PLENARY LECTURES ABSTRACTS

0001
CIRCADIAN ENTRAINMENT BY DIFFERENT DAYLENGTHS: THE ROLES OF DAWN AND DUSK

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In the early days of circadian rhythms research there were two competing views on entrainment by light: through parametric action on the velocity of the endogenous cycle (Aschoff) *versus* non-parametric discrete phase shifts elicited by the lights-on and –off transitions (Pittendrigh). Although the phase shift model became widely accepted, straightforward experimental tests of the alternatives are lacking. In recent years we performed a series of studies in mice to address this issue, using protocols with single and double light pulses of different duration as well as single lights-on and lights-off signals. The results reveal no evidence for a specific role of the transitions *per se*. All features of the circadian system can be explained by velocity changes in response to light, a response that is reduced under prolonged illumination and restored during prolonged darkness. This arrangement allows robust entrainment when the signal is noisy and behavioural withdrawal from light adds to its variance. It leads to stable timing in species never observing dawn and dusk. It even keeps the clock entrained when mice must work for their food and show diurnal (shiftwork) activity. A model for a neuronal pacer network in the SCN shows exactly these properties and predicts seasonal adaptation to daylength.

0002
DAILY RHYTHM OF MICROCYSTIN BIOSYNTHESIS AND NITROGEN-ASSIMILATING ENZYMES IN CYANOBACTERIUM AND ALGAE

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Some cyanobacterial blooms of the genus *Microcystis* (Chroococcales, Cyanobacteria) can be a serious ecological and public health concern due to their ability to dominate the planktonic environment and produce cyclic heptapeptide toxins, named microcystins (MCYs). MCYs inhibit protein phosphatases, especially types 1 and 2A, in a similar way to the action of okadaic acid. Seasonal changes of *Microcystis* species and production of MCYs and aeruginopeptins have been reported previousty. The *psb*A2 gene has exhibited light-dependent and rhythmic expression in *Microcystis aeruginosa* K-81. It has been shown that several physiological and biochemical processes are controlled or at least are influenced by the biological clock in dinoflagellates and cyanobacteria such as, the cyanobacteria *Cyanothece* strain CGD temporally separates nitrogen fixation and photosynthetic activity to protect oxygen-sensitive nitrogenase, and the cyanobacterial KaiB and KaiC proteins (encoded by the gene cluster *kai*ABC) are robustly rhythmical, whereas the KaiA abundance undergoes little if any circadian oscillation in constant light. These findings strongly implicate a circadian regulatory mechanism operating on these metabolic processes are evidence for the importance of circadian rhythms in global metabolic regulation in some cyanobacteria species. Nitrate reductase en nitrate reductase exhibit a circadian expression in different algae. Therefore, some natural peptides, including MCYs and other bioactive oligopeptides, synthetized by cyanobacteria may be influenced by the biological clock. We have isolated and identified for the first time by ESI-MS/MS two MCYs *M. panniformis*. HPLC analyses with *M. panniformis* samples collected during L:D and L:L cycles indicated that the MCY-LR and [Asp^3]-MCY-LR levels were higher during the day. As supported by our data, the biosynthesis of MCYs is controlled by the biological clock. These findings are important to elucidate the mechanisms involved in MCYs biosynthesis as well as their isolation and characterization.
Support: FAPESP, CAPES and CNPq.

0003
FOOD-ENTRAINMENT: CONSENSUS AND CONTROVERSY

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2009 marks the 30th anniversary of the first report that a circadian rhythm of food anticipatory activity persists in rats lacking a suprachiasmatic nucleus circadian pacemaker and light-dark cues (Stephan et al, 1979). Feeding time has since then been shown to regulate the phase of circadian oscillators in many brain regions and in most peripheral organs and tissues. The neural and molecular bases of food anticipatory behavioral rhythms remain to be clarified but progress has been made. The weight of evidence now favors the view that food anticipatory rhythms are mediated by a distributed neural system modulated by peripheral metabolic hormones. Distinct neural circuits may mediate anticipation of other rewards (mating, water, drugs) delivered on circadian schedules. The role of known clock genes in food-entrainable oscillations driving behavioral rhythms is contentious. The mechanisms by which rats and mice anticipate two or more daily meals, and link these with specific feeding places, are unclear. The objective of this presentation will be to review areas of consensus and controversy, and to identify important questions that remain to be addressed in circadian regulation of foraging and physiology.

0004
IMMUNE-PINEAL AXIS: A SHUTTLE BETWEEN ENDOCRINE AND PARACRINE MELATONIN PRODUCTION

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The pineal gland is known to be responsible for the endocrine arm of the internal timing control. The nocturnal peak of melatonin is under direct control of environmental lighting in birds and reptiles and a result of a neuroendocrine connection with the suprachiasmatic nuclei, in mammals. For the best of our knowledge, light was taught to be the only control of melatonin production. Taking into account that melatonin, in concentrations compatible with those found in nocturnal plasma, blocks the transmigration of neutrophils in rodents, a condition which impairs the mounting of a proper innate immune response, we raised the hypothesis that the pineal gland should be also controlled by cytokines and/or inflammatory mediators. In fact, the pineal gland has receptors for the cytokine TNF (TNFR1), the first cytokine to be released at the mounting of an inflammatory response. It also produces this cytokine when stimulated by the lipopolyscaride (LPS) of the membrane of gram-negative bacteria, through the toll-like receptor 4 (TLR-4). Several of the downstream molecules involved in the response to TNF and LPS are also expressed in the pineal gland. Activation of the nuclear factor-kappa B (NF-kB) pathway inhibits *aa-nat* transcription. As a matter of fact, "in silica" study showed that the promoter of the gene for the key enzyme in melatonin synthesis, aryl-alkylamine N-acetyltransferase (AA-NAT) has sequence of nucleotides which binds NF-kB dimmers, and in the pineal gland, the dimmer p50-p50, which lacks the transactivation domain is translocated to the nucleus. Therefore, at the mounting of an inflammatory response nocturnal melatonin production is suppressed. On the other hand, glucocorticoids, by inhibiting NF-kB pathway, were shown to potentiate melatonin production. In the periphery, activated immune-competent cells produce melatonin, which reach very high concentrations in their neighborhood. It is interesting to note that the same pathway (NF-kB), which is pivotal for activating immune-competent cells, is responsible for the synthesis of melatonin. In this case the dimmer translocated to the nucleus is the p50/p65, which has a transactivation domain and induces gene transcription. In a sequential event, melatonin, which is known to block NF-kB pathway contributes to the shut down of the defense response. These events determined till the molecular level in rodents, more probably is the basis for the inverse correlation between TNF and melatonin observed in humans. In summary, the shuttle between endocrine and paracrine melatonin production plays a role in the mounting and shutting down of innate immune responses, and for the first time discloses a mechanism independent of environmental illumination for controlling melatonin synthesis.

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0005

THE POSSIBLE IMPACT OF LIGHT AT NIGHT AND LIGHT INTERFERENCE ON HUMAN HEALTH

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One of the most significant environmental changes that spread world wide in the twenty century is light at night (LAN). No doubt that LAN had a positive contribution to world economy, culture, health services transportation and sports. However it also exposed the human to abolishment of seasonality or to "seasons out of time", while humans as other mammals show seasonality in several variables. The results of recent studies carried out in our center reviled that LAN and Light Interference (LI) have a significant impact on seasonal acclimatization in rodents as well as on human breast and prostate (BC and PC) cancers but not on lung and recto-colon cancers. The results of *in vivo* studies showed that inoculation of BC and PC mice cancer cells to mice and LAN, LI, and melatonin treatment had a significant effect on the proliferation of cancer cells, as assessed by tumor volume. The disruption of our circadian rhythms by LAN and light interference (LI) has impact also on daily rhythms of blood coagulation as we showed in short day (SD) acclimated rats exposed to LI. In such rats daily rhythms of prothrombin time (PT) while LI to SD-acclimated golden spiny mice *Acomys russatus* affected daily rhythms of immune variables. In regards to LAN and LI we should remember that light intensity, wave length and duration of exposure are important variables. The results of a recent study in our laboratory revealed that exposure of mice inoculated with PC cells to blue light at night (~470 nanometer) facilitated the proliferation of the PC cells relatively to mice exposed to long wave length illumination - red light (~700 nanometer). What is the mechanism? Results from our experiments show that LI is a stressor as assessed by the increase of stress hormones (adrenaline and cortisol). Furthermore, a melatonin treatment to mice inoculated with PC or BC cells had a negative effect on cell proliferation. In modern life children and pregnant mothers are exposed to LAN and LI, we are trying to understand if such exposure has an impact on health issues in adults.

0006

IT TAKES MANY TO TANGO: A BRIEF SURVEY OF CHRONOBIOLOGY IN ARGENTINA

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Argentinean chronobiology is currently undergoing an interesting process of growth and transformation, mostly based upon the repatriation of young researchers actively involved in biological rhythm research. In this work we will present a brief history of chronobiological studies in the country, emphasizing the role of precursors in the field (ranging from sleep and melatonin studies to the first reports in liver DNA rhythms). We shall also describe the activities of the most active research groups currently involved in chronobiology in Argentina, scattered throughout the country and working, among others, on the following topics: mammalian and avian retina, circadian gene regulation, circadian rhythms in plants and photoperiodism, neuronal circuitry and regulation of *Drosophila* circadian rhythms, autoctonous models of biological rhythmicity, pineal and melatonin, *C. elegans* rhythms and mammalian entrainment pathways. Finally, we will present some bibliometric data on Argentinean scientific production in chronobiology.

0007

CHRONOBIOLOGY IN LATIN AMERICA

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Although we meet and publish mostly elsewhere around the planet, not in Latin America, we share several characteristics which I will exploit in this communication. Of course I hope to provoke debate. I will start with our common understanding that time is a rather relevant dimension of living matter - devoid of time life is dead, and not the supposedly and perfectly immobile structure that is commonly taught in our schools. I will proceed with considerations on the illusions brought to light by the present globalization, including science - notably our role as scientists in this scenary; time may not be the same everywhere. Then I will ponder on the apparently natural reivindication of a purely Latin American chronoscience. I will conclude with a proposition of a forum designed for a constant debate on possible bridges between our science and our reality, perhaps an online journal with open peer reviews.

0008

LOOKING FORWARD: WHERE ARE WE GOING?

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One of the most interesting and important directions for future research in chronobiology is to integrate the behavioral, physiological and molecular information that has been developed over the past 50 years and use the integrated information to provide insight into the functional importance of temporal structure. Such insight will increase our understanding of almost every biological process and will also open new channels for the treatment of disease. However, to be useful this approach must focus on specific processes and these must be chosen with care; they must be both biologically significant and experimentally tractable. To illustrate the power of such an approach, I will describe our recent work on the timing of ovulation in rodents — a classical problem that was "solved" sixty years ago and has taken its place in every endocrinology text. In the classical account, ovulation is induced by a surge of luteinizing hormone (LH) from the pituitary, the timing of which is dependent on events in the hypothalamus; the ovary is a passive responder. We have shown that this account is incomplete. In fact, there is a circadian rhythm of responsiveness to the ovulation-inducing effects of the LH surge at the level of the ovary itself. Thus the ovary is an active participant in the timing of ovulation, and this new insight is likely to affect the treatment of infertility and perhaps other ovarian pathologies.

THEMATIC DISCUSSIONS ABSTRACTS

THEMATIC SESSION 1 – Timing Symposium

0009

EFFECT OF MUSIC ON SUBJECTIVE TIME ESTIMATION

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We found that 20s-time intervals filled by tonal modulating music elicited retrospective time reproductions in inverse function of interkey distances, with a major impact for sudden modulations. We propose the expected development fraction model (EDF-model) explaining that when modulating music is presented, an expectation of time development is induced in accordance to the traversed interkey distance. Such expected virtual time is supposed to be projected by a music semantic memory and is longer than the perceived stimulus duration maintained by an implicit working memory. If a time estimation is requested, such disproportion or fraction is applied to the implicit working memory duration leading to the time underestimation. A second experiment showed shorter retrospective time reproduction for distant reverse modulating music than for close one. A third experiment showed shorter retrospective time reproduction for cycle of third modulation (versus cycle of fifth one) and for counterclockwise modulation (versus clockwise one). These two experiments additionally confirmed the statement of EDF-model and elucidated some issues of the spatial-temporal cognitive processing of music tonality.

0010

CIRCADIAN VARIATIONS IN SUSTAINED ATTENTION

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Human performance is modulated by circadian rhythms. Efficiency in many tasks increases during daytime and decreases at nighttime and first hours in the morning. It is possible that the observed changes in performance are produced by circadian variations in a basic cognitive process, such as sustained attention. This cognitive process is the capacity to respond efficiently to the environment during prolonged periods (from minutes to hours). There are three indices of sustained attention: general stability of efficiency, time on task stability and short-term stability. The objective of this work was to analyze circadian variations in the indices of sustained attention.
Participants were 9 undergraduate female students, (mean age 18.0 yr, SD=1.31, range 16-21 yr), that attended school from 07:00-13:30 h, Monday to Friday. They were recorded in a con-

stant routine protocol during 28 h; in this protocol feeding, environmental temperature, motor activity and room illumination were controlled. Rectal temperature was recorded each minute, whereas indices of sustained attention were assessed each hour through a continuous performance task (CPT). This task required them to press 1 to any number (except "9") appearing at the center of the computer screen, to press 2 when a "9" appeared, and to press 3 when a "4" appeared after the "9". The CPT had 27 blocks with 20 stimuli each. Three indices of sustained attention were obtained: general stability (standard deviation of correct responses and reaction time within the blocks), time on task stability (linear regression of correct responses and reaction time throughout the blocks) and short-term stability (hit runs and error runs).
Rectal temperature showed circadian variations. Subjective sleepiness and tiredness increased progressively during the recording session, but tiredness did not show circadian variations. All indices of sustained attention declined through the 28 h recording period. But only two indices showed circadian variations: general stability and short-term stability. Time on task stability declined through the recording period (with time awake), but did not show circadian variations.
In conclusion, the indices of sustained attention are crucial for circadian and homeostatic variations. Circadian variations in general stability and short-term stability can modulate variations in the execution of many tasks. Whereas a decline in time on task stability can modulate the execution of many task with time awake.

0011

CIRCADIAN MODULATION OF INTERVAL TIMING IN MICE

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Timing and time perception are fundamental to survival and goal reaching in humans and other animals. To deal with timing, organisms have developed multiple systems that are active over a range of 10 orders of magnitude, the most important being circadian timing, interval timing and millisecond timing. The circadian pacemaker is located in the suprachiasmatic nuclei (SCN) of the hypothalamus, and is driven by a self-sustaining oscillator with a period near to 24 h. The estimation of time in the second-to-minutes range – known as interval timing – involves the interaction of the basal ganglia and the prefrontal cortex. There are several evidences indicating a possible link between circadian and interval timing.
In this work we tested the hypothesis that interval timing is sensitive to circadian modulations. The ability of mice to estimate time intervals of short duration was examined. Animals were trained following the peak-interval (PI) procedure. Briefly, mice were trained in three consecutive phases - pre-training, fixed interval training and peak interval training. Two independent experiments were conducted, in which mice were trained in the middle of the diurnal phase (ZT 5-7) or in the middle of the nocturnal phase (ZT 17-19). Results show significant differences in the estimation of 24-second intervals at different times of day, being more accurate the group trained at night. Interval timing was also studied in animals under constant light (LL) conditions, which abolish circadian activity and temperature rhythms. Mice under LL conditions were unable to acquire temporal control in the peak interval procedure. Taken together, our results indicate a connection between the circadian system and the interval timer.
Currently we are studying the estimation of time throughout the circadian cycle, as well as interval timing in animals subjected to circadian desynchronizations. We also aim to develop theoretical models which tell about the mechanisms involved in interval timing.

THEMATIC SESSION 2 – Breaking the synchrony of the circadian orchestra

0012

EATING AND WORKING IN THE NIGHT: DESINCHRONY AND DISEASE

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Shift- and night – work lead to internal desynchronization characterized by loss of phase relation between behavioral, hormonal, and metabolic rhythms. In the long-term night and shift work lead to cardiovascular and gastric disorders, propensity to obesity, metabolic syndrome and cancer. In order to better understand the mechanisms underlying the internal desinchrony in the night worker, we have developed an experimental model of night-work in rats based on daily schedules of forced activity during the resting phase.
From Monday to Friday rats are placed for 8 hours in slow rotating wheels during the light phase (from 9 AM to 5 PM). During the remaining hours of the day and during weekends rats are returned to their individual home cages in a monitoring system in order to register their spontaneous activity.
After 4 weeks under this "working" schedule the amplitude of daily activity rhythms is significantly diminished and rats voluntarily shift their food ingestion towards "working" hours. Consequently metabolic rhythms are dampened and uncoupled from the SCN, exhibiting peak values during the light phase, when rats are "working", while the activity in the SCN measured with c-Fos and Per1 immunohistochemistry, remains coupled to the LD cycle. Follow up of Fos and Per1 daily cycles in hypothalamic structures indicate that their daily activity shifts to the "working" schedule and thus also is uncoupled from the SCN. Since feeding schedules are strong entraining signals for peripheral oscillators, metabolism and behavior, we explored whether the modified feeding patterns developed by working rats could have promoted the internal desinchrony. By restricting feeding schedules for working rats towards the night, and preventing rats to eat during their "working schedules" the disturbance in metabolic rhythms was prevented and working rats limited to eat during the night showed similar diurnal patterns as *ad libitum* controls. However working and control rats scheduled to eat only during the light phase developed disturbed metabolic rhythms and significantly increased body weight.
Our study evidences the deleterious effects of shifted feeding schedules towards the resting phase and points out the relevance of feeding schedules to prevent internal desinchrony in the night worker.
Support: This study was supported by CONACyT 43950-M; CONACyT 82462 and PAPIIT- UNAM IN-203907.

0013

MARMOSETS UNDER T21: A PRIMATE MODEL FOR FORCED DESYNCHRONIZATION

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Introduction: The suprachiasmatic nuclei (SCN) are the master structure of mammalian circadian system. There are lots of evidences that each SCN cell is an oscillator and that entrainment depends upon coupling degree between them. Knowledge of this coupling mechanism is essential to understand entrainment and expression of circadian rhythms, which will help to provide the development of new treatments to circadian rhythms sleep disorders (CRSDs). Some authors suggest that the dissociation model of circadian rhythm activity of rats under T22 is a good model to induce internal desynchronization, and in this way, enhance knowledge about the coupling mechanism. So we applied the dissociation model to marmosets in order to find a primate model for forced desynchronization.
Methods: With this purpose 6 adult females were kept in individual cages, in a room with controlled temperature and humidity, and sound attenuated. The light intensity during light phase was 146.5 ± 53.3 lx whereas dark phase consisted in total darkness. They were maintained under the symmetric light-dark (LD) cycles T21, T21.5 and T22 during 60, 35 and 48 days, respectively. Motor activity of each animal and vocalizations of all animals were continuously registered.
Results: Two components of circadian motor activity rhythm were seen for all animals under T21, one with the same period of external LD cycle, named light-entrained component (LEC), and the other in free-running, named non-light-entrained component (NLEC). In the other two conditions, only 83.3% (T21.5) and 33.3 % (T22) of the animals showed two significative components. For vocalizations there were observed two circadian components under the three LD cycles periods. These results reflect the activity of at least two different groups of oscillators, being one capable of entrain to the LD cycle while the other can't, depending on LD cycle period. This probably occurred due to an uncoupling between these oscillators inducing an internal desynchronization.
Conclusions: Therefore, as all the animals presented two circadian components in motor activity and vocalizations rhythms only under T21, we suggest that marmosets under T21 can be a good primate model to study internal desynchronization. An animal model with a diurnal primate can be very useful to the development of new treatments to CRSDs, since most of the animal models are with nocturnal rodents.
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0014

A TIME TO KILL AND A TIME TO HEAL. ENTRAINMENT AND INTERACTIONS BETWEEN THE CIRCADIAN AND THE IMMUNE SYSTEMS

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Circadian rhythms are ubiquitous in nature, and control temporal order in most (if not all) physiological systems. Indeed, most immune factors and processes are under diurnal control, orchestrated by the circadian system, although the efferent pathways that control these cycles are not completely understood.
In addition, circadian disorders are usually associated with disease, and temporal disarrangements in immune parameters are closely related to the onset or development of pathological mechanisms. In the last few years we have been working under the hypothesis that the circadian-immune interaction can operate in two directions. First, daily cycles in immune parameters are controlled by the central circadian pacemaker located at the hypothalamic SCN. On the other hand, these same temporal variations might be useful for a fine-tuning of the system by affecting receptors located in the circadian clock. We have provided evidence suggesting that this latter interaction might be mediated by glial cells that respond to peripheral or central cytokines which by different transcriptional activators affect molecular circadian rhythms. In particular, we have shown that an endotoxin (LPS) challenge is able to set the phase of locomotor activity circadian rhythms, reaching the SCN through a Toll-4 receptor activation and pro-inflammatory cytokine signal transduction pathways. Cytokines are also able to affect entrainment and SCN gene expression in vivo and clock gene expression in vitro. We have previously demonstrated that NFkB-related transcription is also involved in entrainment, opening a new window into the regulation of gene expression in relation to circadian rhythmicity. Indeed, the precise knowledge of this circadian-immune interaction might be extremely useful for the understanding of why many infections and illnesses affect circadian rhythms (including the sleep-wake cycle) and greatly decrease the patients' quality of life. Besides a better understanding of the physiological regulation of circadian rhythmicity, the chronobiological basis of the times to kill and the times to heal will therefore provide a novel basis for diagnosis and therapeutical approaches in order to treat disease. When the circadian orchestra is out of tune or rhythm, a precise knowledge of immune-circadian interactions might be helpful for regaining the musical control of the body.

