rats and found a phase response curve similar to that induced by light pulses under free running conditions. They also found that anterior PVT lesions suppress phase advances induced by light pulses at the end of the subjective night, which induced phase delays instead. This leads us to ask whether PVT modulates the clock advance. Yan and Silver (2009) determined the expression of Per1 and Per2 genes in the SCN induced by light pulses at the beginning and the end of subjective night. They mainly showed that when the pulse is applied at the end of the subjective night (CT22) Per1 is expressed in the shell, and when the pulse is applied at the beginning of the subjective night (CT14) Per2 is expressed also in the shell. Since phase changes are related to the change in the expression of clock genes in the SCN, if PVT is necessary for the phase advances then its lesion will modify the expression of clock genes related to phase advances. In order to test this hypothesis, we will determine in male Wistar rats the effect of neurotoxic lesions of PVT produced with ibotenic acid, on behavioral phase shifts and Per1, Per2 and Bmal-1 expression in the SCN induced by light pulses at the end of the subjective night.

PAPIT IN204811
SEXUAL BEHAVIOR AND SEXUAL FUNCTION AS A POTENTIAL "Zeitgeber". ALTERATIONS IN THE SEXUALITY OF NURSES WHO WORK SHIFT-WORK

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The sexual alterations in women are a very common complaint, with an estimated prevalence around 40-43%, with an impact in the quality of life determining side effect to self-esteem, with emotional conflicts and difficulties in personal relationships. Several studies have demonstrated an association between sexual alterations and a low self physical and emotional satisfaction; as well, general well-being in women and their sexuality is closely related with a satisfying quality of life, in which sexual activity has an impact on lots so aspects of their personal life and relationships. As the number of working women is raising, and nursing is one of the places where the biggest percentage is represented by women. The temporal structural of working by day time or night time interferes with the normal behaviors of society, and the biology of the circadian rhythm. It’s this lack of armory that impose some limitations on the social activities of the night time workers, interrupting their psychological and physiological functioning that may tiger a detriment on their social, familiar and couples life, and at the same time, a diminishing on their own well-being. This study focuses on the determination of any association between sexuality alterations and night time work on the nursery staff of a hospital in Bogota dc. Colombia.

STUDY OF VARIABILITY CIRCADIAN BODY TEMPERATURE AND SLEEP-WAKE CYCLE OF THE STUDENT NIGHT SHIFT WORKERS

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Introduction: Currently there has been growing interest in health in developing research on sleep disturbances of the worker who wakes up too early or working at night. Understanding the influence of these factors becomes an important task to clarify the
effects that can result in worker health. From this consideration, we developed an interest in analyzing the patterns of sleep - wake cycle and circadian variation of body temperature in night shift workers of nursing who studies during the day. Materials and methods: data were collected during 30 days in two periods (and school holidays), between nursing assistants and technicians of night shift, daytime students. We used the Questionnaire for Individuals Morning and Evening (HO), Epworth Sleepiness Questionnaire (ESQ), Journal of Sleep and a thermistor (Thermochron iButton ®) for verification wrist temperature every 30 minutes, which is characterized as a safe measurement of temperature, related to the circadian rhythm. Results: 27 subjects participated in the survey, with a mean age of 28 years. As for sleep, it was found they had less to 2:56 pm on days on duty in the school year, compared to rest days: 66.7% had scores higher than 9.0 on the ESS, which features excessive daytime sleepiness. Observed phase relation between the acrophase and the half-sleep phase, but was statistically significant only in the morning subjects in the school year on working days (r = 0.8244), and on vacation with work (r = 0.8843) and vacation without work (r = 0.6004). There were significant differences in the time that the acrophase occurred, when comparing the school year without work and school to work (p <0.0001), with school work and vacation work (p = 0.0244), and work-free vacation with work (p <0.0001). Discussion: You can see in the thermogram, the temperature variation over the day, and no duty on the days the highest temperatures are in the early evening and early hours and days on duty the temperature tends to rise late, close to 12pm, which shows that this sample, even with routine shifts on alternate days, the temperature rhythm of the wrist accompanies the sleep wakefulness cycle.