0015
SCN SUBREGIONS AS INDEPENDENT DIRECTORS
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The master circadian clock located within the hypothalamic suprachiasmatic nucleus regulates an array of diverse circadian rhythms, including the sleep-wake cycle, hormonal rhythms and the phase of peripheral oscillators. This centralized regulation by SCN's clock warranties the synchronization of circadian rhythms to the light dark (LD) cycle through direct retinal input to the SCN, as well as the internal synchronization of circadian rhythms, characterized by a constant phase relationship between different circadian rhythms within an animal. Neurons within the SCN are single-cell circadian oscillators that oscillate in synchrony under 24-h LD cycles. Exposure to 22-h LD, however, leads to the desynchronization between neurons located within the ventrolateral (vl) and dorsomedial (dm) SCN. This forced desynchronization protocol has become a useful tool to dissect out circadian outputs of the master clock that differentially rely on the activity of each of these SCN subregions. Our laboratory has exploited this animal model to better understand the circadian regulation of core body temperature, sleep stages, the release of melatonin, luteinizing hormone and corticosterone, as well as the decoding of photic input by the SCN neuronal network. Taken together, our results represent the first insight into the neural bases of internal desynchronization, a signature feature of most circadian disorders.

0016
THE SYNCHRONY OF LIFE: THE AUTONOMIC OUTPUT OF THE SCN
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The hypothalamus integrates information from the brain and the body; this activity is essential for survival of the individual (adaptation to the environment) and the species (reproduction). As a result, countless functions are regulated by neuroendocrine and autonomic hypothalamic processes in concert with the appropriate behaviour that is mediated by neuronal influences on other brain areas. Within the hypothalamus de suprachiasmatic nucleus (SCN) imposes its rhythm onto the body via three different routes of communication: 1.Via the secretion of hormones; 2. via the parasympathetic and 3.via the sympathetic autonomous nervous system. The SCN uses separate connections via either the sympathetic or via the parasympathetic system not only to prepare the body for the coming change in activity cycle but also to prepare the body and its organs for the hormones that are associated with such change. Herein the SCN is essential to prepare the body for the coming activity and inactivity period by determining the set point of various physiological variables. The hypothesis for our present work is that in view of the role of the SCN in determining these physiological day-night levels the SCN also needs to be informed about the accurate values of these variables. Apart from light, activity and melatonin little is known about the information that is provided to the SCN in order to execute its functions. In this presentation attention will be paid not only to the mechanisms the SCN is using to organize the set point in hormones, glucose, heart rate and temperature but also in how the SCN receives back information about these physiological variables. For example the role of the circumventricular organs in passing circulating information to the SCN will be discussed. In addition the role of the nucleus Tractus Solitarius (NTS) in providing visceral sensory information to the SCN and the role of the SCN in controlling cardiovascular functions is discussed in relation with the post-mortem finding that the activity of the SCN is diminished in people who had a long history of hypertension.
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THEMATIC SESSION 3 – The circadian and sleep phenotypesÇ its relation to health and clock genetics

0017
MOLECULAR AND EVOLUTIONARY ANALYSIS OF CIRCADIAN RHYTHMS IN INSECT VECTORS
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Blood-sucking insects such as sandflies and mosquitoes are vectors of pathogens that cause a number of important diseases. The daily activity patterns of these insect vectors are controlled by the circadian clock. However, despite the medical importance of hematophagous insects, very little is known about the molecular genetics of their biological rhythms. In the model insect species *Drosophila melanogaster*, a number of genes have been implicated in the control of circadian rhythms and the basic mechanism consists of transcriptional negative feedback loops that control the cyclic expression of some of their components. Our group is studying the molecular and evolutionary genetics of circadian rhythms in insect vectors of tropical diseases. Initially, we analyzed the locomotor activity rhythms and clock gene expression in the sandfly *Lutzomyia longipalpis* (Diptera: Psychodidae), the main vector of visceral leishmaniasis in Latin America. We are currently studying the molecular control of circadian rhythms in mosquitoes (Diptera: Culicidae). We are comparing the activity rhythms and circadian expression of the main clock genes in *Aedes aegypti*, a diurnal mosquito that is vector of dengue and yellow fever, and in *Culex quinquefasciatus*, a nocturnal mosquito vector of filariasis. Some of the results our group has obtained studying these insect vectors will be presented.

0018
SLEEP AND CLOCK PHENOTYPES IN WORLDWIDE POPULATION GENETICS STUDIES
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Despite of its complexity, sleep has two straightforward properties – timing and duration, which are influenced by a homeostatic process (how long have we been awake) and a circadian process. Individual circadian clocks entrain differently to environmental cycles (*zeitgebers*, e.g., light and darkness), earlier or later within the day, leading to different chronotypes. To assess chronotype and sleep duration in humans, we ask timing of sleep (independently for the workweek and weekends) using the Munich Chronotype Questionnaire (MCTQ). In the 12 populations we have assessed so far, the distribution of chronotypes and sleep duration form population-specific bell-shaped curves ranging from extreme early to extreme late types (Gaussian distributions are characteristic for multi-factorial traits). Although the genetics of sleep has been addressed in humans, associations between clock genes and sleep duration or timing have never been investigated systematically. To further understand this genetic influence in humans, we are using advanced methods of genomics in association studies with chronotype or sleep duration as phenotypes. Our first results indicate that the investigated traits have a genetic component, and that genes (beyond the known clock and sleep candidate genes) play a role in the molecular mechanisms modulating sleep timing and duration.

0019
PER3 — CLOCK GENE OR SLEEP GENE?
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Whereas invertebrates such as *Drosophila* only have one *Period* gene, mammals such as humans have three paralogues (*Per1, Per2,* and *Per3*). This, as many other gene families, is one of many results of two proposed genome duplication events early in vertebrate evolution (the so-called 2R hypothesis). Typically, in these instances, the duplicated gene products take on different roles. This diversity has been suggested to be a major contributor to the increased anatomic and physiological complexity of the vertebrates. By comparing the neighbouring gene clusters, and intron/exon structure, we were able to reveal evidence for a common origin, followed by duplication, of the human *PER* genes. Data from double knockout mouse models have shown that, whereas *Per1* or *Per2* are each individually to sustain circadian rhythmicity in the absence of all other paralogues, *Per3* is neither necessary nor sufficient for circadian rhythms. Of crucial importance for our work in trying to understand better the function of *Per3* has been a variable number tandem repeat (VNTR) polymorphism in the coding region of the human *PER3* gene. The two alleles of this polymorphism encode either four or five repeats of an 18-amino-acid unit. In our initial study, we found moderate but significant associations between the shorter allele (*PER3*^{4}) and evening preference and delayed sleep phase disorder (DSPD), and between the longer allele (*PER3*^{5}) and morning preference. We subsequently designed a prospective study, where we compared matched volunteers who were homozygous for the two different alleles. No difference were found in amplitude or phase of circadian parameters, including melatonin an cortisol levels and clock gene expression in peripheral leukocytes, disproving our initial hypothesis. Instead, we found considerable differences in sleep homeostasis and tolerance to sleep deprivation between the two groups. The *PER3*^{5} homozygotes had a significantly shorter sleep latency, more slow-wave sleep during baseline sleep, and less non-REM sleep following sleep deprivation. During sleep deprivation, they had a considerably greater decrement in cognitive performance than the *PER3*^{4} homozygotes. Thus, the *PER3*^{5} homozygotes could be described as living under a higher sleep pressure. In conclusion, our findings indicate that the *Per3* gene is more involved with sleep homeostasis than with circadian rhythms. Such a shift in function is entirely consistent with the 2R model of gene duplication.

THEMATIC SESSION 4 – Suprachiasmatic nucleus and circadian rhythms: Some functional aspects

0020
PRESENCE OF SEROTONIN RECEPTORS IN SUPRACHIASMATIC NUCLEUS OF MARMOSET BRAIN (*Callithrix jacchus*)
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The core nucleus of the mammalian circadian timing system (CTS) is the hypothalamic suprachiasmatic nucleus (SCN) which receives a strong serotonergic projection from the raphe. Serotonin is involved in the fine adjustments at several brain centers and in the SCN these actions are reported as been primarily mediated by the receptor type 5-HT$_{1}$. In rodents, there is a consensus that the general 5-HT projection pattern arrives at the ventral portion of the SCN, mismatching the arriving fibers of the retinohypothalamic tract. Confronting these findings, we show here that in a New World primate species, *Callithrix jacchus*, the occurrence of the positive labeling for the subtypes 5-HT$_{1A}$ and 5-HT$_{1B}$ are mostly at the dorsal SCN. We also confirm the dualistic nature of the placement of the 5-HT-positive fibers in the SCN of *Callithrix*, with higher density in their dorsal and ventral contours, with a core devoid of any labeling. The presence of these two types of receptors in the dorsal SCN of marmosets appears to complement the retinal terminal plexus located in the ventral region, confirming the uniqueness of the CTS of the common marmoset as different in relation to the pattern found in other species currently studied. Since the subtypes 5-HT$_{1A}$ and 5-HT$_{1B}$

were preferentially found dorsally, the discussion addresses to a new interpretation of the 5-HT effects in the regulation and synchronization of the CTS itself in New World primates.
Support: CAPES, CNPq, FAPESP, PROPESQ-UFRN.

0021
INTRA AND INTERCELLULAR MODULATION OF CIRCADIAN ENTRAINMENT IN THE SCN: THE ROLE OF NITRIC OXIDE
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The synchronization to light-dark (LD) cycles of the circadian clock is determined by differential phase-shifting established by the own clock phase (i.e., entrainment), which is predicted by phase-response curve (PRC). Photic Glutamate/Ca^{2+} activation of neuronal nitric oxide synthase (nNOs) through phosphorylation by its Calcium-dependent kinase II (CAMkII), leads to the increase of nitric oxide (NO) levels. Intracellular NO levels enhance a GC-cGMP-dependent kinase (PKG) activity, specifically during the late subjective night for light-induced phase advances. Also using Glutamate/Ca^{2+}, a bifurcation in this signal transduction pathway is neccesary for photic-induced delays through NO-dependent activation of Ca^{2+} ryanodine channels. Both mechanisms converge to the phosphorylation of the transcription factor Ca^{2+}/cAMP-responsive element binding protein (CREB), which activates Per1 and Per2 genes in the SCN for clock phase-resetting. At the intracellular level, the availability of both pathways leading to opposite phase-shifts, is an output which depends on the own clock phase. Besides its established role as an intracellular messenger, NO is also a component of the intercellular SCN communication. Extracellular NO is necessary for the photic signal spread into the SCN for the light-pulse-induced advances, but it is not involved for delays. In addition, extracellular NO communication is involved in the entrainment by advances to skelleton photoperiod cycles shorter than 24 hs (T23 cycles), but not in the entrainment by delays to T25 cycles. As well as blocking this non-parametric entrainment to T23 cycles, the extracellular-NO scavenging also inhibits the light-induced Per1 gene activation. These results indicate a direct linkage between extracellular NO signaling and the molecular phase-resetting of the circadian clock. Thus, circadian entrainment depends on the intercellular NO communication of the light messenge within the SCN tissue.

0022
NEURAL CIRCUITRY REGULATING CIRCADIAN SLEEP-WAKE RHYTHM
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Sleep-wake behavior is under control of the circadian pacemaker, the suprachiasmatic nucleus (SCN). Over the last decade, we have identified that for circadian control of sleep-wake behavior, the SCN projects to the ventral subparaventricular zone (SPZ), which then project to the dorsomedial hypothalamic nucleus (DMH). Lesions of the ventral SPZ and DMH abolish circadian rhythm of sleep-wake behavior. The key question is via which circuits the DMH control circadian rhythm of sleep-wake behavior. If we assume that the DMH has to target either sleep or arousal system or both, then we should examine circadian rhythm of sleep-wake behavior following lesions in sleep and arousal structures. At this point, only one sleep promoting area is found in the ventrolateral preoptic nucleus (VLPO). Interestingly, lesions of the VLPO do not significantly affect circadian rhythmicity of sleep-wake behavior, even though sleep is reduced by a half. Lesions of most arousal groups including the basal forebrain cholinergic neurons, locus coeruleus, raphe nucleus, tuberomamillary nucleus, orexin neurons, pontine cholinergic neurons and mesopontine dopaminergic neurons appear not to alter circadian sleep-wake rhythm. We recently have identified a novel arousal neuronal group in the parabrachical nucleus (PB) in the brainstem. All the PB neurons contain vesicular glutamate transporter 2 (VLGUT2) and thus are glutamatergic. To eliminate VGLUT2 gene, we inject an adeno-associated viral (**AAV**) vector containing **Cre** into the PB in flox-VGLUT2 mice. We found that loss of the glutamate signaling in the PB significantly reduces the amplitude of circadian sleep-wake rhythm and wakefulness by about 30%, and unmasks ultradain sleep-wake rhythm. We hypothesize that the DMH controls circadian sleep-wake rhythm via arousal systems including the PB.
Support: NIH NS051609 and NS 062727

THEMATIC SESSION 5 – Synchronization in real world

0023
FOOD INTAKE IN NIGHT WORKERS: A CONFLICT BETWEEN PHOTIC AND NON-PHOTIC SYNCHRONIZERS
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Working at night or in shifts causes changes in feeding behavior, since this process can be concurrently influenced by diverse factors of biological, social and cultural determinants. Since the end of the 1960s there have been studies about eating habits among shift workers. The changes in food consumption in these workers might be explained by the fact that night work is a condition that leads to an imbalance between the meal schedule and intestinal mobility, caused by changes in the quality of food ingested and a reduction in time for breaks at work. Shift workers complain of a lack of appetite, which can be associated with changes in the periodicity of meals. The meal schedule even has an influence on the secretion of digestive juices, and on the absorption and digestion of food. In fact, there appears to be a consensus among researchers in the field that the distribution of total caloric intake over the 24 hours does not vary between different shifts, however, there are differences related to dietary content and caloric distribution over the course of the day among shift workers. Research related to nutrition among shift workers ranges from epidemiological studies which provide evidence for shift work being a risk factor for various diseases such as gastrointestinal and cardiovascular illnesses, for instance, to studies centered on the distribution of food ingestion over 24 hours, particularly on the timing of food ingestion among the workers. Some of these studies include the social repercussions of the meal schedules as well.

0024
SHEDDING A LIGHT ON ADOLESCENT SLEEP DEPRIVATION
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Adolescents experience excessive daily sleepiness because they get an insufficient amount of sleep. An adolescent requires an average amount of daily sleep in the range of 8.5 to 9.25 hours. Studies of adolescents in several countries have shown that the student's average sleep period is reduced during school days to around 7 hours due to a tendency of students to delay bedtime while maintaining the same waking time to adhere to school schedules. A partial sleep deprivation that averages 1-2 hours per day during school days is enough to trigger symptoms of sleepiness, leading to a reduced school performance. It is already known that excessive sleepiness impairs concentration and learning capacity. For many years, the phase delay was attributed exclusively to social factors: greater independence to organize their own schedules, access to technology (TV, video games, computers, and the Internet), and a greater demand for social outings and events. In the early 1990s, it was demonstrated that pubertal stages of development were associated with a delay in sleep times. According to the classification proposed by Tanner, more mature adolescents have more delayed sleeping times than less mature adolescents, regardless of the age factor. In order to identify the possible social factors involved in the expression of the biological rhythmicity during adolescence, we have carried out studies with rural populations, some of which do not have electric power at home, These results reinforce the major influence exerted by social factors upon the adolescent phase delay. The magnitude of this delay in urban populations, particularly those that have access to technological resources, is greater and, consequently, sleep deprivation during school days is also greater. Changes associated with pubertal maturation render the individual more susceptible to delays in sleep time. It is as if the same stimuli have a different effect depending on the function of the puberal stage. In more advanced puberal stages, the adolescent's body is more sensitive to stimuli capable of promoting delays in the biological rhythms. We can thus conclude that not all adolescents are sleep deprived, only those who are exposed to contemporary society's technological advances.

0025
CIRCADIAN RHYTHMS IN URINARY CORTISOL AND 6-SULFATOXY MELATONIN IN BRAZILIAN NURSES ON DAY AND NIGHT SHIFTS
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Objective: To study the degree of disruption of the circadian periodicity of urinary cortisol and melatonin in nurses working on either day or night shifts.
Methods: Nineteen young adult healthy women holding only one job participated in this study. The night workers (n=12) worked 12h night shift (19:00-07:00) followed by 36h off, having one extra off day twice a month, resulting in three consecutive free nights. Day workers (n=7) worked 6 days a week 6 hours per shift, 6 worked during the afternoon shift (13:00 to 19:00) and one worked at morning shift (07:00-13:00), all having one free day per week. Participants wore an actigraph to monitor activity-rest times and filled out a daily log to register perceived sleep quality. Nurses were classified as more tolerant (n=8) and less tolerant (n=4) to night work, being considered less tolerant ones those who exhibited at least two of these parameters: fatigue, sleep disturbances, sleepiness, insomnia, minor psychic disorders, and reduced availability of free time off work. Urine was collected during waking hours in two different times of four consecutive days to measure 6-sulphatoxymelatonin (6SOHMel) and cortisol. The data were analyzed by the single cosinor for each subject during each study period and for the days on night shift and the days off. Each group during each of the examined time spans was analyzed by ANOVA and cosinor.
Results and Discussion: Day workers and night workers on working days and on days off showed a circadian rhythm for cortisol excretion (acrophase between 12:00 and 13:30). ANOVA indicated lower cortisol excretion in the night workers on the day of work (day workers vs. night workers at work, p=0.0012) with recovery on the days of rest (night workers at work vs. off work, p=0.0412). The sum of all cortisol values was lower in the less tolerant night workers than more tolerant ones (p=0.0473). Examining separately days on night work and days off, the lower values appear to be due to lower values on work days, although in the separate groups statistical significance was only marginal (p=0.054). In the day workers, a circadian variation for 6SOHMel excretion was confirmed by ANOVA, but not by cosinor (p=0.101) with an acrophase around 08:16. The night workers showed a circadian rhythm validated by cosinor on nights workers (p=0.001) and on days of rest (p=0.002) with acrophases at a 08:04 and 08:08 respectively, corresponding to the morning urine collection. The circadian mean was significantly lower than the day workers in the days of work (p<0.0001) and remained so in the days off work (p<0.0001). The sum of all 6SOHMel values was lower in the less tolerant workers than more tolerant ones (p=0.0426).
Conclusion: Night workers with alternating days off, have no phase adaptation of cortisol or melatonin to the night shift. Sup-

pression of melatonin in shiftworkers is thought to be one of the mechanisms leading to an increased cancer incidence. Subjects with lower tolerance of shift work show lower cortisol and 6SOHMel excretion, but this needs to be better evaluated.
Support: CAPES, CNPq, the HealthPartners Research Foundation, Grant # 99-130, Fogarty International Center, Mount Sinai School of Medicine, NY, USA.

0026
EFFECT OF SUNLIGHT EXPOSURE IN THE MORNING ON SLEEP-WAKE CYCLE AND DAYTIME SLEEPINESS OF ADOLESCENTS
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Introduction: Adolescents present biological and social pressures to a phase delay that is counterposed by early school schedules. At vacation, the sleep schedules change making it difficult the return to school. As a consequence they have short sleep duration and sleep irregularity between school and no school days leading to daytime sleepiness and cognition performance deficit. As light promotes phase advances between the end of rest and the start of activity in humans, problems can be minimized by exposure to morning sunlight.
Objective: To evaluate the effect of sunlight exposure in the morning on the sleep-wake cycle (SWC) and sleepiness of adolescents in the return to the classes after mid-year vacation.
Methods: 83 high school students volunteers aged 15 ± 0.5 took part in 2 stages: 1ª) Characterization about sleep knowledge and habits; 2ª) SWC and sleepiness evaluation for two consecutive weeks after mid-year vacation. The SWC was assessed by sleep log and the sleepiness levels were evaluated by the Karolinska Sleepiness Scale (KSS), on 5 times: wake-up time, 8h, 11h, 14h and bedtime. Besides, subgroups underwent a psychomotor vigilance test on weekdays at 8h and 11h. At the second week after mid-year vacation the sunlight intervention group (n=45) attended the first class (7:15-8:00 h) at shadow in an outdoor environment exposed to sunlight (11.500 ± 4.800 lux) while the control group (n=38) stayed in usual classroom (256 ± 96 lux).
Results: The intervention group anticipated the bedtime from 23h48min to 23h25min and increased sleep duration in 16 minutes on weekdays. The sleepiness levels, measured by the KSS, showed no difference between the two weeks for both groups, but the PVT detected a decrease in reaction time at 11hs in the intervention group. Naps frequency diminished on weekdays from 46% to 31% on the intervention group. Irregulatity remained for bedtime, but decreased to wake-up time for both groups.
Conclusion: sunlight exposure in the morning advanced the SWC and increased alertness at 11hs. Thus, this intervention can be used as a school procedure to help students to adapt their sleep-wake cycle on the return to school.

0027
EVALUATION OF BRIGHT LIGHT USE TO REDUCE SLEEPINESS IN BRAZILIAN UNIVERSITY STUDENTS
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Introduction: Former studies carried out among young workers have shown high sleepiness levels during evening school attendance. In addition, several studies have shown that exposure to bright light prevents sleepiness during night work. However, as far as we know, the effects of bright light exposure is still unclear during evening hours among college students.
Aim: To evaluate the effects of bright light exposure on sleepiness during evening hours among college students.
Methodology: Twenty-seven healthy College students, all males, with ages ranging from 21 to 24 years old, working during the day and studying in the evening, participated in this study. During three weeks the students filled out daily activities logs, including sleep duration, wore actigraphs and recorded levels of sleepiness three times in the evening (19:00; 20:30; 22:00) using the Karolinska Sleepiness Scale. In a crossover design, on the second and third weeks, the students were exposed once to white bright light during 20 minutes (8.000 lux) at 19:00h or at 21:00hours. Salivary melatonin samples were collected before and after light exposure. A repeated measure ANOVA was performed to evaluate the effects of light exposure on sleepiness. Melatonin levels (increased or decreased after light exposure) and sleep duration before light exposure (≤ 6 hours or > 6 hours) were considered as factors. LSD Post Hoc test was used.
Results: The percentage of individuals with melatonin suppression compared to baseline after light exposure at 19:00h was 52.2%, and at 21:00h, 57.9%. The results showed a reduced level of sleepiness at 22:00h when bright light exposure was presented at 21:00h (F=4.01; p=0.03); the mean values of sleepiness at 19:00h were 4.17 ± 1.64, at 20:30h=4.45 ± 1.13; and at 22:00 h= 4.36 ± 1.29. When bright light exposure was presented at 19:00h, the mean values of sleepiness were: at 19:00h= 4.73 ±1.27, at 20:30h= 5.08±1.24) and at 22:00h=5.43 ±1.62). No significant differences on sleepiness were observed after bright light exposure at 19:00h.
Conclusions: The study showed an effect on sleepiness when light exposure was presented at 21:00h. This result might suggest that bright light exposure close to sunset would not produce a perceived reduction of sleepiness.
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0028
COMPARATIVE ASPECTS OF THE EYE AND RELATIONSHIP WITH TEMPORAL NICHE
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Evolutionary attempts in modify the ocular structure may appear relatively quickly in the geologic time span, suggesting that eyes are able to modify face to environmental pressures. Adaptive mechanisms to the temporal niche are present in the visual system of most of vertebrates, which involve modifications in ocular dimensions and design, in the retinal cells distribution as well as in the organization of neurochemical circuits related to the retinal resolution or detection of changes in the luminance levels. The symmetry of the perceived environment is reflected in the arrangement of retinal cells. Nonoverlapping topographies of different classes of photoreceptor and retinal ganglion cells have indicated that there is parallel processing, where different components of the visual scene may be sampled by distinct subsets of cells. On the other hand, the quality of the optical system may limit the amount of information that can be made available to the brain since the retina can only encode information that is present in the image. Therefore, adjustment of focus is particularly important if an animal has eyes adapted for use under low- or high-light conditions. Together with knowledge of the position of the eyes in the head, the shape and size of the lenses, pupil and distribution of photosensitive retinal cells can identify adaptations of each species to the temporal niche. Animal eyes that are primarily used under low-light conditions, for example, usually have optical systems of short depth of focus, such that chromatic defocus may lead to considerable blurring of the images. In some vertebrates, this problem is solved by multifocal lenses having concentric zones of different focal lengths, each of which focuses a different relevant spectral range onto the retina. In combination with these multifocal lenses the presence of a slit pupil avoids the loss of wellfocused images at relevant wavelengths even in bright light. For maximum light-gathering ability, the eyes of nocturnal and crepuscular vertebrates have pupils that are large relative to the focal lengths of the optical systems. Moreover an increase in the eye size and in the retinal magnification factor improve the retina sensitivity without reduce its resolution when compared with eyes of diurnal animals. Thus, although the main characteristics of the eye can be preserved in the phylogeny, the history of each family or genus add specific features that can be more involved with the lifestyle within a temporal niche than with the relationship among the species.

0029
MELATONIN MODULATES VISUAL FUNCTION AND CELLS VIABILITY IN THE MOUSE RETINA
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The role played by melatonin in the mammalian retina is not well defined. Melatonin receptors are expressed by several retinal cell types, including the photoreceptors, suggesting that melatonin receptors may be involved in the regulation of photoreceptor physiology. Previous studies have reported that melatonin influences the membrane conductance of dark adapted frog photoreceptors, acts directly on the rod photoreceptors to increase dark adaptation, and potentiates rod signals to ON type bipolar cells in fish retina. Melatonin is also involved in the modulation and circadian regulation of the electroretinogram in many species. No previous study has investigated the functional roles of specific melatonin receptors in the mouse retina. This lack of data is due to the fact that the vast majority of mouse strains do not produce melatonin and many that do so carry a mutation that leads to the rapid degeneration of photoreceptors (e.g., C3H *rd1*). In the present study, we have crossed mice with targeted deletion of the *MT1* melatonin receptor gene ($MT1^{-/-}$ mice) onto the $C3Hf^{+/+}$ background. These mice make melatonin but do not develop retinal degeneration. We then investigated the effect of disruption of the *MT1* gene on retinal structure and function. MT1 receptor transcripts were localized in photoreceptor cells and in inner retinal neurons and ganglion cells. A diurnal rhythm of scotopic ERG responses was observed in WT mice, with higher a- and b-wave amplitudes at night, but this rhythm was absent in mice lacking MT1 receptors. Injection of melatonin during the day increased the scotopic threshold and the amplitude of the a- and b-waves in the WT mice, but not in the $MT1^{-/-}$ mice. These data demonstrate that melatonin and MT1 receptors regulate visual processing in the mouse retina, enhancing visual responses at night when melatonin is released. We then investigated the effects on MT1 receptors on retinal morphology at three different ages (3, 12 and 18 months). We did not observe any difference between MT1-/- and C3Hf+/+ at 3 months of age, whereas at 12 months $MT1^{-/-}$ mice have a significant reduction in the number of photoreceptor nuclei in the outer nuclear layer. No differences were observed in the number of inner retinal neurons or ganglion cells. At 18 months the loss of photoreceptor nuclei in the outer nuclear layer was further accentuated and, surprisingly, we also observed a significant reduction in the number of ganglion cells. Our data demonstrate the functional significance of melatonin and MT1 receptors in the mammalian retina and create the basis for future studies on the therapeutic use of melatonin in retinal diseases associated with decreased retina sensitivity to light and degeneration of retinal cells as occur in age related macular degeneration and glaucoma.

0030
SHEDDING LIGHT ON NON-VISUAL PHOTORECEPTION
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Melanopsin is the photopigment of intrinsically photosensitive retinal ganglion cells (ipRGCS). ipRGCs mediate photic responses such as the entrainment of circadian rhythms, the pupillary light reflex, and the acute suppression of elevated nocturnal melatonin that do not require the formation of visual images. Photoactivation of melanopsin triggers a phosphoinositide signaling pathway similar to the phototransduction cascade observed in the rhabdomeric photoreceptors of invertebrates. Blue light ($_{max}$ ~ 480 nm) is the optimal stimulus of melanopsin. In recent years blue light has been implicated in a series of responses including effects on heart rate, sleep, and alertness. We have initiated studies to explore the role of melanopsin in previously undescribed effects of light. These studies will be described.

0031
PROTEIN AND mRNA RHYTHMS IN CHICKEN EMBRYONIC RETINAL CELLS AND THEIR MODULATION BY LIGHT AND GLUTAMATE
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The avian circadian system is composed by the retina, the mammalian homolog region of the supra-chiasmatic nucleus (SNC) and the pineal gland. The retina itself shows many rhythmic physiological events, such as movements of photoreceptor cells, opsin expression, retinaldehyde re-isomerization, melatonin and dopamine production and release among others. Altogether these rhythmic events are coordinated to predict environmental changes in light conditions during the day, optimizing retina function. In this work we investigated the expression of the melanopsin genes, *Opn4x, Opn4m,* as well as *Clock*, *Per2*, *N-Acetyltransferase* and *Tyrosine Hidroxylase* genes in chick embryo retinal cells in constant dark, in 12L:12D photoperiod, or in constant dark, in the presence of glutamate. These genes represent key components of a circadian system regarding photoreception, an oscillator and neurochemical outputs. Primary cultures of chicken retina from 8-day-old embryos were prepared at ZT0, and seeded at the density of 10^7 cells per 25cm^2 culture flask. The cells were kept in a humidified incubator in a 5% CO_2 atmosphere at 40ºC in constant dark, or in 12L:12D for 5 days, or in constant dark for 5 days and treated with 100uM glutamate for 12h starting at ZT0 of the fifth day in vitro. The medium was exchanged every 72 hours at ZT0. Total RNA extraction was performed along 24 hours every three hours starting at ZT0 of the sixth day. The samples were submitted to RT-PCR followed by quantitative PCR for mRNA quantification. To analyze the OPN4x expression in these cells we performed immunocitochemistry analysis with anti-chicken melanopsin antibodies (immunopurified, Bethyl Laboratories, USA) developed in rabbit. We also quantified the protein levels of melanopsin, clock and tyrosine hydroxylase by Western Blot. mRNA quantification showed no rhythm of transcription for any gene in cells kept in constant dark. However under a 12L:12D photoperiod all genes started to express phase variations. The present data show evidences that mRNA transcription and protein synthesis in chicken embryonic retinal cells are modulated by light and glutamate. We also demonstrate that those cells are able to entrain circadian rhythms in culture and that glutamate may take part in this process.
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0032
CIRCADIAN OSCILLATORS AND NON-VISUAL PHOTORECEPTORS IN THE CHICKEN RETINAL GANGLION CELLS
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In vertebrates, retinal ganglion cells (RGCs) convey photic information to the brain regarding ambient illumination conditions whereas a subset of these cells may act as non-visual photoreceptors regulating a number of non-image forming functions. In blind birds lacking functional visual photoreceptors, light still regulates pupil responses and the entrainment of daily rhythms; remarkably, photic synchronization is lost after enucleation. RGCs also contain oscillators displaying daily rhythms in gene expression, and lipid and melatonin biosynthesis under constant conditions. Moreover, primary cultures of immunopurified embryonic RGCs are able to generate self-sustained rhythms in gene expression and melatonin biosynthesis. Rhythms in melatonin synthesis were synchronized to the LD cycle. Thus, some RGCs in the cultures were intrinsically photosensitive (ip) acting through a cascade similar to that of rhabdomeric photoreceptors involving phospholipase C activation. Cultures of RGCs expressed the mRNAs for the Gq protein, two isoforms of the photopigment melanopsin (Opn4x and Opn4m) and the photoisomerases RGR and peropsin. Around 15-20% of the cells expressed the Opn4x protein and exhibited significant changes in intracellular Ca^{+2} mobilization after light exposure. Besides, light stimulation causes the activation of the phosphoinositide (PIP) cycle with the increase in intracellular levels of inositol 1,4,5-trisphosphate (IP_3) and the differential activation of PIP kinase activities. Changes in IP_3 levels and Ca^{+2} mobilization by light were reverted by the administration of the PLC inhibitor U73122 (10 µM). Results indicate that oscillators and photoreceptors are present in the same population of embryonic RGCs, and that these features appeared very early in development even before the rest of cell types have became totally differentiated.

POSTERS ABSTRACTS

A – Sleep-Wake Cycle

0033
CIRCADIAN RHYTHMS IN HUMAN SELF-MONITORING
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Introduction: Circadian rhythms in performance can be due to variations in one or several basic cognitive processes. One of these processes is self-monitoring, a component of executive functions. This cognitive process refers to the capacity of people to adjust their responses according to the results of their own actions; it is crucial to detect and correct errors in performance. The objective of this study was to identify possible circadian rhythms in self-monitoring.
Methods: Participants were three undergraduate students, 1 male (age = 19y) and 2 females (both age = 17y), without health or sleep problems. They were recorded in a constant routine protocol for 29 h; in this protocol room temperature (24±1ºC), light exposure (maximum 5 lux), physical activity (reclined) and feeding were controlled, and participants remained awake. Rectal temperature of the participants was registered each minute, and they responded to a tracking task every 1:40h. In this task, a 50 pixels circle was displayed for 180ms, with a fixed inter-stimulus interval, selected at random from 200 to 730ms. The circle follows a fixed linear path across the screen. After 22-33 stimuli, the inter-stimulus interval and path changed. The participant had to use the mouse to click inside the circle once each time it appeared on screen. Self-monitoring was measured as adjustments of the participant's response to changes in the task.
Results: There were circadian variations in self-monitoring, with a decrease in efficiency and an increase in latency during night and first hours in the morning (daytime: percent of correct responses 88.96%, 87.92%, 88.08%; median response latency 90.40ms, 140.10ms, 139.20ms; nighttime: percent of correct responses 81.82%, 54.66%, 53.54%; median response latency 113.88ms, 214.00ms, 170.63ms).
Conclusion: Impairment of self-monitoring can produce more errors and more accidents during the night and early morning.

0034
INFLUENCE OF THE SCHOOL SCHEDULE IN THE SLEEP/WAKE CYCLE IN PRESCHOOL CHILDREN IN A PUBLIC SCHOOL
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Introduction: Ontogenetic, individual and cultural changes modifies the sleep/wake cycle in infancy. Moreover, this phase is marked by changes in the psychosocial environment, such as the school schedule, which modify the pattern of sleep. The aim of this study was to evaluate the influence of the school schedule in the sleep/wake cycle in preschool children in a public school according with gender.
Methods: Participated 19 children that attended to school on the morning (9 boys and 10 girls), aged 4-6 years. The research was conducted in two stages: 1st - meeting with parents, delivery of the consent forms and characterization of the habits of sleep, with the application of sleep habits questionnaire and economic classification, and 2nd - characterization of patterns of sleep by sleep log by 7 days and observation of behavior in the classroom by 5 days (first observation: 07:35-08:20 a.m.; second observation: 09:45-10:35 a.m.). There were observed the frequency of yawning, rubbing the eye, stretching and stooping on the desk. The questionnaires were filled by parents.
Results: During the school days, the time in bed duration decreased (boys: 29min and girls: 56min) and the children woke-up earlier (boys: 70min and girls: 75min) than weekends (t test, $p < 0.05$). The frequency of yawn and stretch was higher at the first interval of observation (Wilcoxon, $p < 0.05$). The girls slept and woke-up earlier in the week and went to bed earlier in the weekend than boys. Moreover, the girls have a higher frequency of nap than boys (t test, $p < 0.05$).
Conclusion: From the preliminary results, we suggest that the preschool children showed signs of sleep partial deprivation associated to extension and reduction of sleep, mainly in girls, probably due to the school morning schedule. However, is necessary to extent the sample to confirm this hypothesis.

0035
IDENTIFICATION OF CHRONOTYPES OF STUDENTS FROM UNDERGRADUATE COURSES OF BIOLOGICAL SCIENCES AND GEOGRAPHY AT THE UNIVERSIDADE DO ESTADO DO RIO GRANDE DO NORTE, BRAZIL
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Introduction: The synchronization between the environment and the individual's internal body clock is required for the normal function of any organism. Many of the learning difficulties and adaptation problems to school activities or work can be a consequence of existing differences in the chronobiological characteristics among individuals. We analyzed the circadian typology of students from undergraduate courses of Biological Sciences (diurnal) and Geography (nocturnal) at the Universidade do Estado do Rio Grande do Norte, northeastern Brazil.
Methods: We submit a specific questionnaire with the aim of identifying the students' chronotype. Central and dispersion tendencies measures were calculated according to undergraduate

course and gender (Student t). Ninety five university student volunteers (33% men; 67% women) of Biological Sciences (47) and Geography (48) filled the Brazilian version of the Chronotype Questionnaire (CQ), the portuguese translation of the Horne and Östberg's Morningness–Eveningness Questionnaire (MEQ). CQ score distribution was correlated to the normal curve (range=27-74; mean=51.0; s.d.=10.0).
Results: It was verified a heterogeneity of chronotypes in university students (n=95) with individuals moderately matutine (21.0%), intermediate (61.0%) and moderately vespertine (18.0%) being found. There was no gender-by-chronotype association (p=0.233). CQ means were significatively different (p=0.004) when undergraduate Biology students (54.0±8.7) were compared to Geography students (48,2±10.4). We verified that 25.5% students in the Biological Sciences course were moderately matutine, 68.1% were intermediate and 6.4% were moderately vespertine (n=47). By the other hand, Geography students were 16.6% moderately matutine, 54.2% intermediate and 29.2% moderately vespertine (n=48).
Conclusion: Based on these results, we conclude that there was a predominance of the intermediate chronotype, there were differences between the students' chronotypes of Biology (diurnal) and Geography (nocturnal) courses and that most volunteers study in a schedule suitable for their chronotype. Our results reinforce the value of knowing biological rithms and chronotypes for the planning of work and study activities.
Support: CNPq/ FAPERN/ UERN

0036
EXPLORATORY STUDY ON CHRONOTYPE, SLEEP HABITS AND PRESENCE OF SLEEPINESS IN TEENAGERS WHO STUDY IN THE MORNINGS AT A PUBLIC SCHOOL LOCATED IN BOGOTA, COLOMBIA
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Introduction and Objective: To examine the relationship among chronotype, sleep habits and somnolence in a group of teenagers who study in the morning at a public school located in Bogota, Colombia.
Methods: An exploratory study was carried out including a questionnaire that was designed in order to gather information taking into account social and demographic variables and their relationship with the sleep pattern. In order to evaluate the chronotype of the students, a Composite Morningness Scale (CSM) was applied. Furthermore, an assessment of the presence of somnolence was carried out by means of an Epworth scale of somnolence (ESE-VC).
Results: 72 teenagers participated in this study (38 women-34 men) with an average age of 15.57 (between 15 and 18 years old). All of them are students at the CED JUAN LOZANO Y LOZANO Junior High school in the morning shift (the arrival time is 6:30 a.m, dismissal time 12:15 p.m). A diurnal chronotype of 30.6% was found as well as a neutral chronotype of 65.3% and a matinal or vespertine chronotype of 4.2%. It could be determinate an average of 7.32 +- 0,9 daily sleep hours. An inverse correlation was found between the CSM and the ESE-VC (P<0,05), as well as between the ESE-VC and the sleep hours (P<0,01), and also a direct correlation between the CSM and the sleep hours (P<0,01).
Conclusion: a strong association was found among the chronotype, sleep hours and the presence of somnolence in teenagers who study in the morning. It is also evident that teenagers with Morningness tendencies can express themselves with extreme values barely compatible with school schedules making difficult the academic performance.

0037
SEASONAL DIFFERENCES IN BIOLOGICAL RHYTHMS OF BRAZILIAN NATIVE INDIANS
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Introduction/Objective: Compare summer and winter data of wrist temperature and activity/rest pattern of adult indians who live in a settlement without electricity.
Methods: Participants were 22 Guarani adult indians aged 18 to 80. The settlement "Boa Vista" is near the Atlantic coast in southeast Brazil (23º21'S; 44º51'W). Photophases during data collections were approximately 13h in summer and 11h in winter. Wrist temperature (WT) were collected every 30min along three consecutive days with thermistors (Thermochron,, iButton type DS 1291H). Rest/activity (RA) was registered with actimeters (MicroMini Motionlogger, - Ambulatory Monitoring, Inc.) for 7 consecutive days, in bins of 1minute. The data were analyzed with the COSINOR method and the parameters acrophase, amplitude, MESOR were compared in two moments. The Pearson moment correlation test was applied to analyze the changes in COSINOR parameters according to age. The onset, offset and duration of rest between seasons were compared with one-way ANOVA.
Results: WT: All subjects showed a significant circadian rhythmicity (COSINOR: p<0.0001). The amplitude values were higher and MESOR values were lower in winter than in summer. Although submitted to distinct photophases, acrophase values did not differ according to season. RA: the indians showed a phase advance in winter compared to summer values (onset winter: 21h23 and onset summer: 22h42; F=18.69, p<0.00004) (offset winter: 06h12 and offset summer: 07h18; F=23.98, p<0.00001) without difference for rest duration. The phase relation between WT and RA varies according to the season: □Ψwinter = 11h13 and □Ψsummer = 12h46.
Conclusion: The adult indians showed WT rhythms with seasonal difference, specifically in the amplitude and MESOR. The RA was linked to specific characteristics of Guarani routine. In winter the indians' activities began near the sunrise and during summer they used to phase-delay their rhythms probably due to social demands involving nighttime activities during summer.

0038
NOT ALL ADOLESCENTS ARE SLEEP DEPRIVED: A STUDY OF BRAZILIAN RURAL POPULATIONS
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Introduction: Several studies have shown that during weekdays adolescents generally sleep on average considerably less than the recommended 9h/day. This sleep deprivation has been attributed in great part to a sleep phase delay, which occurs for biological and socio-cultural reasons. The aim of this study was to depict some environmental factors related to adolescents sleep length.
Methods: A total of 1140 students (569 males), aged 10-19 years, attending two schools of rural regions of Paraná State in southern Brazil, completed a questionnaire about their sleep habits. Demographic data were also obtained. Prevalence ratios (PR) were estimated for more than nine hours of sleep on weekdays. Sleep duration in adolescents with and without electric lighting at home was compared by means Kruskal-Wallis test.
Results: Average sleep duration was 9.63(±1.64)h during weekdays and 10.14(±2,42)h during weekends. The prevalence of adolescents with more than nine hours of sleep during weekdays was 58.3%. On multivariate analysis, age, work and bedtime were factors associated with sleep duration. The prevalence of more than nine hours of sleep was lower in older students (16-17years) than in younger students (10-11years)(PR=0.93; CI95%:0.87-0.99) and lower in workers than in non-workers (PR=0.92;CI95%:0.89-0.96). Adolescents with later bedtimes (10pm) showed shorter sleep duration when compared to those with earlier bedtimes (9pm)(PR=0.79;CI95%:0.76-0.84). Adolescents without electric lighting at home showed longer sleep duration on weekdays (p<0.001) and on weekends (p<0.01) when compared to those with electric lighting at home.
Conclusion: In contrast with data previously reported, a high prevalence of adolescents with more than nine hours of sleep during weekdays was found. Data on populations living in less industrialized regions reinforce the idea that technological advances are associated with the negative impact of the sleep phase delay in adolescents.