PR.4 LASC82
CHARACTERIZATION OF SOCIAL FACTORS ASSOCIATED WITH DIURNAL SLEEPINESS IN KINDERGARTEN CHILDREN

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The sleep-wake cycle (SWC) of children is influenced by biological and social factors. However due to current lifestyle, social changes increase and have a negative impact on
sleep patterns. The media use, family environment and school starting time are some of the social factors that can exert negative effects on SWC of children, which can lead to irregularities in the bedtime and wake up time, partial sleep deprivation and daytime sleepiness. These changes are not expected in kindergarten children with such intensity, who naturally go to bed and wake up early. In a previous study we found daytime sleepiness during classes in children between 4-6 years that attended school in the morning. Therefore, this study aims to identify the social factors related to diurnal sleepiness in kindergarten Brazilian children that attend school in the morning. We hypothesized that the children with higher economic status and use the media (TV and computer) have higher levels of daytime sleepiness. The research will involve 20 schools (10 public and 10 private), located in 4 areas of the city of Natal, totaling 300 children. The sample will be transversal with children between 4-6 years, from both genders. The research will be done in two stages: 1st stage, meeting with parents to delivery of the consent form and characterization of the habits of sleep, with the application of sleep general habits questionnaire and economic classification, and 2nd stage, characterization of sleep pattern by sleep log by 7 days and observation of behavior in the classroom by 5 days by focal sampling method. In this method, it will be observed behaviors related to sleepiness (yawning, rubbing the eye, stretching and stooping on the desk) in 3 groups of children per class for 10 min for each group. All questionnaires will be filled out by parents.

PR.5  
EFFECTS OF ADVANCE AND DELAY OF THE SLEEP PHASE ON THE COMPONENTS OF ATTENTION

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An advance or a delay of the sleep phase produces an increase in sleepiness, as well as lower performance in arithmetic calculations, memory tasks, and vigilance tasks. This decrement in performance could be due to impairment in a basic cognitive process, such as attention. Attention includes four components: tonic alertness, phasic alertness, selective attention and sustained attention. The objective of this project is to determine the effects of an advance and a delay of the sleep phase on the components of attention. The hypothesis of this study is that people with an advance of the sleep phase will show
lower performance in all the components of attention. Sixty undergraduate students will participate, 30 female and 30 male, between 16 and 18 years of age. This study will have two stages; in both stages the participants will respond to a continuous performance task (CPT) to assess all components of attention. In the first stage, all the participants will sleep from 24:00 h to 08:00 h, for seven days. At 12:00 h of the seventh day, they will respond the CPT. In the second stage, the participants will be divided in 3 groups: the first group will remain in the same sleep schedule (24:00 h to 08:00 h), the second group will advance their sleep phase 2 hours (22:00 h to 06:00 h), and the third group will delay their sleep phase 2 hours (02:00 h to 10:00 h). Each group will keep its sleep phase for 5 consecutive days, and responses to CPT will be registered each day at 12:00 h. An Analysis of Variance will be used to compare the components of attention among the groups.

PR.6 LASC90
EFFECTS OF COFFEE INGESTION ON THE COMPONENTS OF ATTENTION AFTER A 24 H SLEEP DEPRIVATION

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Sleep deprivation decreases performance in many activities. This decrement in performance may be due to impairment in attention. This cognitive process is the capacity to respond to environmental demands and has four components: tonic alertness, phasic alertness, selective attention and sustained attention. Coffee is a central nervous system stimulant that reduces sleepiness, fatigue and improves performance in different cognitive tasks. The objective of this project is to determine the effects of ingesting coffee on the components of attention in a 24 h sleep deprivation. Coffee ingestion is expected to improve all the components of attention. Participants will be 60 undergraduate students, from 18 to 22 years old that consume coffee on a regular basis. The components of attention will be assessed with a Continuous Performance Task (CPT). For two weeks, participants will keep a sleep diary and will abstain from drinking caffeine or alcoholic beverages. Then, the participants will be divided in three groups and they will be registered in the laboratory for two consecutive days. The first day, the three groups will respond the CPT at 12:00 h (without sleep deprivation). Afterwards, groups 1 and 2 will remain awake in the laboratory for 24 h, while group 3 will sleep at home. At 11:30 h of the second day, group 1 will drink a cup of coffee, while groups 2 and 3 will drink a cup
of water. The three groups will respond again the CPT at 12:00 h. An Analysis of Variance will be used to compare differences among groups.

PR.7  
EFFECTS OF A 15 MINUTES NAP ON THE COMPONENTS OF ATTENTION

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A 15 minutes nap improves performance in sleep deprived persons. This improvement could be due to an effect on attention, a basic cognitive process that has 4 components: tonic alertness, phasic alertness, selective attention and sustained attention. The objective of this project is to determine the effects of a 15 minutes nap on the components of attention. The hypothesis of this study is that a nap will improve all the components of attention in sleep deprived people. Participants will be 30 undergraduate students, from 17-21 years of age, without health or sleep problems. They will keep a sleep diary for 2 weeks, and then they will sleep from 23:00-07:00 for 5 days. The fifth day they will respond a Continuous Performance Task at 12:00 h in the laboratory. The next night, they will sleep only 4 hours, from 23:00 h to 03:00 h. They will respond a Continuous Performance Task at 12:00 h. From 13:00 h to 13:30 h, participants will eat lunch in the laboratory. After this, participants will be randomly divided in two groups: a nap group, and a no-nap group. The nap group will take a 15 minutes nap, from 15:30 h to 15:45 h, at the laboratory, in total darkness and without noise. The no nap group will stay awake during this period in the same conditions. Immediately after this period, both groups will respond the Continuous Performance Task. Data will be analyzed using Analysis of Variance.
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