0039
REASONS TO BEDTIME AND WAKE-UP TIME IN PRESCHOOL CHILDREN IN A PUBLIC SCHOOL
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Introduction: Social exigencies as early school starting time and the precocious usage of entertainment devices are altering sleep/wake patterns of younger children. These activities that are being done near the bedtime can interfere negatively on their development, especially in school-aged children. The aim of this study was compare the reasons to bedtime and wake-up time in preschool children according to gender.
Methods: 29 Brazilian children (18 females and 11 males), aged 4-6 years, that attended to school on the morning in a public school of Natal-RN/Brazil were evaluated through sleep habits questionnaire answered by the parents, who were asked to assess the motivations to bedtime and wake-up time of their children in school and weekend days.
Results: On school days, the motivation to bedtime most frequently reported by the parents was "ordered by parents" (38.5%) and to wake-up time, "school starting time" (92.6%). On weekends, "playing" (26.9%) was the most reported to bedtime and "not being sleepy" (51.8%), "go to the church" (22.2%) and "sauntering" (18.5%) to wake-up time (Chi-square, p<0,05). The bedtime and wake-up time were earlier and the time in bed was lower on school days when compared to weekend (Wilcoxon, p<0,05). Comparing gender, on school days the most observed reason for bedtime for boys was "feeling sleepy" (66.7%) and for girls, "watching TV" (43.7%) and to wake-up time, "not being sleepy" (22.2%) for boys while for the girls there was no significant difference among the motivations. On weekends, the most reported reason for bedtime for girls was "watching TV" (50.0%) and "playing" (31.2%) and for boys it was "feeling sleepy" (55.5%); to wake-up time the most informed for girls was to "go to the church" (18.7%) (Chi-square, p<0,05). For boys, the analysis of the data revealed no significant differences among the options. Some additional results were that the boys showed later bedtime (Mann-Whitney, p<0,05) and a trend to lower sleep duration (Mann-Whitney, p=0,09) from Saturday to Sunday when compared to girls. Besides, the frequency of nap was higher in girls (61.1%) than in boys (Chi-square, p<0,05).
Conclusion: According to the preliminary results, the entertainment and social activities in school and weekend days influence the sleep/wake cycle in preschool children and these activities are different according to gender, but for confirm these hypothesis is necessary increase the sample.

0040
SLEEP HABITS DIFFERENCES BETWEEN 11TH AND 12TH GRADE ADOLESCENTS
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Introduction: The Adolescence is characterized for later sleep schedules influenced by biological and social factors. With the passage of school grades, the adolescents are under an increase of the load of school and social pressure that would contribute to the sleep delay. This factor together with the school time in the morning are related to sleep partial deprivation with negative consequences to the adolescent health.
Objective: To compare the sleep habits of Brazilian adolescents from 11th to 12th grade. **Methods:** Students from two private schools in Natal/RN participated with 158 adolescents from 11th

grade (age between 14 and 17 years) and 213 students from 12th grade (aged 15 and 18 years). The "Health and Sleep" questionnaire was applied to characterize the sleep habits and pattern. The sleep habits were compared using the chi-square and the sleep-wake schedules by using the t-test for independent samples. For both, the level of significance considered was 5%.
Results: From Monday until Thursday and on Sunday the bedtime was seen to be later for the 12th grade students compared to that for 11th grade students. The reasons for the late bedtime reported by most students were TV and computer using, but on weekends the 12th grade students reported "to study" and "to go to the parties" with higher frequency. The wake-up schedules showed no difference between the two groups. On weekdays, the reasons to wake-up time reported by most students were the "school schedule" and on the weekends, "not being sleepy". In addition, on weekends the 12th grade students report to wake-up to study with a higher frequency. The students from both grades took naps in common times, but the 12th grade students wake up earlier and spent less time sleeping. Moreover, this group reported greater participation in extra-school courses and lesser practice of exercise.
Conclusion: At the 12th grade the students sleep later and the report of bedtime and wake up time due to study increases on weekends. Besides, the length of the nap decreases due to increased participation in extra-school courses. The decrease in physical activity can be considered as a negative effect of high school load.

0041
EFFECT OF THE WORK SCHEDULE ON THE SLEEP-WAKE CYCLE OF HIGH SCHOOL TEACHERS OF PUBLIC SCHOOLS
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Introduction: The sleep-wake cycle is influenced by the interaction of several environmental stimuli. Among the social stimuli a factor that influences the sleep-wake cycle is work schedule. The teacher's work schedule is differentiated from the majority of workers because they have work inside and outside the classroom. Thus the excess of extra-class tasks can promote partial sleep deprivation which can compromise the health and damage the personal and professional life. Therefore the aim of this study is characterize the sleep-wake cycle of high school teachers of public schools in Natal-RN comparing them regarding the work schedule.
Methods: The research was conducted in 3 public schools with 27 high school teachers aged between 27 and 66 years. The study was performed in two stages: I. Meeting with teachers to explain the research and deliver the consent forms. At this meeting, the teachers received the "Health and Sleep" questionnaire to assess the general habits of sleep. This questionnaire was collected about 3 days after this meeting. II. They filled the sleep diary by 15 days. Teachers were divided by the time of starting and ending of work in 4 groups (n): G1 (7) – Start= 7:00h-8:30h/End= before 19:00h; G2 (5) – Start= 7:00h-8:30h/End= after 21:00h; G3 (4) – Start= after 10:00h/End= before 19:00h and G4 (6) – Start= after 10:00h/End= after 21:00h.
Results: The teachers showed later bedtime and wake-up time and a decrease in time in bed (±57min) in the week (t test, $p < 0.05$). There was a difference between groups in bedtime (G1 ≠ G2, G1 ≠ G4, G2 ≠ G3, G3 ≠ G4), wake-up time (G1 ≠ G3, G1 ≠ G4, G2 ≠ G3, G3 ≠ G4) and time in bed (G1 ≠ G2, G1 ≠ G3, G2 ≠ G3, G3 ≠ G4) according to the time of starting and ending of work. The G2 and G4 have later bedtime than G1 and G3 (±55min). The groups G1, G2 and G4 have earlier wake-up time than G3 (±1h09min) and G3 have higher time in bed duration than the groups G1,G2 and G4 (±1h43min) (Kruskal-Wallis, $p < 0.05$).
Conclusion: From the preliminary results, we suggest that the sleep-wake cycle is influenced by the work schedule of high school teachers and there is an extension in bedtime duration on the weekend probably due to a partial deprivation of sleep during the week. However, is necessary to extend this sample to confirm this hypothesis.

0042
SLEEP COMPLAINTS AMONG EX-NIGHT WORKERS - ARE THERE RESIDUAL EFFECTS OF NIGHT WORK ON SLEEP?
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Introduction: Data on persistent effects of night work on sleep are scarce and controversial. The aim of this study is to in vestigate possible persistent effects of night work on sleep among femalenursing personnel.
Methods: A cross-sectional study was carried out with female registered nurses and nurse assistants at three hospitals. A multidimensional questionnaire was used including information on work schedules throughout occupational life. Daytime workers (N=680) were classified into two groups: exclusive daytime workers, corresponding to those with no experience on night work (N=281) and former night workers (N=399). Former night workers were divided into two groups in accordance with the time spent on nocturnal work (from 3 to 9 years or 10 years or more). They were also classified in two groups according to self-reported sleep problems (difficulty falling asleep, difficulty maintaining sleep, early morning awakening, general complaint on insomnia (corresponding to reporting any of the above-mentioned problems), and unsatisfactory sleep. Binomial logistic regression was used to evaluate the association between the night work experience and referred sleep problems (exclusive daytime work as the reference group).
Results: Significant associations were observed between the experience on night work and three out of the five studied sleep problems. Adjusted odds-ratio (and CI95%) for difficulty maintaining sleep, insomnia, and unsatisfactory sleep were 1.71 (1.05-2.79), 1.51 (1.01-2.25) and 1.59 (1.07-2.35), respectively, after controlling for potential confounders (age, schooling degree, income, presence of children, marital status, professional and domestic worked hours/week, professional category, type of contractual employment, hypertension, body mass index, alcohol consumption, and smoking habits). Compared to workers who never worked at night, workers who have spent 10 years or more on nocturnal work have 2.16 times a greater chance of having nocturnal sleep interrupted (OR=2.16), and have 1.87 times more chances of having a general complaint of insomnia. In relation to dissatisfaction with sleep, workers who spent between 3 and 9 years in nocturnal work had 1.62 times (OR=1.62; 1.02–2.58) greater chances of referring to dissatisfaction with sleep than those that never worked at night.
Conclusions: Results suggest that there are residual consequences of night work on some of the studied sleep problems, particularly for those who have spent 10 years or more no nocturnal work. Those data can subsidize discussions on the adoption of legislative actions to limit exposure to night work.
Support: Faperj; CNPq; Mount Sinai School of Medicine (Program on Occupational Health)

0043
SLEEP-WAKE CYCLE DURING DAYLIGHT SAVING TIME TRANSITION: A PILOT STUDY
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Introduction: In the beginning of Brazilian daylight saving time (DST), the social clock is advanced by one hour, often inducing a circadian rhythms adjustment. The objective of this study is to compare sleep-patterns and mood before and after DST transition and identify.
Methods: Fifty four volunteers, freshmen undergraduate students, participated in the pilot study. They answered the Horne-Östberg questionnaire to assess chronotype and kept sleep logs during two consecutive weeks, one before and one after the beginning of DST to record sleep-wake patterns, sleepiness and mood. Sleepiness was assessed every day at 8am, 12pm, 6pm and 8pm by means of a visual analogue scale (VAS). Mood variables (anxiety, fatigue and anger) were obtained by the same means. Data before and after DST transition were compared using *t*-test.
Results: The mean score of chronotype was 47.05±12.92. Subjects showed later sleep times (before=11:36pm / after=12:14am, $p < 0.01$) and wake up times (before=07:12am / after=08:13am, $p < 0.01$). Subjects also showed an increase of sleepiness at 8am ($p<0,01$). Differences on mood variables were not found.
Conclusion: The results suggest that there is no adjustment of sleep-wake times in the first week after DST beginning.

0044
SCHOOL SCHEDULE PROVOKES TO IRREGULARITY IN THE SLEEP- WAKE CYCLE
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Introduction: Most brain and body functions are influenced by sleep quality. Sleep-wake irregularity can lead to cognitive and physical impairment, and increase the risk of pathologies development. Taking this into consideration, the aim of this work was to evaluate the sleep-wake pattern in medical students under natural conditions.
Methods: All volunteers (n = 32) filled questionnaires with their personal data, and health status. The students also answered the Pittsburgh Sleep Quality Index (PSQI), the Portuguese version of the Horne and Östberg Morningness/Eveningness Questionnaire (HO), the Health and Sleep Questionnaire and Epworth sleepiness scale (ESS). Activity rhythms of nine volunteers were monitored with actigraphs set to record in a 2 min-interval for 14 days, including two weekends. During this period they filled a sleep diary. During the experiment the students had classes everyday starting at 7 a.m., except on weekends. Actigraphic analyses were performed on Actiware-Sleep, version 3.4.
Results: The volunteers were 18 men e 14 women (19.69 ± 1.9 years old). Analysis of the questionnaires showed values of 48.94 ± 8.65 (HO), 6.88 ± 2.06 (PSQI) and 7.2 ± 2.85 (ESS). Data were compared between weeks and weekends using the paired t-test. Data from questionnaires were different between weekdays and weekends for bed time ($p < 0.001$) and get up ($p < 0.001$). The students went to bed and got up earlier on weekdays than on weekends. Actigraphic data showed the same differences between weekdays and weekends ($p < 0.007$ for bed time, and $p < 0.001$ for get up time). Time in bed was not different between weekdays and weekends ($p > 0.31$ for questionnaire data, and $p > 0.38$ for Actigraphic data). According to students' reports the main reason for getting up (90.6 %) on weekdays was the need to be in the class in the morning ($X^2 = 66.02$, $p < 0.001$).
Conclusion: The medical students had irregular sleep-wake cycle. This irregularity may be provoked by the classes' onset, because the students had to wake up too early on weekdays due to school schedule.
Support: Capes, CNPq and FAPERN.

0045
WEEKLY SCHOOL TIME IS ASSOCIATED WITH SOCIO DEMOGRAPHIC, LIFE-STYLE AND SLEEP-WAKE VARIABLES AMONG WORKING COLLEGE STUDENTS
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Introduction: Literature has indicated that unhealthy lifestyles, health symptoms (including sleep patterns) and work factors are

associated with poor academic performance. We hypothesize that time spent in college is also affected by these mentioned variables. The aim of this study is to assess the associated factors with weekly school time.
Methods: This is a cross-sectional study carried out in a public college, in São Paulo, Brazil. All working students aged 21-26 years attending a college institution in the evening (19:30 -22:30h) were invited to participate in this study. Inclusion criteria were: 6 or more daily working hours and attending daily evening classes, 2 hours or more, from Monday to Friday. Eighty-two students agreed to participate. They answered a comprehensive questionnaire about socio-demographic, life-style, working conditions and health symptoms. In the second step, all participants wore an actigraph for 7 consecutive days to obtain information about sleep length, sleep efficiency, sleep latency, nocturnal awakenings and naps. During the same week, they also filled out a daily activity protocol to obtain information about time spent on the following activities: college attendance, commuting time, work, extracurricular activities, and leisure time. The statistical analysis consisted in descriptive statistics (frequency distributions, means and standard deviation), Shapiro-Wilk test to detect its normality, and a multivariate linear regression analysis. A stepwise forward selection was performed. In all analysis was considered α=5%.
Results: The linear regression analysis showed an association between time spent in college and being a female (increased 141.09 minutes on time spent in school), reported drinking habits (decreased 188.31 minutes on time spent in school), higher weekly working time (for each minute of work time spent in school decreased 0.29 minutes), shorter sleep length during work days (for each minute of sleep during work days time spent in college decreased 1.45 minutes) and high levels of sleepiness on Saturday (increased time spent in school 185.24 minutes). The model was adjusted by sleep onset and age.
Conclusions: Socio-demographic, life-style, sleep length during work days, higher sleepiness on Saturdays and higher working times significantly affect time spent in College and may have negative effects on academic performance.
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0046
SEX DIFFERENCES IN SLEEP AND REACTION TIME OF WORKING COLLEGE STUDENTS

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Introduction: Former studies have shown an important interference of socio-demographic and life-style factors, health symptoms and working conditions on sleep and sleepiness among working high school students . These studies also have shown that as result of the double journey this population seems to be chronically sleep deprived. It might lead to daytime sleepiness and low performance at school and at work. The objective of this study is to detect sex differences in sleep length, sleepiness and performance of working college students, in connection to workdays and free days.
Methods: The study was conducted in a public college in São Paulo, Brazil. The participants were engaged in full-time jobs (6 hours or more) from Monday to Friday and attended evening classes (19:30–22:30h). The study group consisted on 40 males and 37 females, aged 21-26 yrs. The participants wore for seven consecutive days an actigraph and filled out sleep logs to obtain information about their sleep- wake cycle. Karolinska Sleepiness Scale (KSS) and Psychomotor Vigilance Task (PVT) were used to obtain data of sleepiness at three different moments: wake up time, mean of the whole day and at bedtime. An ANOVA yielded main effects of sex, day and time of day and interactions effects using Huynh–Feldt whenever possible. Time of the day was evaluated just for sleepiness and PVT measures. The analyses were made separately for working days (Monday to Friday) and free days (Saturday to Sunday).
Results: The analysis found sexes differences in the sleep length, sleepiness and performance. In both work days and free days a main effect of sex was found: females slept about 40 min more than males. The analysis also showed a day effect for working days as students slept more on Friday night. The sleepiness analysis revealed, upon awakening time on work days females were sleepier than males. During free days, females were sleepier at bedtime compared to males. Regarding performance, on work days, a main effect of sex was found, as reaction time was slower for females compared to males. During free days sex effect disappeared.
Conclusion: Females sleep length was longer than males during the whole week, but even so, they seemed to be sleepier and showed a poorer performance. The restricted time to sleep during the work days possibly leads to a sleep rebound during free days in both sexes.
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B – Health, Disease and Aging

0047
EVENING CHRONOTYPES HAVE POOR SLEEP QUALITY IN EARLY CLASS STARTING TIME

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Introduction: The sleep-wake cycle of medical students is affected by their academic demands and class starting times. In spite the different sleep needs and characteristics, such as cronotype, they have to follow the same schedules. Therefore, it is necessary to know the relationship between cronotype and sleep quality.
Methods: 234 students from 1st to 4th year of medicine´s course of Federal University of Rio Grande do Norte were assessed. The Pittsburgh Sleep Quality Index was used to evaluate the subjective sleep quality. Scores of seven components were added to confer a global punctuation, which varies from 0 to 21. Punctuations below 5 indicate good sleep quality, 5-10 indicate poor sleep quality and above 10 indicate disturbance of sleep. The Epworth Sleepiness Scale was used to evaluate daytime sleepiness. The scale presents a maximum value of 24 points, and values above 10 indicate extreme daytime sleepiness. The Horne and Östberg (1976) questionnaire for "eveningness - morningness" dimension, was used to classify the participants in extreme morning (emt), morning (mt), intermediates (it), evening (et) and extreme evening types (eet). The punctuations are from 30 to 16 for eet and from 70 to 86 for emt.
Results: The cronotype had a normal distribution, as in the total sample (12eet, 32met, 133it, 45mt and 03emt), regardless sex and period. The sleep quality showed value higher than 5 from 2nd to 4th years and less than 5 in the one of the first year; having as average 5,6 (SD= 1,1). The daytime sleepiness showed homogeneity among the periods, having average of 8,9 (SD=0,6). The cronotype and the sleep quality presented a linear relation (p<0,001) - et had a worst sleep quality than mt – with a specific significant relation between the components C1 (subjective sleep quality, p=0,02) and C3 (sleep duration, p<0,001). There was no significant relationship between cronotype and daytime sleepiness, although it was observed a predominance of excessive daytime sleepiness in emt.
Conclusion: The most medical students show a low sleep quality, worst on the eet. The cronotype have influence on the sleep quality, probably due to the early class time in the 2nd to the 4th year and not so early in the 1st year, the et students have a better sleep quality in the 1st year decreasing along following years. In addition, it appears the evening types have some difficulty to adaptat to the college schedule.

0048
SLEEP QUALITY INFLUENCES POSTURAL CONTROL IN THE ELDERLY

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Introduction: Sleep/wake cycle disturbances occur in over half of adults aged 60 and older. This inability to have restful overnight sleep results in a low quality sleep, which may be associated with impaired balance ability and increased risk of falls in older people.
Objective: The aim of this study was to evaluate the relationship between sleep/wake cycle and postural control in the elderly.
Methods: Twenty volunteers (67.2 ± 7.14 years-old) participated in this study. Participants wore wrist actimeters (Ambulatory Monitoring. Inc.) during 7 consecutive days and answered *Epworth* (ESS) and *Pittsburgh* (PSQI) questionnaires. In the second data collection day, postural stability was assessed using a force platform (AMTI, OR6-7) to determine the antero-posterior and medio-lateral amplitudes, trajectory and velocity of the centre of pressure. The elderly people remained on the force platform by 60 seconds in four different conditions: normal stance/eyes open, normal stance/eyes closed, narrow stance/eyes open, narrow stance/eyes closed. The correlation coefficient of *Spearman* was applied to identify association between sleep and postural control variables.
Results: It was observed a positive correlation between sleep quality (PSQI) and medio-lateral amplitude of centre of pressure in the normal stance/eyes closed (r=0.48;p<0.05) and the narrow stance/eyes open (r=0.47;p<0.05). No correlations were observed between sleep/wake patterns and postural control variables.
Conclusion: The findings of this study suggest that sleep quality influences some postural control variables in older people and may make them more prone to falls as there is a close relationship between balance and the risk of falls.

0049
CAN POSTURAL CONTROL PERFORMANCE BE AN INDICATOR OF A TRUCK DRIVER'S SLEEP DEPRIVATION?

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Introduction: Long-haul drivers work irregular schedules due to load delivery demands. In general, driving and sleeping occur at irregular times and, consequently, partial sleep deprivation may emerge and result in sleepiness at the wheel. Many methods have been tested to measure sleepiness while driving in order to avoid accidents; however, there is a need to find a way to estimate sleepiness before driving. Recently, postural control performance has been suggested as an indicator of sleepiness in laboratory studies. Nevertheless, to our knowledge no previous studies have been conducted measuring postural stability with truck drivers chronically exposed to sleep deprivation. This study aimed was to investigate whether postural stability in the anteroposterior and mediolateral planes differs in truck drivers before and after a night's work.
Methods: Eight male truck drivers working at night (NG), mean age=34.8 yo; SD=4.4 yo, volunteered to participate in this study. The drivers' postural stability was assessed immediately before and after an approximately 450 km journey. Two identical force platforms were used to record a number of postural variables (AMTI, OR6-7) at departure and arrival sites, and included: amplitude, frequency and speed of anteroposterior and mediolateral movements of the centre of pressure. The control group was made up of nine day truck drivers who were measured before and after a day's work (DG; mean age=38.4 yo; SD=6.3 yo). Drivers were evaluated in two conditions: eyes open and eyes closed during 60s in a comfortable standing position. A repeated measurements ANOVA was applied to identify differences between groups (NG vs. DG) and within-group (before and after work). The analysis was done for the two test conditions (eyes open and eyes closed)

and the significance level set at p=0.05.
Results: An interaction effect of time of day and type of shift in both conditions: eyes open (p<0.01) and eyes closed (p<0.001) for amplitude of mediolateral movements was observed. No differences were observed related to the other postural variables.
Conclusion: Postural stability, measured by force platform, seems to reflect sleep deprivation, suggesting it could be a simple and fast indicator of sleepiness at the workplace. Nevertheless, it is not clear what postural variable best indicates sleep deprivation and other studies are required.

0050
ANALYSIS OF WRIST TEMPERATURE FOR CHRONOBIOLOGICAL STUDIES IN NEWBORN BABIES
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Introduction: Temperature rhythm is known to evolve from an ultradian to a circadian pattern in newborn babies earlier than sleep/wake cycle. However, studies following this evolution are limited by difficulties in collecting temperature data in longitudinal series. Recently, a new method for recording wrist temperature continuously was described in adults, allowing the inference of central temperature rhythm. The aim of this study was to investigate the use of wrist temperature in term and preterm newborns for chronobiological research and its relation to axilar and environmental temperature.
Methods: Seventeen newborn infants were studied: 4 term babies in the first week of life and 13 preterm newborns betwwen 34 and 36 weeks of post-conceptional age. All subjects used a skin thermistor, Thermochron, attached to the wrist, continuously for 10 to 14 days, which registered temperature every 10 minutes. Axilar temperature was mesured for 14 babies every 4 hours during the whole study and environmental temperature was recorded with the ibbuton also every 10 minutes, continuously. Babies who were in incubators also had their environmental temperature mesured by the own incubator thermistor every 4 hours. Data of wrist, axilar, environment and incubator temperatures (WT, AT, ET and IT, respectively) were compared through Pearson correlation. These data were also submitted to a Lomb Scargle periodogram and a Cosinor analysis, investigating the presence of circadian rhythmicity. Acrophases of significative circadian rhythm observed for each variable were compared in order to investigate a phase relation.
Results: WT and AT had a significative correlation for all babies, except one. WT and ET were correlated while babies were maintained inside incubators, but not for babies out of them. However, incubator temperature measured by the own incubator system doesn't present a significative correlation with WT or AT. WT presented a significative circadian rhythm for all infants, except one term newborn, while AT had circadian rhythm for about 60% of subjects. Most infants had a WT circadian rhythm different from 24 hours. Incubator temperature measured by our thermistor also exhibited circadian rhythm for most subjects, but WT and ET acrophases were not related. Comparing WT and AT acrophases, we found that AT follow WT acrophase in about 2 hours.
Conclusion: WT recorded by skin thermistor is a good alternative for studying temperature rhythm in term and preterm infants and seems to be independent of environmental temperature at least for babies out of incubators.

0051
ASSOCIATED FACTORS WITH FATIGUE AMONG WORKING COLLEGE STUDENTS
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Introduction: Former studies showed negative health effects among adolescents, when combined work and study. To assess factors associated with perceived fatigue among College working students.
Methods: This is a cross sectional study carried out in a public College of São Paulo, Brazil. All students aged 18-26 years old, who worked at least six hours per day, attended evening classes (19h30min to 23h:10min) were invited to participate. Two hundred seventy three students voluntarily accepted to participate in this study. They answered a comprehensive questionnaire about living and working conditions (including detailed set of questions on occupational stressors, the work-control Karasek model, and other working conditions), sleep disturbances, reported sleep duration, and health symptoms. A Brazilian adapted version of the Yoshitake fatigue scale (Yoshitake, 1975) was included in the survey. Mean age of participants was 22 years old, and did not differ of the whole population. Initially, the data were submitted to descriptive statistics (frequency distribution, means and standard deviation), followed by hierarchical linear regression analysis. Four levels were tested in the model: first - socio-demographic, second - life-style, third - working conditions and fourth - health conditions variables. A stepwise forward selection was performed. In all analysis was considered α=5%.
Results: According to the linear regression model, fatigue scale increased 16.79 points for females (p<.01) and showed morningness as a protective factor (ß1=-.23; p=.05); regarding working conditions fatigue was associated with active jobs (ß1=12.11; p<.01) or high strain jobs (ß1=11.00; p<.01), and those with medium (ß1=6.11; p=.02) or low social support (ß1=8.12; p<.01) (second and third tertile, respectively). A longer sleep length was a protective factor (ß1=-.07; p<.01) and reporting symptoms of sleep disturbances increased 5.99 points on fatigue scale (p<.01). The model was adjusted by age, sharing family expenses and daily working time.
Conclusions: College and work demands associated with a number of factors can pose a significant school and work conflict on students, interfering with their studies as well as their health and wellbeing. Psychosocial factors as work demands, work control and social support seem to be relevant factors which should be taken into account to decrease fatigue at work, mainly among females who work and study.
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0052
HOT FLASHES AND CIRCADIAN RHYTHMS OF BODY TEMPERATURE, REST/ACTIVITY AND MOOD IN POST-MENOPAUSAL WOMEN
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Introduction: Menopause marks the end of fertility in women. Besides mood changes at the end of the menstrual cycles in middle-aged women, some start presenting hot flashes.
Objective: verify possible correlations between peripheral temperature, rest/activity and mood rhythms and the presence of hot flashes in post-menopausal women.
Methods: We studied three groups of women, two of which comprised patients from the Hospital das Clínicas de São Paulo: i) post-menopausal women with hot flashes (n=13), on average 55 (±4) years old; ii) post-menopausal women without hot flashes (n= 6) 59 (±1.5) years old. A control group featured 10 women on average 36 (±4) years old. For 30 consecutive days volunteers recorded motor activity of the arm, wrist skin temperature and hot flashes episodes by Tempatilumi; in addition, the subjects kept a sleep diary, and two visual analog scales (anxiety and joy) for self-evaluation of mood states every 3 hours and in the moment of a hot flash. All subjects completed the Horne-Ostberg questionnaire of chronotypes. Data for motor activity, temperature, hotflash occurrence and mood were submitted to spectral analysis. Rhythmic parameters were determined by the Cosinor or Rayleigh method. The program El Temps was used to draw actograms to visual inspection of the variable over time. Comparisons between groups were quantified by the t-test, or nonparametric alternatives where necessary. Correlations between variables were quantified by Pearson's r or Spearman's Rho. A significance level of 5% was assumed in all trials.
Results: In 5 women with hot flashes we notice the presence of a significant circadian component in the occurrence of hot flashes, together with periods greater than 24 hour. Visual inspection of hotflash timeseries suggest an acrofase of hotflashes in the late afternoon, between 16 and 18h.
Conclusion: Initial analyses of hotflash occurrences correspond to findings in the literature. The next steps aim at identifying possible correlations with other physiological variables like body temperature, and motor activity, as well as with the recorded behavioural observables.

0053
INTAKE OF ENERGY AND MACRONUTRIENTS DURING WORKING DAYS AND DAYS-OFF AMONG WORKING COLLEGE STUDENTS
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Introduction: The daily intake of energy and macronutrients can be affected by a number of factors during waking time, including number and duration of daily activities. Evening College students usually have a double shift- they work during the day and study in the evening hours. This can contribute to unhealthy diets and irregular mealtimes. The aim of this study is to estimate the percentage contribution of energy and macronutrients in the predetermined intervals along the 24h-period among College students during workdays and weekends (free-days).
Methods: A cross-sectional study was conducted among 26 working college students (14 males and 12 females), aged 21 to 26 years, who worked at least six hours per day, attended evening classes (19:30h to 23:10h) in a public College of São Paulo, Brazil. Activity rest records were assessed during 7 consecutive days using Ambulatory Monitoring Actigraph. The dietary intake was estimated by seven-day food records. The nutritional contribution of each food was computed using Nutrition Data System for Research software. One hundred eighty- two food records were assessed. The day (24-h period) was divided in 4 intervals: 1) 5h|--11h, 2) 11h|--14h, 3) 14|--19h:30min and 4) 19h:30min|--5h. Descriptive and mean comparisons were performed using SPSS for windows, version 13.0. A 5% level of significance was used in Kolmogorov-Smirnov test to detect normality and Student t-test to compare workdays and weekends food intake.
Results: The mean daily intake of energy, fat, carbohydrates and protein were respectively: 2,244.5 Kcal (sd 829.8); 84.1(sd 32.5); 275.2(sd 120.2); 91.2(sd 34.0). The average daily percentage contribution of energy, fat, carbohydrates and proteins showed statistical significance (p<.05) between weekdays and weekends, in intervals 1, 2, and 3, respectively in the following items- a) energy: 21.3% and 12.0%; 32.6% and 29.9%; 28.1% and 42.3%; b) fat: 19.9% and 10.2%; 36.4% and 22.8%; 27.5% and 42.7%; c) carbohydrates: 24.3% and 14.7%; 28.1% and 20.0%; 29.2% and 42.4%; d) protein: 18.2% and 11.3%; 38.3% and 21.6%; 26.6% and 42.7%.
Conclusions: The study found lower percentage of energy and macronutrients in the interval 1 and 2, and higher, in the interval 3 when compared the dietary intake during the weekend and weekdays (workdays). During the weekends there were a reduction/omission of some meals, such as breakfast and lunch. This is probably due to later waking times during weekends as

a sleep rebound.
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0054
IS SCHOOL SCHEDULE A RISK FACTOR FOR OBESITY IN ADOLESCENTS?
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Introduction: Adolescents show a sleep phase delay. In contrast, schools from 5th grade often anticipates school starting times. As a result, there is a partial sleep deprivation, which has been associated with an increase in body mass index. One could suppose that morning school schedule might be a risk factor for obesity in adolescents. The aim of this study is to compare sleep-wake patterns, body mass index (BMI) and waist circumference (WC) of adolescents submitted to two school schedules, morning and afternoon.
Methods: 68 students (29 boys and 39 girls) from two public schools in Curitiba (PR), aged from 12 to 16 years, participated in the study. 31 students attended afternoon schedules (1:00pm to 5:30pm) and 37 morning schedules (7:20am to 11:45am). They filled out a questionnaire about their sleep habits. Waist circumference (WC), height and weight were measured and body mass index (BMI) was calculated.
Results: Sleep variables, BMI and WC were compared using the Kruskal-Wallis test, considering school schedule, morning or afternoon, the independent variable. Table 1 shows the results. Afternoon shift students showed later sleep times and had longer sleep duration when compared with morning shift students, although there were no differences in BMI and WC.
Conclusion: The results confirm the role of school times in the partial sleep deprivation which adolescent attending earlier school starting times are submitted. The hyphotesis that school schedules are involved in body mass index gain was not confirmed.

0055
THE RELATIONSHIP BETWEEN SLEEP AND OBESITY IN ADOLESCENTS
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Introduction: The consequences of adolescents obesity is well documented in many systems and the most common and harmful are metabolic syndrome and cardiovascular disease. Recently, some studies have suggested that sleep deprivation could play a role in obesity occurrence. We aimed investigate the relationship between sleep patterns and excess of body weight in adolescents.
Methods: between August and October of 2008, adolescents (301 boys and 287 girls) from both sexes (mean age 12,6±0,9) who attended afternoon classes from 1pm to 5.30 pm, participated in the study. The variables measured were body mass index (BMI), waist circumference (WC) and body fat percentage. Bedtime and wake time on school days and weekends were evaluated by a questionnaire. Sleep duration were calculated from these data. Nonparametric correlation analyses were used to investigate associations between sleep and anthropometric variables.
Results: Mean sleep duration was 10 hours with no significant differences between school days and weekends (p>0,53). There was an inverse correlation between sleep duration and three anthropometric variables. For bedtime and wake time there were no significant associations. When analyzing by group, significant associations between waist circumference/BMI (r=-0.11 for both) and sleep duration occurred only in boys.
Discussion: the results evidence a tendency that who sleep less is probably more susceptible of being with a higher BMI, WC and body fatness. However, the power of associations are too small and it contrasts with strong correlations observed in previous studies. The longer sleep duration of these adolescents, related to afternoon school schedule, suggests an absence of sleep deprivation in many of them and could explain, at least partially, the results.
Conclusion: These results are part of on ongoing follow-up study which will investigate changes in sleep-wake patterns and body weight after the transition to morning schedules. These data will be compared with those from adolescents that will keep the same school schedule.

0056
SLEEP QUALITY AND INSOMNIA COMPLAINT IN STROKE
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Introduction: The presence of a brain injury can lead to appearance of changes in sleep-wake. However, have not been identified the complaints of sleep disorders and their relationship with the quality of sleep, besides what mechanism of regulation of sleep may be affected after a stroke. The aim of this study was to investigate the quality of sleep, complaints of sleep disorders and associated factors in stroke patients from the Physical Therapy services in Natal-RN.
Methods: A transversal study was developed with a sample of 70 subjects, 40 patients (57 ± 7 years), 11 ± 9 months after injury, and 30 healthy subjects (52 ± 6 years), evaluated with the Pittsburgh Sleep Quality Index (PSQI) and Sleep Habits Questionnaire. The data were analyzed by Chi-square test, t'Student test and logistic regression analysis.
Results: The patients showed on average poor quality of sleep (patients: 6,3 ± 3,5; healthy subjects: 3,9 ± 2,2; p=0,002), increased sleep latency (p=0,019), greater length of sleep (p=0,039) and more dysfunction during the day (p=0,001), i.e. more daytime sleepiness. Regarding complaints of sleep disorders (dyssomnias and parasomnias), the complaint of insomnia was the most prevalent (patients: 37,5%; healthy subjects: 6,7%; p=0,007). By regression analysis, the latency and complaint of insomnia were the predictors of the quality of sleep (*OR*= 0,120; p= 0,036). In addition, the female gender (*OR*= 11,098; p= 0,036) and the complaint of broken sleep (*OR*= 32,040; p= 0,003) were considered risk factors for the complaint of insomnia.
Conclusion: Through these found results, it was suggested that stroke can affect the homeostatic process of regulation of sleep, with the lowest latency and the absence of the complaint of insomnia considered protection factors to the quality of sleep. We suggest that poor sleep quality and insomnia be given priority assessment during clinical diagnosis.

0057
EFFECTS OF HIGH-CARBOHYDRATE AND HIGH-PROTEIN DIET ON NIGHT SECURITY GUARDS' SLEEPINESS
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Introduction: Studies indicate that night work is associated with metabolic alterations. These include obesity, elevated serum lipids, hypertension among others. In addition, a few laboratory studies have been performed to evaluate the effects of different diets on sleepiness. It has been suggested that there is a decrease of alertness with a high-carbohydrate diet. This study aims to compare the effects of high-protein and high-carbohydrate night meal on sleepiness among night security guards, according to their body mass index.
Methods: Twenty-four night security male guards who work from 00:00 to 08:00h (Monday to Friday) volunteered to participate on this study. The guards average age was 30.8 yo (SD= 5.5 yo); body mass index was 28.7 kg/m^2 (SD= 4.0 kg/m^2) and waist was 106.2 cm (SD= 10.4 cm). They wore an actigraph for three weeks, five days a week (Monday to Friday), to estimate their sleep-wake cycle, which was confirmed by a daily sleep log. Karolinska Sleepiness Scale (KSS) was used to self-record sleepiness every three hours from waking up to going to bed. The first week was considered the baseline and the workers had the night meal they usually ate at work. In the second week, the night meal was exchanged by a high-carbohydrate diet, and in the third week the content of the night meal was high-protein. The workers did not know about the contents of the meals. An ANOVA considering time as repeated measurement (before and after the night meal), and body mass index as a factor (above 30 kg/m^2 and below 30 kg/m^2) was performed for each condition. Pos-hoc tests were calculated whenever necessary.
Results: The analysis found a time borderline effect for the baseline week (p=0.05). It was also found an interaction effect between time and body mass index for the week with high-carbohydrate meal (p<0.05). The workers with body mass index above 30 kg/m^2 were sleepier than the others after eating the high-carbohydrate meal. There were no significant effects observed for the week with high–protein night meal.
Conclusion: The results suggest that night meal content does have an effect on night worker sleepiness. However, this effect seems to be modulated by weight.
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0058
EVALUATION OF ACUPUNCTURE EFFECTS ON SLEEP-WAKE CYCLE IN PATIENTS WITH CHRONIC PAIN – PRELIMINARY RESULTS
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Introduction: The analgesic effects of acupuncture are widely recognized, although its mechanisms are not fully established. Moreover, little is known about its influence on sleep-wake cycle. The objective of this study is to evaluate the effects of acupuncture on sleep-wake cycle in patients aged between 40 and 70 years complaining of chronic muscular or articular pain.
Methods: Six people aged between 48 and 61 years were undergone to 10 sessions of electroacupuncture, during 5 weeks. The selected acupoints were: GB-20 (*Fengchi*), GB-13 (*Benshen*), LI-4 (*Hegu)*, EH-6 (*Neiguan*), ST-36 (*Zusanli*), SP-6 (*Sanyinjiao*), LR3 (*Taichong*) and KI3 (*Taixi*), and frequencies were 3 Hz and 100 Hz. Before and after the treatment, volunteers answered to Epworth Sleepiness Scale (ESS) and Pittsburgh Sleep Quality Index (PSQI). Besides, they wore actimeters and filled out sleep-logs for seven consecutive days. Sleep-wake cycle variables before and after the treatment were compared by Wilcoxon test. **Results:** An increase of sleep duration (before: 6h11min; after: 6h48min) was observed (p<0,05). Effects of acupuncture treatment were not detected on other sleep parameters such as wake episodes, onset, offset, sleep efficiency, ESS and PSQI scores.
Conclusion: These preliminary results suggest that acupuncture somehow influence sleep-wake cycle. Data from a larger sample and comparisons with *sham* acupuncture treated patients are required and have been collected in an ongoing study.

C – Learning, Memory and Attention

0059
DIFFERENT BRAIN STEM LATERALIZATION BETWEEN DEPRESSION AND MANIA IN BIPOLAR DISORDER PATIENTS
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Introduction: Bipolar disorder show cyclic changes of mood with characteristic sleep-wake, motor and hormonal circadian abnormalities, suggesting that suprachiasmatic nuclei dysfunctions should play a role on it physiopathology. Previous research on major depression showing right side vestibular hypo-activity suggests a functional relationship between mood and vestibular activity. In order to elucidate if this vestibular pattern is related

or not with mood states, we studied vestibular activity in bipolar disorder patients during successive different mood states between mania and depression.
Methods: We studied two bipolar disorder patients during successive depressive and manic states using electronystagmographic records after rotatory stimulation. Right and left Slow Phase Velocity (SPV) of nystagmus was calculated in order to quantify vestibular activity and right/left SPV ratio to appreciate differences in vestibular lateralization during depression and mania.
Results: We recorded 4 episodes of depression and 4 of mania. Compared with healthy people, in depression we found a 41.6% decrease in right SPV, 16.7% increase in left SPV and during mania we found a 30.4% decrease in right SPV and a 37.6% decrease in left SPV. The mean right/left SPV ratio in depression was 0.7±0.1 (mean +SD, 4 records). This was significantly lower than the 1.4±0.1 found in manic phases (4 records) ($p< 0.05$, Mann-Whitney test).
Conclusion: Different patterns of vestibular dysfunctions were found in mania and in depression in bipolar disorder patients. Right side hypo-activity and left side hyper-activity were observed in depression, while asymmetric hypo-activity (less activity at left side than right) was observed during the manic states. During the depressive phase in bipolar disorder patients we found that the vestibular abnormal activity was similar to those of major depression patients previously studied by our group. There is a need to study the pathophysiologic meaning of these findings in order to understand the relationship between lateralized vestibular brain stem nuclei and mood states, and the mechanism underlying rapid changes in vestibular lateralization. Circadian rhythms of hormonal secretion and sleep-wake cycles also should be further studied during both mood states in order to ascertain the relation between lateralized vestibular abnormalities and suprachiasmatic nuclei function.

0060
EFFECTS OF 24-H SLEEP DEPRIVATION ON SUSTAINED ATTENTION
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Introduction: Sleep deprivation produces sleepiness, tiredness, and errors in school and work performance. These difficulties in performance could be due to a decrease in basic cognitive processes such as attention. This cognitive process is the capacity to respond to the environment and has four different components: tonic alertness, phasic alertness, selective attention and sustained attention. Sustained attention is the capacity to respond efficiently to the environment during prolonged periods (from minutes to hours). 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 assess the effects of 24-h sleep deprivation on sustained attention.
Methods: Participants were 20 undergraduate students (9 male, 11 female), mean age=17.80±1.06 (16-20) years. They were divided in two groups: control (5 males, 5 females) and 24-h sleep deprived (4 males, 6 females). Participants of the control group were allowed to sleep, whereas participants of the sleep deprived group remained awake during one night. Cognitive performance was assessed in both groups at 06:00, 08:00, 10:00 and 12:00 h. Attention was measured with a continuous performance task.
Results: Compared to the control group, the sleep deprived group showed less general stability of efficiency (standard deviation of correct responses, control group 1.37±0.23, sleep deprivation group 2.29±0.21, U=16, $p<0.001$; standard deviation of reaction time, control group 40.93±3.35, sleep deprived group 53.38±3.17, U=19, $p<0.05$); lower time on task stability (control group -0.22±0.11, sleep deprived group -0.41±0.13, U=12, $p<0.05$); and less short term stability (error runs of 2 to 3 errors control group 10.77±3.30, sleep deprived group 22.23±3.07, U=19, $p< 0.01$); error runs of more than three errors (control group 2.77±1.59, experimental group 10.93±2.84, U=17, $p<0.05$).
Conclusion: In conclusion, sleep deprivation for 24 h reduces all indices of sustained attention during the morning; this can reduce the efficiency at work and school, and increase the probability of accidents during the morning.

0061
INFLUENCE OF THE SLEEP IN THE FUNCTIONAL PERFORMANCE IN STROKE PATIENTS: A PILOT STUDY
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Introduction: It is known that sleep plays an important role in the consolidation of motor memory. Recent studies have shown that the presence of sleep between the training of a motor task and retention test promotes a better learning than in the presence of only wake between training and testing. These findings have also been found in patients who suffered a stroke, however, how this sleep characteristics can interfere with motor learning during the rehabilitation, then we are studding what is the relationship of sleep wake/cycle with the functional performance in individuals after stroke.
Methods: The sample was composed of 11 individuals (63,4 ± 8,8 years) with stroke motor sequel in the chronic state (16,9 ± 10,2 months after injury). The tools for assessment of sleep was the Pittsburgh Sleep Quality Index (PSQI), the questionnaire of Horne and Ostberg (HO), the Epworth Sleepiness Scale (ESS) and the actigraphy, whose used variables were: total hours of nighttime sleep (TST), sleep latency (SL), and sleep efficiency (SE). The Functional Independence Measure (FIM) and the Berg Balance Scale (BSE) were the measures used to evaluate the performance of individuals. The National Institute of Health Stroke Scale (NIHSS) and the Mini-Mental State Examination was used to evaluate the neurological impairment and cognitive state, respectively. Data were analyzed using Spearman's linear correlation with the significance level set at 5%.
Results: A negative correlation was observed between the sleepiness (score of ESS) and equilibrium function (score of BSE) ($p < 0,035$). But we did not find any correlation between sleep parameters and functional performance (FIM).
Conclusion: These findings suggest an association between sleepiness and balance in stroke patients and support the need for further studies.

0062
EFFECTS OF 24-H SLEEP DEPRIVATION ON PHONOLOGICAL AND VISUOSPATIAL COMPONENTS OF WORKING MEMORY
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Introduction: Sleep deprivation affects different cognitive processes. Working memory is a cognitive process that temporarily maintains information to perform multiple complex tasks such as reading, learning and reasoning. This cognitive process has two components: phonological and visuospatial storages. The phonological storage component is important for the processing of verbal information, whereas the visuoespacial storage is relevant for the processing of visual tasks, such as driving a car, space navigation and solving mathematical problems. The objective of the present study is to determine the effect of 24-h sleep deprivation on the components of working memory.
Methods: 20 undergraduate students participated, age 17.80±1.06, (16-20) years; they attended classes in the morning shift. They were divided in two groups. A control (non-sleep-deprived) group and a 24-h sleep-deprived group. Both groups were recorded in the laboratory at 06:00h, 08:00h, 10:00h and 12:00h. The phonological memory task consisted in a set of 4 upper-case letters displayed simultaneously on the monitor´s screen, followed by one lower-case letter; the participants had to indicate if the lower-case letter matched one of the 4 upper-case letters previously displayed. The visuospatial memory task was similar, but instead of letters, 3 dots were displayed simultaneously in different places of the screen, followed by a circle; the participants had to indicate if the circle appeared in any of the places where the dots were previously shown.
Results: In the phonological storage component of working memory, there was a reduction of correct responses with time of day, in both groups ($F= 3.8953$, $p< 0.05$). In the visuospatial storage component of working memory, the sleep deprived group showed an increase in reaction time ($F= 9.470$, $p< 0.01$), there was also an increase in reaction time with time of day, in both groups ($F= 4.366$, $p< 0.01$).
Conclusion: In conclusion, 24-h sleep deprivation impairs the visuospatial storage component of working memory during morning hours; this can decrease efficiency in many activities, such as driving a car, space navigation and solving mathematical problems.

0063
CIRCADIAN VARIATIONS IN A RESPONSE TO AN ALARM AS A CONCURRENT TASK
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Introduction: Human performance depends on basic cognitive processes such as alertness. Alertness is defined as a cognitive activation that makes a person capable to produce general responses to the environment. This basic activation is necessary to support higher cognitive processes such as memory and attention. Alertness can be assessed through the readiness to respond to an alarm. The objective of this study was to analyze circadian variations in alertness.
Methods: Participants were two female students, age: 17 years that attended school on a morning shift schedule. They were recorded in a constant routine protocol during 30 h; in this protocol feeding, environmental temperature, motor activity, recumbent position and room illumination were controlled. Rectal temperature was recorded each minute. Alertness and performance were recorded every 100 minutes, alertness was measured using an alarm task and performance was measured using a continuous performance task (CPT). Both tasks were recorded concurrently during 12 minutes. Participants used the dominant hand to respond to the CPT and the non dominant hand to respond to alarm task. Alarm task required to hold a key pressed during 12 minutes, if the key was released an auditive alarm appeared indicating the participant to press the key again. There were circadian variations in rectal temperature with a highest level during the afternoon and a lowest level during the early morning.
Results: Performance on the alarm task showed circadian variations. During daytime the key was hold press all time (no releases), the highest frequency of key releases occurred during nighttime and early morning. Reaction time to the alarm was classified in: short (RT less than 900 ms) and large (RT more than 1 sec) responses. During night, short RT was more frequent and indicated that participants were capable to perceive the alarm and respond fast to it, while large RT were less frequent and indicated an impairment in the capability to react to the alarm. These data suggest that during the nighttime, an auditory stimulus is not capable to activate the alerting system. Large RT can be related to microsleeps.
Conclusion: The results of this study may affect activities such as driving a car, as well as on many working situations during nighttime and early morning.

0064
EFFECTS OF SLEEP REDUCTION DURING THE WEEK IN SUSTAINED ATTENTION
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Introduction: The majority of the people living in cities works 5 days a week (Monday to Friday) and rest during weekends (Saturday and Sunday). People working in this schedule tend to sleep less during weekdays, compared to weekends. Sleep reduction during weekdays may affect cognitive performance. Attention is a basic cognitive process, with 4 components: tonic alertness, phasic alertness, selective attention and sustained attention. Sustained attention 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 analyze the effects of sleep deprivation during the week in sustained attention.
Methods: Participants were 19 undergraduate students attending school at a morning shift (07:00-13:00 h). 11 students were registered during weekdays (weekday group), whereas 8 students were recorded during weekends (weekend group). All participants kept a sleep-wake diary during 11 days. Then, each participant was recorded in the laboratory from 20:00 h to 02:00 h. Sleepiness, tiredness and responses on a Continuous Performance Task (CPT) were recorded at 2 h intervals. This task required the participant to press 1 to any number (except "9") appearing at the center of the computer screen, to press 2 when a "9" appeared, and to press 3 when a "4" appeared after the "9". The CPT had 27 blocks with 20 stimuli each. Three indices of sustained attention were assessed: general stability (standard deviation of correct responses and reaction time), time on task stability (linear regression of correct responses and reaction time) and short-term stability (hit runs and error runs).
Results: Compared to the weekend group, the weekday group slept less the night preceding the recording session (weekday group 6:16±0:34 h, weekend group 9:28±1:24 h, U=0, p<0.0001). The reduction in sleep was due to differences in waking time (waking time: weekday group 05:25±0:24 h, weekend group 09:11±1:05 h, U=0, p <0.0001; bedtime: weekday group 23:08±0:41 h, weekend group 23:42±1:05 h, U=32.50, NS). Both groups showed changes in all indices of sustained attention with time of day. Compared to the weekend group, the weekday group showed less stability and an increase in isolated hits at 02:00 h (SD of reaction time F=3.64, p<0.01; isolated hits F=4.85, p<0.05).
Conclusion: In conclusion, sleep reduction during the week impair sustained attention at night. This can decrease efficiency in many activities, such as driving a car or working at night.

D – Photic and non-photic synchronization

0065
DOES SCHEDULED GLUCOSE INDUCE ANTICIPATORY ACTIVITY IN RATS?

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Introduction: Food-entrained rhythms have been identified in several species. However, the entrainment mechanisms underlying the expression of these rhythms are not completely described. It has been reported that glucose phase-shifts the food-entrainable oscillator in rats. We aimed to test whether scheduled glucose ingestion is sufficient to induce anticipatory activity, studying the expression of the motor activity rhythm in rats.
Methods: Male and female Wistar rats (n = 10) were used. They were entrained to a 12h:12h LD cycle with ad-lib food for 3 days. Food was withheld on day 3 at ZT 12. From the 4th to 13th day the animals received glucose solution (50 %) in plastic flasks (20 mL/day/animal) between ZT 06-09, and then meal (glucose solution) was ommited for two days. The motor activity was monitored by infrared motion sensors positioned 15 cm above the cage lids connected to a personal computer where data were collected each 5 min using *Aschoff* software (E. T. Santos, A. Maitelli, J. F. Araújo, Universidade Federal do Rio Grande do Norte, 2000). Motor activity data were normalized by the daily mean. Rhythms analyses, which included actogram, periodogram, waveform and cosinor, were performed on *El Temps* software (A. Díez-Noguera, Universitat de Barcelona, 1999).
Results: The rats anticipated mealtime. Locomotion between ZT 04-06 averaged over days 1-2, over the last two days (12-13) of restricted glucose and on meal omission 2 were statistically different (ANOVA for repeated measures, F (2,18) = 12,440, $P < 0.0005$). A post-hoc Tukey test revealled that activity on days 1-2 was lower than on days 12-13 and meal omission 2 ($P < 0.001$ and $P < 0.002$, respectively) but no difference between days 12-13 and meal omission 2 ($P > 0.94$). The motor activity rhythm of six animals seemed to fully entrain to mealtime. Averaged acrophase over days 1-2 and 12-13 occurred 16.97 ± 0.42 h and 8.84 ± 0.47 h after lights-on, respectively, and 9.66 ± 0.67 h on meal omission 2. ANOVA revealed significant difference between these days (F (2, 18) = 95.84, $P < 0.000001$). A post-hoc Tukey test showed difference between days 1-2 and days 12-13 ($P < 0.001$) and meal omission 2 ($P < 0.001$), but no difference between days 12-13 and meal omission 2 ($P > 0.42$).
Conclusion: Daily glucose ingestion induces anticipatory activity in rats and possibly also entrains the circadian pacemaker in the suprachiasmatic nucleus of the hypothalamus.
Support: CNPq, FAPERN.

0066
CIRCADIAN MOTOR ACTIVITY RHYTHM OF *Callithrix jacchus* UNDER T CYCLES SHORTER THAN 24 HOURS

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Introduction: Some behavioral and physiological alterations are resulted from disturbances in circadian system, like metabolic syndrome and sleep disorders. Some authors suggest that the dissociation model of motor activity in rats under T22 is an excellent model to study these disturbances. Then, with the purpose to evaluate if this model can be applied to diurnal primates, we observed the motor activity of marmosets under LD cycles shorter than 24h.
Methods: Motor activity of six adult females, in individual cages, was continuously registered. They were isolated in a room with controlled temperature and humidity, sound attenuated, under the symmetrical light-dark (LD) cycles T24, T21, T21.5 and T22, during 14, 60, 35 and 48 days, respectively. The light intensity during light phase was 146.5 ± 53.3 lx whereas dark phase consisted in total darkness. There were analyzed the percentage of variance (VSP) of each female motor actrivity rhythm, by Periodogram Sokolove-Bushell, and the percentage of activity during light phase.
Results: Motor activity rhythm was expressed with two circadian periods under the three T cycles shorter than 24 hours: one entrained to external LD cycle, named light-entrained component (LEC) and other in free-running, named non-light-entrained component (NLEC). But all the animals presented both components only under T21. In the other two conditions, only 83.3% (T21.5) and 33.3 % (T22) of the animals showed two significative components. The value of VSP of LEC was 8.2 ± 1.1, 14.2 ± 2.5, 24.8 ± 8.0, and of NLEC was 12.0 ± 5.3, 10.9 ± 3.9, 11.9 ± 1.5 to *T*21, *T*21.5 and *T*22, respectively. Despite that, most of activity happened at the light phase in relation to total distribution of activity, being 86.7±4.3% (T21), 94.0±3.2% (T21.5) and 91.4±6.4% (T22).
Conclusions: The occurrence of two circadian components in the motor activity of all the females under T21 is a strong evidence that this LD cycle period promotes internal desynchronization in this species. Therefore we suggest that marmosets under T21 can be a good animal model to study internal desynchronization in diurnal primates.
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0067
ALERT STATE IN *Callithrix jacchus* UNDER LIGHT-DARK CYCLES SHORTER THAN 24 HOURS

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Introduction: Some quotidian situations, like summer time, shift work, among others, promote desynchronization among the endogenous oscillators that can lead to disturbs in circadian rhyhtmicity. These disturbs can generate diseases, including circadian rhythms sleep disorders. Some authors suggest that the dissociation model of rats under T22 is a good model to force internal desynchronization. Then, in order to find a primate model of internal desynchronization we evaluated the effects of light-dark (LD) cycles shorter than 24 hours on alert state of marmosets.
Methods: Six adult females, in individual cages, were maintained under the symmetric light-dark (LD) cycles T21, T21.5 and T22 during 60, 35 and 48 days, respectively, in a room with constant temperature and humidity, and sound attenuated. The light intensity during light phase was 146.5 ± 53.3 lx whereas dark phase consisted in total darkness. The vocalizations (an alert indicator) of all the animals were continuously recorded by a microphone installed in the room, connected to a computer.
Results: There were observed two circadian components for vocalizations frequency and duration in the three external LD cycles, being one synchronized to the LD cycle, named light-entrained component (LEC), and the other an average of endogenous period of all females, named non-light-entrained component (NLEC). The percentage of variance (VSP) of vocalizations frequency was 16.6%, 13.9%, 10.7% for LEC and 9.3%, 4.0%, 4.2% for NLEC; and of vocalizations duration was 15.9%, 12.4%, 11.4% for LEC and 9.8%, 4.6%, 4.3% for NLEC. The occurrence of vocalizations at the light phase was of 95.2%, 95.0%, 98.3% for frequency and 95%, 95.9%, 98.8% for duration. All the values were given for T21, T21.5 and T22, respectively.
Conclusions: Expression of two circadian components in vocalizations rhythmicity of marmosets under T cycles shorter than 24 hours indicates that alert state is driven by at least two different groups of circadian oscillators, being one capable of entrain to the external LD cycle while the other can't, depending on T cycle. Both components were more evident and similar in T21 than in T21.5 and in T22. Based on this data, we suggest that marmosets under T21 can be a good animal model to force internal desynchronization. This can be very helpful to provide new treatments to disturbs in circadian rhythmicity, including cognition deficit.
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0068
HEPATIC GLUCONEOGENESIS IN A RESTRICTED FOOD SCHEDULE DURING THE EXPRESSION OF THE FOOD SYNCHRONIZED OSCILLATOR

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Introduction: A continuous glucose contribution is essential for life, being the brain's main fuel and the unique source of energy that cells that lack mitochondria can use. If the glycemia falls, like in fasting or between meals circulating glucose recovers by two processes: (1) glucogenolysis, contributing short term glucose from the reserves of glycogen (first12-24h of fasting); and (2) the gluconeogenesis, forming glucose from some aminoacids and from other molecules such as glycerol and lactate. Nevertheless, if the fasting period is last too long the organism also obtains energy from acetyl-CoA produced by the degradation of the free fatty acids (FFA) and ketonic bodies (KB). In previous studies, animals under restricted food schedule (RFS) and expressing of the food entrainment oscillator (FEO). Showed, high levels of

circulating FFA and KB, partial reduction of hepatic glycogen, increased ATP and an oxidized redox state, suggesting a rheostatic adaptation of the liver metabolism. Hence, the aim of this project was to explore the process responsible of maintaining the levels of glycemia and the regulation of the hepatic gluconeogenesis, during the expression of FEO.
Methods: We analyzed the key enzymes of the gluconeogenesis in cellular hepatic fractions: glucose-6-phosphatase (G6Pase; microsomes) and the phosphoenolpyruvate carboxykinase (PEPCK; cytosol), by measuring enzymatic activity and by Western blot.
Results: We found significant differences in the glycemia, the enzymatic activities and the expression of G6Pase and PEPCK during circadian characterization, showing a phase shift around the food access and presentation of bimodal patterns. In addition, we found higher levels of glucose and lower levels of genetic expression (amount of protein) and of activity (enzymatic activity) of the G6Pase and the PEPCK (before accessing to the food), in comparison with a simple fasting (24 and 48h).
Conclusion: These results reinforce the idea of a rheostatic state by the animals that express FEO, during the control of the hepatic gluconeogenic process. Our results suggest that hepatic metabolism depends more in lipid oxidation than in biochemical formation of glucose during the fasting period of FEO expression.

0069
CARACTERIZATION OF THE MOTOR ACTIVITY RHYTHM IN A COMMON MARMOSET FAMILY
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Introduction: Most studies about the ontogeny of biological rhythmicity are based on the idea that the circadian periodicity is installed during infancy and remains the same until the beginning of the senescence. Although some studies have exploited the circadian rhythmicity in the transition phase between the infancy and adult stages, they not explored the peculiarities of this development phase that prepares a subject to the adult stage. The diurnal rodent *Octodon degus* shows a phase delay in activity onset in the puberty that remains until adult stage.
Methods: In order to compare the phase relation of the motor activity rhythm between juveniles marmosets and parents, two juveniles and their parents were maintained under artificial light conditions (light-dark cycle: 12:12h) and the activity was monitored by Actiwatch (MiniMitter) during twenty days.
Results and conclusion: The activity onset occurred earlier and activity offset occurred later in adults than in juveniles (Tukey, $p<0.05$). Consequently, phase active duration was higher in the adults in relation to juveniles. If the activity onset advance observed in pubertal animals (preliminary data observed in our laboratory) and in adults compared to pre-pubertal animals is confirmed in more animals, it is possible to suggest that this advance begins in the puberty and remains until the adult stage. The phase angle of activity onset and offset was smaller in adults than in juveniles (Tukey, $p<0.05$). Since the activity total was smaller in adults than in juveniles, this result may be consequence of the play behavior that involves juvenile displacement increase. In relation to 24h and 8 h components of motor activity rhythm, there was no difference between adults and juveniles, possibly because the circadian component becomes stable near five months age. Although the evidence that motor activity rhythm showed difference between adults and juveniles, the evaluation of more animals is necessary to confirm this hypothesis.

0070
THE PUBERTY MODULATES THE CIRCADIAN ACTIVITY RHYTHM OF COMMON MARMOSETS?
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Introduction: The study of circadian activity rhythm during puberty may contribute to understand endogenous mechanisms involved with sleep-wake cycle phase delay exhibited by human adolescents. The common marmoset have been used as animal model in many biomedical researches, however the circadian rhythmicity during puberty was not yet exploited. A previous study with pubertal marmoset observed that the activity onset occurred earlier and the total activity increased after puberty onset. However, it was not possible to affirm that the results just due to puberty, because the animals were maintained under natural environmental conditions.
Methods: To evaluate the effect of puberty onset on circadian activity rhythm, the activity of a pair of twins (2 females) were continuously monitored by Actiwatch (MiniMitter) between the 4th and 6th months of age. The animals were maintained with their family in a cage under artificial light conditions (light-dark cycle: 12:12h). Fecal steroids were monitored twice a week to determine the puberty onset.
Results and conclusion: According with the preliminary result, the percent of spectral power of the 24 h and 8 h components remained stable along the phases. The other parameters of the motor activity did not show difference between the animals (ANOVA, $p>0.05$). However, the animals showed difference between the phases (one pre-pubertal and three puberty with 15 days each) in all observed parameters (ANOVA, $p<0.05$), except for active phase duration (ANOVA, $p>0.05$). The activity onset occurred earlier in the first puberal phase compared with the pre-puberal and second puberal phases (Tukey, $p<0.05$). Consequently, the $_{on}$ was lower in this phase (Tukey, $p<0.05$). The activity offset occurred later and $_{off}$ smaller in the pre-puberal phase in relation to the other phases (Tukey, $p<0,05$). The total activity increased along the phases (Tukey, $p<0.05$). There was not interaction between the animals and phases (ANOVA, $p>0,05$), indicating that the animals showed similar distribution in motor activity rhythm parameters at all phases of development. The changes observed in motor activity rhythm may be related to the maturation process in puberty. However, the activity onset advance observed in these twin pairs and in the previous study of our laboratory were differed from that was observed in humans, *rhesus* monkey females and *Octodon degus,* that show a delay of phase. The evaluation of more animals is necessary to confirm our hypothesis.

0071
EFFECT OF FOOD SCHEDULE ON THE HYPOTHALAMUS AND DEVELOPMENT OF METABOLIC SYNDROME IN RATS SUBMITTED TO NIGHT WORK MODEL
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Introduction: The light-dark cycle provides the most powerful environmental cyclic stimulus, under which practically all organisms have evolved and adapted. To survive in a cyclic environment the organisms developed a circadian system that induces cyclic changes in the physiology and behavior. In mammals this system consists of a biological clock and multiple organs that constitute peripheral oscillators. The circadian system allows the organisms to express physiological processes and feeding at the appropriate time of the day or night. Nevertheless, recent studies report evidence that the type and the time of food can drive peripheral oscillators out of phase from the biological clock causing a internal desynchronization (ID), characterized by the loss of phase relation between behavioral, hormonal and metabolic rhythms. Shift- and night –work lead to ID and at long term lead to cardiovascular and gastric disorders, propensity to obesity, metabolic syndrome and cancer. To better understand the mechanisms underlying ID and in order to propose strategies to prevent this disturbance, we have developed an experimental model of night-work in rats based on schedules of forced activity.
Methods: From Monday to Friday rats are placed for 8 hours in slow rotating wheels during their inactivity phase. During the remaining hours of the day and during weekends rats are returned to their individual home cages placed in a monitoring system in order to register their activity, and their feeding patterns.
Results: After 4 weeks under this "working" schedule rats diminished their nocturnal activity and voluntarily shifted their food ingestion towards "working" hours. Metabolic rhythms were dampened or uncoupled from the suprachiasmatic nucleus (SCN) activity, which remained fixed to the LD cycle and promoted increased body weight and abdominal fat accumulation. Follow up of Fos and Per1 daily cycles indicated that in hypothalamic structures activity had shifted to the "working" schedule and thus was uncoupled from the SCN. Since feeding schedules are a strong entraining signal for metabolism and behavior, we explored whether the changed feeding patterns such as developed by working rats could have promoted the internal desynchrony. Food restricted to the dark phase (the normal activity phase) reverted all metabolic disturbances to the normal range and prevented body weight increase, demonstrating the important contribution of feeding habits to prevent internal desynchrony and obesity in the night worker.
Conclusion: Feeding schedules are relevant to prevent internal desynchrony in the night worker and may explain metabolic disturbances arising from disturbed feeding patterns.
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0072
RELATION BETWEEN THE TEMPORAL PATTERN AND ALIMENTARY ACTIVITY OF TWO SPECIES OF FISH
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Introduction: The circadian rhythms in fish has an endogenous control being able to be synchronized by diverse factors, as light-dark cycle, change of temperature, presence of predator, availability and competition for food, and others. This work aims to verify the existence of temporal pattern in alimentary activity and the relation between this index and the frequency of capture for fishes *Plagioscium squamosissimus* and *Astyanax* cf. *lacustris*, in Piató Lagoon (5°32'04.9"S/36°56'41.8"W), municipality of Assu, Rio Grande do Norte, Brazil.
Methods: The capture of animals has occurred monthly for 24 hours, with catching to each four hours (00:00, 04:00, 06:00, 08:00, 12:00, 18:00 e 20:00 hours), using seine nets located in three different points. The alimentary activity was analyzed using the Index of Stomach Repletion, that is calculated as the ration of the weight of the stomach and the weight of the fish. **Results and conclusion:** The results for alimentary activity showed to similar pattern *Astyanax* cf. *lacustris* and *Plagioscium squamosissismus* an increase around 04:00 e 20:00 hours of significantly (ANOVA $p<0,05$), featuring such twilight-nocturnal about to the breeds studied. The association with frequency of capture time showed a strong relation with the pattern of alimentary activity, what it indicates that both the species use the phase of lesser luminosity leave of it to exert its activities locomotive in food search.

0073
OBESITY INFLUENCES IN THE ENTRAINING OF LOCOMOTOR ACTIVITY IN THE VOLCANO MOUSE *Neotomodon alstoni*.
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Introduction: Obesity is often related with atypical activity patterns in mammals, some of this problems are related with circa-

dian regulation. The volcano mouse *Neotomodon alstoni*, endemic of central Mexico, develop obesity in most organisms in captivity, obese animals develop symptoms equivalent to the metabolic syndrome and makes this species interesting to study circadian behavior. The aim of the present work is to elucidate whether each obesity affect in the entraining of locomotor activity in the volcano mouse to schedule changes and to know if activity displays similar phase response shifts when exposed to a 1 h light pulse (200 lux) in the controls and obese volcano mouse.
Methods: Male adult mice *N alstoni*, were used. Freely moving locomotor activity was recorded by means of infrared light beams in a no-running wheel environment. Two experiments were set, first, controls (45±10 g) and obese (70 ±10 g) mice were exposed to LD 12:12 photoperiod and 6 h advances and delays were set every 10 days. Obese animals require larger number of days to fully entrain to either advances or delays in photoperiod and diurnal activity is more intense in obese mice. A second experiment consisted on animals exposed to LD (12:12) for at least 10 days, thereafter, mice were maintained under constant darkness conditions. Ten days later, a light pulse was given at different circadian times (CT) and phase shifts were estimated. In the obese animals phase delayed and advanced significantly reduced compared with control animals and the phase stability decreased in obese animals.
Results and conclusion: Our results indicate that the photic entrainment in mice *N alstoni* seems to be affected by the overweight conditions, and that this species could be of particular interest to study the effect of obesity upon circadian physiology.
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E – Invertebrate Models

0074
CIRCADIAN RHYTHMS IN *C. elegans*: LOCOMOTOR ACTIVITY, METABOLISM AND STRESS TOLERANCE

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Introduction: *C. elegans* is a model organism widely used in diverse areas of research but not well characterized in chronobiological studies. We have recently designed an automated system to track individual nematodes and demonstrated the existence of circadian activity rhythms in both LD (light : dark, 12 h : 12 h) and DD (constant darkness) conditions, periods were found to be of 24.2±0.44 h and 23.1±0.40 h respectively. In addition, circadian periods were temperature-compensated and could also be entrained by temperature cycles. Mutations in clock gene homologs induced circadian phenotypes with altered periodicities.
Methods: In order to determine if the worms are able to detect different light wavelengths, we have studied phototaxic responses, and found a phototaxis index of 0.7 ± 0,09 towards the green wavelength of light (520nm). Another approach to uncover rhythmic outputs was the study of stress tolerance behaviors. We found that *C. elegans* showed rhythmic stress tolerance patterns for oxidative and osmotic stress with peaks at ZT 12 (lights on) and ZT 0 (lights off), respectively. Stress-related gene expression was determined by sqRT-PCR and confirmed by RealTime-PCR: gpdh-1 and gpx showed a significant diurnal variation. We have also studied circadian rhythms in metabolic variables, such as food consumption and defecation.
Results: Food consumption rate (determined by decreasing OD600 of *E. coli* OP50) was shown to be rhythmic and a peak was found in the evening (ANOVA, p<0.0001). Defecation rhythms also showed to be governed in a circadian manner. These results show that control animals have a 24 h period in the frequency of the ultradian defecation rhythm (ANOVA, p< 0.0001). Furthermore, the defecation behavior of the JT73 mutant strain depicts a normal rhythmic pattern on a circadian level (ANOVA, p<0.01). Finally, we have detected aaNAT activity and melatonin in this nematode. aaNAT exhibited a diurnal variation peaking at ZT12, and melatonin could serve as a possible circadian output signal.
Conclusion: In summary, our results show that several different circadian outputs can be recorded in *C. elegans;* in particular, the circadian rhythm of locomotor activity can be entrained to environmental signals. These data might be a basis for the screening of putative circadian mutants in this species.

0075
DAILY VARIATIONS IN ABIOTIC AND BIOTIC STRESS TOLERANCE IN *C. elegans*

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Introduction: Circadian rhythms control several behaviors through neural networks, hormones and gene expression. One of these outputs in invertebrates, vertebrates and plants is the stress resistance behavior.
Methods: We first studied the circadian variation in abiotic stress resistance of adult *C. elegans* cultured in liquid survival medium as well as the genetic mechanisms that underlie such behavior. Measuring the stress resistance by tap response behavior we found a rhythm in response to osmotic (NaCl LC50=340mM) and oxidative (H_2O_2 LC50=50mM) shocks, with a minimum at ZT0 (i.e., lights off) and ZT12 (lights on), respectively. In addition, the expression of *glutathione peroxidase* (*C11E4.1*) and *glycerol-3-phosphate dehydrogenase* (*gpdh-1)* (genes related to the control of stress responses) also showed a circadian fluctuation in basal levels with a peak at night. Moreover, in the mutant *osr-1* (AM1 strain), a negative regulator of the *gpdh-1* pathway, the osmotic resistance rhythms were masked at 350mM but reappeared when the strain was treated with a higher NaCl concentration. We then studied the response to biotic stress. In order to do so we studied the response of *C. elegans* to a soil dwelling bacterium, *P. fluorescens*. These bacteria elicit at least two types of deleterious effects in *C. elegans*. Slow-killing depends on the GacS pathway. Fast-killing (similar to what has been described for *P. aeruginosa*) depends strongly on hydrogen cyanide production.
Results: The response observed in *C. elegans* nematodes to fast paralytic killing varies along the day and its sensitivity is higher during the night, at ZT12 (lights off). This behavior correlates well with HCN tolerance, which is higher during the day, at ZT0 (lights on). This innate immune response to *P. fluorescens* and *P. aeruginosa* might depend on the stress response pathway of *C. elegans*.
Conclusion: This work demonstrates for the first time that in the adult nematode *C. elegans* stress responses vary throughout the day, and suggests an underlying rhythmic gene expression that governs these behaviours.

0076
PIGMENT-DISPERSING-FACTOR AND CORAZONIN IMMUNOREACTIVITY IN THE CENTRAL NERVOUS SYSTEM OF THE STINGLESS BEE (*Melipona quadrifasciata*)

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Introduction: The stingless bees colony comprises a queen, workers of different ages that perform different tasks and males only present before the queen's fecundation. Among the workers, only the oldest ones – foragers – keep a direct contact with the light/dark environmental cycle. Biological rhythms are present and are controlled by the circadian system located in the central nervous system and in the retrocerebral complex. This system is composed by a web of multiple oscillators coupled to input pathways from the sensorial system and output pathways. The output pathways consist of nervous, neurohormonal and hormonal components. Several studies suggest the participation of the Pigment Dispersing Factor (PDF), a neuropeptide, in the output pathyways of the circadian system and in the synchronization of central oscillators. Another neuropeptide, Corazonin, seems to be envolved in downstream output pathways.
Methods: The objective of this study was to identify putative circadian pacemakers of the stingless bee, *Melipona quadrifasciata*. The central nervous system and the retrocerebral complex of foragers were analyzed throughout immunocitochemical assays. An antibody against *Drosophila melanogaster* PDF and another against *Apis mellifera* Corazonin have been used. Analysis and an accurate recognition of the brain's areas of *M. quadrifasciata* were previously made by the central nervous system mapping.
Results: PDF and Corazonin immunoreactivity (-ir) were present in the central nervous system of *M. quadrifasciata*. PDF-ir was localized in the cells and processes of the optic lobe, in all of its three neuropils: *lamina*, *medulla* and *lobula*. In the brain, the localization of PDF-ir cells suggests the presence of this neuropeptide in the *pars lateralis* (one of the largest clusters of brain's neurosecretory cells). These results are distinct from previous descriptions of the honeybee's system. Corazonin-ir was found in the brain of the stingless bee in two groups of cells localized in the protocerebrum (anterior portion of the brain), that apparently also belong to *pars lateralis*. Corazonin was found in the glands of the retrocerebral complex - *corpora cardiaca* and *corpora allata* - as well. Its presence in these glands has been recently reported in other insect species.
Conclusion: The specific immunoreactivity of both PDF and Corazonin indicates the localization of circadian pacemakers and its efferences in the central nervous system of the stingless bee. These assays are the first studies that aim to identify the components and the organization of the circadian system of the stingless bee.
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0077
SEASONAL INFLUENCE ON THE BEHAVIOR OF WORKERS AND ARCHITECTURE OF THE *Melipona quadrifasciata* COLONY (HYMENOPTERA; APOIDEA; MELIPONINI)

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Introduction: *Melipona quadrifasciata* is a tropical eusocial stingless bee. In this species, the workers (sterile females) are responsible for the physical maintenance of the colony. The structure of the nest comprises the regions: 1) brood chamber - in the center of the nest, where the oviposition by the queen (fertile female) occurs. Nurses (young workers) build brood cells. 2) pots region - food storage region, where intermediate age workers - builders - construct pots to store honey and pollen. 3) entrance - consists in a hole, used in the orientation of foragers (old workers) returning from the field and in a tunnel that guarantees constant darkness inside the nest.
Methods: In this work, seven colonies of *M. quadrifasciata* have been eye-inspected, during two years. In each inspection, the regions of the nest were measured and the workers activity registered. To evaluate behavior, ethograms were built.
Results/Conclusion: The hive structure shows different proportions and characteristics according to the season. In the end of summer/beginning of autumn, the pots area and the number of pots increase. Their maximum size and number are reached by the end of autumn. These increases are related to the amount of food stored and accumulation of reserves are a form of anticipation for wintertime when flowering is at its lowest level. This anticipation assures the colony survival during winter and it occurred in both years of observation. Foragers activity is low or absent during the same interval. In contrast with the pots area, the brood chamber area is enlargemented during summer and to the increase in size corresponds a addition in oviposition episodes. Even though experiments under controlled environmental conditions will be necessary, the results indicate rhythmic changes in the proportions of the nest's areas and in the castes' activity along the year. In addition to variations in the structure, preliminary data indicate the existence of circadian and ultradian components in the daily activity of builders. Their activity pattern is in antiphase with that of foragers. Possibly this is because the population within the colony increases during the dark as foragers remain inside the hive at night.
Results allow to demonstrating the existence of an internal temporal organization in the colony of *M. quadrifasciata*. How much

of it is due to endogenous rhythms is not known yet, however ultradian, daily and seasonal components are present both in the activity of the castes and the structure of the colony.

0078

THE ENTRAINMENT OF DEEP-WATER BENTHIC SPECIES TO MONOCHROMATIC LIGHT INTENSITY CYCLES MIMICKING INCREASING DEPTHS: THE CASE OF THE NORWAY LOBSTER, *Nephrops norvegicus* (CRUSTACEA: DECAPODA)

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Introduction: The entrainment of deep-water benthic species represents a not yet understood aspect of marine chronobiology. Species with wide depth range of distribution, encompassing shelves and slopes show entrainment for monochromatic 480 nm blue light, since this is the only wavelength invariantly present down to the inferior limit of the twilight zone (1000 m depth). Different populations experience entrainment to light-dark cycles (LD) of markedly different intensities. The Norway lobster, *Nephrops norvegicus,* inhabit shelves and slopes, is territorial spending its life close to the same burrow. Field and laboratory studies show that animals emerge with a timing that is depth dependent: at night on shallow shelf, at crepuscular hours on lower shelf, and at daytime on the slope.
Methods: In this study, our aim was to assess the entrainment capability of *Nephrops* in the laboratory, using two monochromatic (480 nm) light-dark cycles (LD) of intensities mimicking the shelf and the slope. With a new IR actograph prototype endowed with a burrow, we could measure rhythms in door-keeping behaviour (DK) as well as proximal- and distal emergence (PE and DE, respectively). Two groups of animals were exposed to LD of 0.1 lux (N = 8) mimicking the slope, and to 10 lux mimicking the shelf (N = 10) (LD1). Animals were then transferred to constant darkness (DD), and then re-exposed to the same LD (LD2).
Results: At 0.1 lux, all behavioural activity was diurnal and entrainment stronger, since similar activity levels appeared either confined during photophase at LD1, the expected photophase at DD, as well as during the re-imposed photophase at LD2. Conversely, under 10 lux a weak entrainment was found with no dominant diurnal or nocturnal DK activity at LD, or with aparent nocturnal PE and DE activities due to exogenous masking by light.
Conclusion: Present data indicate that the determination of diurnal/nocturnal niche in *Nephrops* is a process which depends on light intensity, reflecting slope and shelve depths in the field, due to balance in circadian/masking modulation of behaviour.

0079

CIRCADIAN PROTEOME OF THE MOSQUITO *Aedes aegypti* (DIPTERA: CULICIDAE), THE DENGUE AND YELLOW FEVER VECTOR

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Introduction: Circadian rhythms have been described in almost all organisms, but very little is known about their molecular control in hematophagous insect vectors. As the circadian clock drives activity and feeding behavior in these vectors, understanding the molecular machinery of the clock may add important information to the dynamics of vector-borne disease transmission. We are currently studying the circadian proteome of *Aedes aegypti*, vector of dengue and yellow fever viruses, in order to identify the proteins that are differentially expressed in a circadian pattern.
Methods: We analyzed the mosquito proteome through the two-dimensional electrophoresis (2D-E) gel approach. In the first dimension, we used a 4-7 pI interval to focalize the proteins from female heads collected every four hours in constant dark. In the second dimension, the focused proteins were resolved in a 12% SDS-PAGE. The resulting 24 gels (four replicates per time-point) were analyzed using Image Master Platinum 5.0 software.
Results: A preliminary analysis revealed that from 315 detected spots approximately 11% (35 spots) displayed a pattern that suggests circadian variation and, therefore, were identified by MALDI-TOF-TOF mass spectrometry. It is of interest to note that the mRNA from *ca.* 30% of these identified proteins had been previously identified in *Drosophila* circadian microarray assays. After identification, proteins were assembled according to their functions.
Conclusion: Ongoing studies are validating these results. We hope that the analysis of the circadian proteome of *Aedes aegypti* will contribute to our understanding of the coordination of some clock controlled output processes in this disease vector.

0080

COMPARATIVE ANALYSIS OF THE ACTIVITY PATTERNS OF *Aedes aegypti* AND *Aedes albopictus* (DIPTERA: CULICIDAE)

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Introduction: The mosquitoes *Aedes aegypti* and *Aedes albopictus* are two important vectors of dengue virus. In Brazil, *Ae. aegypti* plays a crucial role in dengue transmission whereas *Ae. albopictus* is still considered a potential vector, despite its important role in Southeast Asia.
Methods: In this study, we compared the activity patterns of *Ae. aegypti* and *Ae. albopictus* in the laboratory in light-dark and constant dark conditions. We also tested the effect of insemination and blood-feeding. The activity pattern of both mosquito species was studied using a modified version of the Drosophila Activity Monitor (TriKinetics).
Results and conclusion: In LD12:12, females of both species show a bimodal activity pattern with a peak after lights-on and a second more pronounced peak in the end of the photophase. However, *Ae. albopictus* presents more nocturnal activity than *Ae. aegypti***.** In constant darkness, the morning peak disappears in both species and *Ae. aegypti* shows a shorter free running period than *Ae. albopictus*. Blood-feeding significantly decreases female activity in both species.

F – Cellular and Molecular Mechanisms

0081

SCREENING FOR POLYMORPHISMS IN THE *AANAT* GENE AND THEIR ASSOCIATION WITH DIURNAL PREFERENCE

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Introduction: Arylalkylamina N-Acetyltransferase (AANAT) is the rate-limiting enzyme of melatonin synthesis pathway. Polymorphisms in the *AANAT* gene are likely to alter melatonin rate of synthesis and causes shifting in circadian rhythm of individuals. **Purpose:** To perform a screening of the *Aanat* gene to identify which polymorphisms are present in Brazilian sample and seek associations with diurnal preference.
Methods: Individuals with extreme diurnal preference were selected according Horne-Ostberg questionnaire scores. DNAs are amplified by Polymerase Chain Reaction and analyzed in Denaturing High Performance Liquid Chromatography (DHPLC). Segregated association tests for each SNP or haplotypes blocks were performed
Results: A total of six polymorphisms were found. Two still not reported in data banks or in the literature. All polymorphisms in the *Aanat* gene found in this sample, except by one, presented very low frequencies, which maximum frequency 6%. The only one which is found more often, the C-263G is localized in the promoter region exactly at a putative biding site for the transcription factor SP1. This polymorphism is a Single Nucleotide Polymorphism (SNP), a C to G change in the position -263. A -263G allele is more present in evening preference volunteers.
Conclusions: We observed that, except by one SNP in the promoter region, the frequency of variation of these SNPs in the *AANAT* gene are extremely low which indicate that it is very much conserved. There is a borderline association between SNP C-263G in the promoter region and extreme diurnal preference. This polymorphism is in the transcription factor SP1 biding site indicates that it can modulate the rate of transcription of *AANAT* gene with consequences in the temporality of the melatonin secretion curve.

0082

ANALYSIS OF CLOCK GENE CIRCADIAN EXPRESSION IN TWO MOSQUITOES

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Introduction: Mosquitoes show a range of activity patterns, from diurnal, to crepuscular and nocturnal. Although activity and blood-feeding rhythms are controlled by the circadian clock, it is yet unknown whether such species-specific differences in behaviour are directly controlled by core clock genes rather than reflecting differences in how the information of the central clock is translated into output signals.
Methods: To begin addressing this question we initiated a comparative analysis of the circadian expression of clock genes in two important mosquito vectors of tropical diseases, *Aedes aegypti* and *Culex quinquefasciatus*, which show very different locomotor activity patterns. Under controlled laboratory conditions *Ae. aegypti* is mainly active in the photophase, while *Cx. quinquefasciatus* activity is largely restricted to the scotophase. Using quantitative Real-Time PCR, we analysed the expression of several clock genes of these two species in light-dark (LD) and constant dark (DD) conditions.
Results and Conclusion: The results show generally conserved circadian expression patterns for all genes except the mammalian-like *cryptochrome- 2 (cry2).*

0083

IS *PER3* POLYMORPHIC IN CHIMPANZEES?

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Introduction: Clock genes are conserved between eucoelomate animals. However, where invertebrates such as *Drosophila* have one

copy of each clock gene, owing to gene duplication, vertebrates has gene families containing two or three members. Thus, whereas *Drosophila* has one *Period* gene, mammals have three (*Period1, Period2*, and *Period3*). In all species studied, variability in circadian parameters has been associated with polymorphisms and mutations in clock genes. The human *PER3* gene contains a variable number tandem repeat (VNTR) polymorphism in the coding region, where a 54-bp unit encoding 18 amino acids is repeated either four or five times. This polymorphism has been associated with diurnal preference and Delayed Sleep Phase Syndrome (DSPS), in studies performed both in the UK and in Brazil, and has been shown to associate with sleep homeostatic parameters both in the UK and in the USA. The VNTR in the *Per3* gene appears to be exclusive to primates, but the number of repeated units differs between species. Some species, such as the gorilla and the Brazilian marmoset *Callithrix jacchus* have intraspecific polymorphism as found in humans, with 3/4 or 6/7 tandem copies, respectively.
Methods: In order to investigate if an intraspecific polymorphism exists in the chimpanzee, we analyzed 42 samples of DNA extracted from individuals born in captivity after crossings between parents from different populations.
Results/Conclusion: All DNA samples showed four repeat of the 54-bp unit, with no evidence for polymorphism. We conclude that the intraspecific polymorphisms found in humans and gorillas have probably occurred after the divergence of these species from their common ancestors with the chimpanzee. We are continuing the analysis of different primate species in order to understand better how the VNTR has expanded, and whether it influences circadian and sleep phenotypes in primate species other than humans.

0084
NOCTURNIN IS A DEADENYLASE INVOLVED IN THE POSTTRANSCRIPTIONAL CONTROL OF CIRCADIAN GENE EXPRESSION

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Introduction: 5-10% of the transcripts 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 decay has not been established. We have characterized in mouse the unique ribonuclease reported which expression is circadian regulated, *Nocturnin* (*mNoc*). We showed that recombinant GST-mNOC is a deadenylase, a poly(A)-specific ribonuclease. Deadenylation triggers degradation or translational silencing of mRNAs. We then studied *mNoc* expression in NIH3T3 cells in response to stimuli that synchronize circadian clocks in cultures. *mNoc* was acutely induced by FBS and TPA, whereas forskolin and dexamethasone had no effect. This induction exhibited a typical immediately-early gene response.
Methods: We generated an antibody and found that mNOC has a robust circadian expression pattern in mouse liver and kidney peaking at night. We then studied the subcellular localization and showed that mNOC is present in the cytoplasm and did not co-localize with P-bodies or stress granules, subcellular domains where a number of factors involved in mRNA cytoplasmic processing accumulate.
Results and Conclusion: Polysome analysis showed that mNOC is not associated with ribosomes or polyribosomes. Our data suggest that mNOC is involved in the posttranscriptional regulation of mRNAs whose levels decline at night or in response to stimuli.

0085
DAY-NIGHT VARIATION IN THE AVERSIVE CLASSICAL CONDITIONING AND ZENK PROTEIN EXPRESSION IN THE HIPPOCAMPUS OF PIGEONS (*C. livia*)

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Introduction: Many studies suggest that these behavioral processes are affected by the circadian timing system. The procedures of classical aversive conditioning and analysis of the conditioned freezing response are useful for the study of behavior and the underlying neural mechanisms. Previous studies showed the induction of Zenk expression in the hippocampus of pigeons after training in classical aversive conditioning.
Methods: This study investigated day - night variations in the acquisition and retrieval of aversive classical conditioning and in the expression of Zenk protein in the hippocampus of pigeons. The *Experiment I* evaluated the conditioning to the context in pigeons attributed to conditioned (COND), control (CC) or naive groups (N). The *Experiment II* investigated the aversive conditioning to the tone in groups of pigeons that received tone-shock pairing (PS), unpaired tone and shock (NPS) or tone alone (TS). In both experiments the photoperiod was used with pulses of light (15 min) at ZT00 and ZT12 and the sessions conducted at ZT02 and ZT14. The results of the *Experiment I* indicated between groups differences in the occurrence of freezing ($p<0.05$). The analysis of the Zenk-positive cells in the hippocampus showed a significant increase in the HPV of the COND ZT02 group compared with the other groups ($p<0.05$), indicating a significant time difference when compared to COND ZT14 ($p<0.05$). Higher density of Zenk-positive cells was found in the HpVM of COND ZT02 group compared to HpVL ($p<0.001$) and to HpVM of other groups ($p<0.05$). *Experiment II* data showed higher occurrence of exploratory risk assessment in PS and NPS groups ($p>0.05$) as compared to TS ($P<0.5$). The occurrence of freezing in the PS groups was different from TS groups ($p <0,05$). Higher Zenk-positive cells densities in HpV than in HpD were observed in the animals trained with paired or unpaired tone and shock stimulation ($p <0.05$). These PS and NPS groups showed no significant differences in the density of labeling of Zenk-positive nuclei in HpVL as compared to HpVM ($p>0.05$).
Results and Conclusion: Data from both experiments showed different behavioral patterns during the exposure to the conditioned aversive context and to the conditioned aversive tone. The variations in Zenk expression indicate differential activation of the hippocampal regions during retrieval of aversive memory. The day-night variation in the conditioned freezing to the context as well as in the Zenk expression in the hippocampus suggests that these processes may be modulated by the circadian timing system.
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0086
COMPARISON OF *Callithrix jacchus* PER3 GENE AGAINST OTHER PRIMATES

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Introduction: Period 3 gene (Per3) is lacking of characterization and full annotation in *Callithrix jacchus*, although in other primates it is known (e.g. Human). Mutations in the Period genes have strong effects on circadian rhythms and are associated with human circadian rhythms sleep disorders. And a major effort is given for a Variable Number of Tandem Repeats (VNTR) in exon 18 of hPer3. This VNTR is also associated with the diurnal preference phenotype (morningness/eveningness) and with DSPS. In marmoset the VNTR contains 7 repeats and differs from others primates (e.g. in general others primates can range in 3-5 repeats). However, the role of this Per3 VNTR is unclear. Here, we present the full characterization of Per3 gene which includes definition of exons-introns regions, search for polymorphism, identification and analysis of transposable elements and noncoding RNA, and the comparison of this VNTR with others primates.
Methods: To search for length polymorphisms in VNTR region were analyzed DNA samples of 70 marmosets, 33 capuchin monkeys, one Goeldi´s marmoset. In addition, we are analyzing two red howler monkeys and one wooly monkey. The DNA was extracted from blood. To get the sequence of marmoset´s cDNA Per3, the primers were design based on human, chimpanzee and rat cDNAs. Total RNA was extracted from liver of a marmoset and the cDNA was made from this. After a marmoset Per3 draft contig became available in UCSC genome browser we started used the draft sequence to help with the intronic regions. But, this contig is incomplete and have differences of our exemplar. Now we started to look for SNPs in 50 marmosets by DHPLC technique in exonic regions.
Results and conclusion: The entire cDNA sequence showed 4173 bp and the identity of marmoset cDNA with the human and marmoset of UCSC Per3 was 82% and 92%, respectively. More than 70% of intronic fragments were sequenced. These sequences showed up 81-89% of identity with the related sequence of the hPer3. Phred/Phrap/Consed package has been used to assembly all the gene sequence. RepeatMasker and RNAz were used for identification of TE and ncRNA, respectively. In the VNTR region, only one marmoset presents a length polymorphism (7 and 6 repeat), while the Capuchin monkeys carry two copies and Goeldi, six, but there was no polymorphism's variation in these two species. Which the Per3 sequenced and characterized we will be able to study gene-environmental interactions in an animal model as marmoset.
Support: AFIP, FAPESP (CEPID).

0087
COMPARISONS OF THE VNTR POLYMORPHISM IN THE HUMAN PER3 GENE, DIURNAL PREFERENCES AND SLEEP AMONG WORKING COLLEGE STUDENTS

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Introduction: In humans Per3 VNTR polymorphism consists of 4 or 5 repeats of exonic sequence of 54bp. This work aimed to investigate the associations between sleep variables and 1) VNTR polymorphism in Per3 gene and 2) diurnal preferences score, during workdays and free days.
Methods: Subjects were students engaged in full-time jobs from Monday to Friday and attending evening classes at a public College in São Paulo, Brazil. A hundred students (males n=55, females n=45), from 21 to 26 years old, filled out a questionnaire about sleeping times (during working days and free days) and the Horne-Östberg (HO) questionnaire for diurnal preferences. They also provided a salivary DNA sample. The length polymorphisms in the hPer3 gene were genotyped using polymerase chain reaction. The χ^2 test and two way ANOVA were used to assess proportions and mean differences between VNTR polymorphism in Per3 gene and sleep variables (sleep onset, sleep offset and sleep duration), during work days (Monday to Friday) and free days (Friday to Sunday). The same procedure was performed with the diurnal preference score.
Results: 40.0% students were homozygous 4-repeat (4/4), 51.0% heterozygous (4/5) and 9.0% homozygous 5-repeat allele (5/5). About diurnal preferences score, 19% were considered as evening type, 66% as intermediary type and 5% as morning type. There was no association between Per3 VNTR polymorphism and diurnal preference score (χ^2=3.84, p=.428). During work days there was no significant difference between VNTR polymorphism in Per3 gene and sleep onset (p=.169; p=.717), sleep offset (p=.665; p=.514) and sleep duration (p=.087; p=.852). During free days the sleep onset was later in evening type with genotype 4/5 (03:15h) than morning type of the same genotype (01:15h) ($F=2,163$; $p = 0,009$). Regarding diurnal preferences score, during work days, there was no significant difference on sleep onset

(p=.218), sleep offset (p=.117) and sleep duration (p=.767). During free days, the sleep onset among evening types was delayed in 79.9 minutes compared to morning types and 68.1 minutes compared to indifferent types (p=.008). The sleep offset among evening types was also delayed compared to morning types (107.0 minutes) and indifferent types (79.7 minutes) (p=<.001). There was no significant difference between diurnal preference score and sleep duration during free days (p=.729).
Conclusion: During free days on weekends when there is no social constrains to sleep onset and offset times the different chronotypes express different sleep profiles that are associated in part with Per3 VNTR genotype.
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0088
LIGHT EFFECTS ON MELATONIN RECEPTOR (MEL 1C) AND MELANOPSIN (*OPN4M* AND *OPN4X*) GENE EXPRESSION IN *Xenopus laevis* MELANOPHORE
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Introduction: Many organisms use environmental clues, such as photoperiod length, to regulate physiological processes. Biological rhythms are associated with light-dark cycle (LD), important for all species that have some type of photosensitive pigment. Many ectotermic vertebrates adjust their body color to the background in order to mimic the environment through the migration of pigment granules within skin chromatophores. The pigment translocation may be regulated by light, and by neural and endocrine systems, including melatonin, the pineal gland hormone, which is one of the links between the environment and the organism, since it is always released at night. Studies on the response of *Xenopus laevis* melanophores to light led to the discovery of a new photopigment, melanopsin. Melanopsin is present in the retina of all vertebrate groups, including humans.
Methods: In this work we used quantitative PCR to investigate the expression of the melanopsin genes (*Opn4*m and *Opn4*x) and the melatonin receptor gene (Mel 1C) in melanophores of *Xenopus laevis* submitted to 12 hours of light and 12 hours of darkness (12L:12D) regimen. Cultured melanophores were seeded ($2x10^6$ cells) in $25cm^2$ culture flasks, and kept at 25ºC in 12L:12D for 5 days. Total RNA extraction was performed along 24 hours every four hours starting at ZT0 of the sixth day. The samples were submitted to RT-PCR followed by quantitative PCR for mRNA quantification of melatonin receptor (Mel 1C) and melanopsins (*Opn4m* and *Opn4x*).
Results and conclusion: We demonstrated a tendency to increase the levels of mRNA for the melatonin receptor (Mel 1C) during the escotophase, with greater expression at ZT20. Similar results were seen for the mammalian-like melanopsin (*Opn4m*). Unlike *Opn4m*, there was a peak of expression of *Opn4x* at the onset of light, with subsequent decline during the photophase. In summary, the expressions of all three genes are apparently synchronized by the light-dark cycle.
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0089
mRNA RHYTHMS OF MELANOPSINS AND ENDOTHELIN RECEPTOR IN *Xenopus laevis* MELANOPHORES AND THEIR MODULATION BY LIGHT
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Introduction: The daily rhythm of activity is characteristic of all living organisms, which have the ability to orient in space and time, and can distinguish between linear and cyclic time. The light-dark cycle is very important for species that have some type of photosensitive pigment. Pigment cells of heterothermic vertebrates respond with pigment migration to a variety of agents, including endothelins (ETs). In amphibians, ET-3 promotes pigment granule dispersion in *Xenopus laevis* melanophores. *Xenopus laevis* melanophores also respond to light with melanin granule dispersion, due to the presence of two melanopsins.
Methods: In this work we used quantitative PCR to investigate the expression of the melanopsin genes (*Opn4*m and *Opn4*x) and the endothelin receptor gene in melanophores de *Xenopus laevis* submitted to in 12L:12D regimen. Cultured melanophores of *Xenopus laevis* were seeded ($2x10^6$ cells) in $25cm^2$ culture flasks in 2% fetal bovine serum-supplemented medium, and kept at 25ºC in 12 hours of light and 12 hours of darkness (12L:12D) for 5 days. Total RNA extraction was performed along 24 hours every four hours starting at ZT0 of the sixth day. The samples were submitted to RT-PCR followed by quantitative PCR for mRNA quantification of melanopsins (*Opn4m* and *Opn4x*) and endothelin receptor (ETc).
Results: mRNA quantification showed increased expression of the gene for the non-mammalian melanopsin (*Opn4x*), during the light phase, peaking at ZT0 (lights on). Although not statistically significant, a trend to increase during the dark phase was observed for *Opn4m* mRNA. Under similar conditions there was no temporal variation of ETc mRNA along 24h.
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0090
DIURNAL CLOCK GENE EXPRESSION IN THE OLFACTORY BULB OF NEWBORN RABBITS
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Introduction: In the European rabbit (*Oryctolagus cuniculus*) the once-daily nursing acts as an effective non-photic entraining signal for the newborn circadian system relevant for pups' survival. Rabbits only nurse for a few minutes once every 24 h and previous studies have shown that the pups, blind at birth, display endogenous circadian rhythms in behavior (locomotor activity), physiology (temperature) and molecular (clock gene expression in the suprachiasmatic nucleus) entrained by this regular daily event. Furthermore, the young are able to anticipate the time of nursing, and this persists even during fasting conditions.
Methods: In order to understand the neural organization of the rabbit's early circadian system, we investigated the 24-h pattern of expression of the clock genes *Per1*, *Cry1* and *Bmal1* in the olfactory bulb (OB; a potential circadian oscillator in mammals). We used 43 pups from seven litters maintained in constant darkness and entrained by nursing, allowing to the newborn pups the access to 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-h intervals so as to obtain eight groups (n=5–6 pups/group) distributed evenly across the 24 h before the next scheduled nursing. Profiles in the expression of the clock genes *Per1*, *Cry1* and *Bmal1* were determined using *in situ* hybridization in sections containing the OB.
Results: We report for the first time: 1) that *Per1*, *Cry1* and *Bmal1* are all expressed in the OB of the newborn rabbits, 2) the expression take place mainly in the mitral cell layer, and 3) the expression of the clock genes shows diurnal rhythmicity in the OB.
Conclusion: Our findings suggest that the OB is a potential part of the newborn rabbit's circadian system. Nevertheless, is important to dilucidate if the OB of newborn rabbits displays an endogenous circadian rhythm and if is possible to synchronize it by non-photic cues associated with the mother's daily nursing visit.
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0091
ANALYSIS OF TRANSGENIC DROSOPHILA CARRYING CLOCK GENES FROM THE SANDFLY *Lutzomyia longipalpis* (DIPTERA: P*sychodidae*)
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Introduction: Hematophagous insects such as sandflies and mosquitoes are vectors of many important diseases. However, despite their medical importance little is known about the molecular genetics of their circadian rhythms.
Methods: As part of a molecular and functional analysis of clock genes of blood-sucking insects, we are using P-element-mediated transgenesis to transform *Drosophila melanogaster* with circadian genes from the sandfly *Lutzomyia longipalpis*, vector of American visceral leishmaniasis.
Results: Preliminary results indicate that the expression in *D. melanogaster* of *L. longipalpis timeless* and *cycle* genes alters the pattern of locomotor activity of wildtype flies and we are currently extending this analysis to clock mutants. These results suggest that sandflies and fruitflies might share enough similarities in these genes so that the expression of the *L. longipalpis* circadian genes in *Drosophila* affects its pattern of locomotor activity.
Conclusion: These data support the use of *D. melanogaster* as a host system for *in vivo* studies of *L. longipalpis* clock genes.

0092
INTERNAL CIRCADIAN DESYNCHRONY FOLLOWING 8 H ADVANCE OF THE LIGHT:DARK CYCLE IN PER2::LUC MICE
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Introduction: Circadian rhythms regulate most physiological processes. Adjustments to circadian time, or phase-shifts, are necessary following international travel and on a more frequent basis for individuals who work non-traditional schedules such as rotating shifts. Since the disruption that results from frequent phase-shifts is deleterious to both animals and humans, we sought to better understand the kinetics of resynchronization of the mouse circadian system.
Methods: We have previously examined responses of mice to 6 h advances of the light:dark (LD) cycle as well as following 12 h delay shifts to resynchronize following LD cycle reversal. Here we measured responses to an even more disruptive phase-shift, an 8 h phase advance. Mice bearing a luciferase reporter gene for mPer2 were subjected to an 8h advance of the light cycle, and molecular rhythms in suprachiasmatic nuclei (SCN), thymus, spleen, and esophagus explants were measured periodically for 1 week following the shift. We then subjected some mice to a second 8 h advance and measured rhythms from the same tissues in the subsequent week.
Results: We observed significant internal desynchrony following these LD shifts. While the SCN normally maintains a circa 8 h phase separation from these peripheral tissues, this separation was drastically reduced following subsequent 8 h advances. Four days after the second 8 h advance, the peripheral tissues were widely dispersed in phase.
Conclusion: This protocol provides an approach to disrupt internal phase coherence, allowing better understanding of health effects from internal circadian desynchrony.

0093
***In situ* HYBRIDIZATION OF CLOCK GENES IN BRAINS OF *Aedes aegypti* (DIPTERA: *Culicidae*)**
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Introduction: The components of the circadian clock responsible for generating and sustaining molecular rhythmicity in insects are well characterized in the model system *Drosophila* as well as the neuronal circadian circuitry involved. However, little is known about the intrinsic timekeeping mechanism and the neuronal clusters in other Diptera such as the mosquito *Aedes aegypti*, **vector** of dengue and yellow fever viruses.
Methods: In this study, we are using *in situ* hybridization to localize in the mosquito brain the transcripts of the clock genes *period*, *cycle* and *cryptochrome-2*.

Results and conclusion: Analysis of *in situ* hybridization images using digoxigening-labeled antisense riboprobes in different zeitgeber times, shows expression of these three genes in distinct regions of the brain such as the antennal lobes of deutocerebrum, in the three neuropilar regions of the optic lobes (lobula, lamina and medulla) and several sites of the protocerebrum. In addition, we found preliminary evidence for differential labeling by the three probes.

0094
ALPHA-MSH EFFECTS ON RHODOPSIN AND MC1R GENES IN B16 *Mus musculus* MELANOCYTES

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Introduction: In vertebrates, skin color is given by pigments, synthesized and/or stored in cutaneous pigment cells. The vertebrate color change is mainly regulated by α-MSH. Its action is associated with melanosome dispersion or melanin synthesis, processes which lead to body darkening, whereas melanin aggregation or synthesis inhibition results in skin lightening. Opsins, such as melanopsin and rodopsin, may be expressed in skin pigment cells, besides being present in the retina, and mediate photoresponses such as cell proliferation and melanosome dispersion.
Methods: The aim of this study was to investigate the temporal expression of rhodopsin and of the receptor MC1R, as well as the effects of 10^{-8} M α-MSH for 24 hours on these parameters, in *Mus musculus* B16 melanocytes, kept in 12L:12D. Cells were seeded (10^6 cells per 25cm^2 flasks) in 2% fetal bovine serum-supplemented medium and kept in 12L:12D for 5 days (lights on at 9 AM). In the beginning of the light phase of the 5th day, the cells were treated with 10^{-8}M α-MSH for 24 h. In the beginning of the 6th day, the hormone was removed and total RNA was extracted every 4 h, during 24 h.
Results and conclusion: Using real time PCR (quantitative) we demonstrated that α-MSH does not modulate MC1R mRNA levels, as compared to the control untreated group, although a tendency to reduction was evident. On the other hand, for rhodopsin mRNAm, we observed a statistically significant decrease of the transcript level, as compared to the control group. The temporal expression analysis of those genes revealed that they are not under rhythmic control neither in the hormone-treated group nor in the untreated group, due to the absence of temporal variation along the 24h in each condition.
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0095
RHYTHMS OF PHOSPHATIDYLCHOLINE BIOSYNTHESIS IN SINCHRONIZED CULTURE OF FIBROBLASTS

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Introduction: The circadian system that controls temporally physiology and behavior is composed of central pacemakers located in the brain and a network of peripheral oscillators distributed throughout the body. We have reported that the biosynthesis of phospholipids oscillates daily in synchronized fibroblast cultures under an intrinsic clock control involving the expression of the clock protein mPer1. However, it is unknown how the synthesis of phosphatidylcholine (PC), the most abundant eukaryotic cell lipid, is regulated along the day in cell cultures. The main pathway of PC synthesis in all mammalian nucleated cells is the CDP: choline pathway -via of Kennedy- with CTP: phosphocholine cytidylyltranferase (CCT) as key enzyme. In mammals, there are two genes encoding for CCT: *Pcyt1a* that encodes the CCTβ protein from alternative transcripts termed CCTβ1 and CCTβ2 and *Pcyt1b* gene that encodes the CCTβ2 and CCTβ3 proteins from differentially alternative spliced mRNA CCTβ2 and CCTβ3. CCTβ is predominantly a nuclear protein in most cell types and is also found associated with the Endoplasmatic Reticulum (ER) and Golgi. In contrast, CCTβ is excluded from the nucleus and is primarily localized to the ER compartment and Golgi complex. CCTβ protein is ubiquitously expressed in nucleated cells and is tightly regulated at both transcriptional and post-transcriptional levels. However, CCTβ proteins are most abundant in brain and gonads and little is known about the molecular mechanism involved in the transcriptional regulation of its gene.
Results and conclusion: Here, we show that the synthesis of PC in synchronized cultures of NIH 3T3 cells exhibits a daily variation with higher levels at 6.5 h post serum shock. Similar changes were found in total CCT activity presenting higher levels at 6.5 h and 35 h post stimulation. We found detectable mRNA levels for all the CCT isoforms by RT-PCR in the cultures and a daily variation in some of those mRNA isoforms. Preliminary data show a daily variation in CCTβ protein levels by Western Blot. The results suggest that the biosynthesis of PC in cultured fibroblast display a daily variation that is accompanied by similar changes in CCT enzyme activity as well as in mRNA and protein levels for the isoform β.

G – Mathematic Models

0096
DOMINATING DATA DIVERSITY AND DELUGES: COMPUTATIONAL APPROACHES TO INTEGRATIVE DATA MANAGEMENT AND ANALYSIS IN CHRONOBIOLOGY

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Introduction: Over the last decade, technological advances in data acquisition and storage have significantly increased the diversity and the volume of chronobiological data sets. As a consequence, the appropriate and efficient extraction of knowledge from these data sets often requires the application of an entire system of advanced analysis algorithms. Data-rich research in chronobiology is therefore very likely to increasingly benefit from interdisciplinary collaborations between experimental biologists and scientists with a background in mathematics and computation. In the present work we will summarize the results and the analysis strategies chosen in three recent, international, interdisciplinary collaborations between experimental groups in Brazil studying biological rhythms in animals and humans, and the Systems Biodynamics Group at ITQB-UNL in Portugal.
Methods: All projects featured the simultaneous *in vivo* measurement of a multitude of variables, like wrist or body temperature, or activity levels, for time spans between 3 and 32 days, and sampling rates between 2 h^{-1} and 60 h^{-1}. Analyses were performed by taylor-made cascades of scripts for data pre-processing, analysis and graphical representation, written in the R Language for Statistical Computing. Data storage was based on spreadsheets and data sharing realized by email.
Results: While the featured approach yielded some advantages over the use of stand-alone software packages, it became clear that data and workflow management could still be improved by the deployment of correspondent web-based data pipelines for integrated storage and processing. A current parallel project at ITQB-UNL on data integration in microbial ecology sketches how collaborative computational tools and concepts from the semantic web can improve the scientific workflow in multi-partner projects, foster the integration of data sources from diverse origins, and improve the connection of experimental data with numerical modeling and simulations.
Conclusions: Integrative computational tools for collaborative data analysis, storage, sharing, and integration have a large potential for extracting the maximum knowledge from diverse and voluminous data sets in chronobiology. Notwithstanding this potential, it should be remarked that informatics alone, without the corresponding interpersonal communication among team members, might not be sufficient for the long-term success of interdisciplinary team sciences.

0097
MATHEMATICAL MODEL OF PHOTOENTRAINMENT BASED ON SPATIOTEMPORAL HETEROGENEITY OF THE SUPRACHIASMATIC NUCLEI SUBREGIONS

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Introduction: The coordinated activity of multiple oscillators in the suprachiasmatic nuclei (SCN) regulates mammalian physiology and mediates entrainment of circadian rhythms to daily light-dark cycles. Some studies have shown that long photoperiods reduce the coupling between ventral region (VR) neurons, whereas dorsal region (DR) neurons broaden the duration of their activity. In this work we aimed to describe, by means of mathematical modeling, the spatiotemporal characteristics of the circadian rhythm in the SCN.
Methods: A mathematical model composed of two groups (VR e DR) of coupled oscillators was developed by using different photoperiods. A decreased coupling between the oscillators simulated the effect of light observed experimentally in the VR and in the DR. The oscillators were entrained by the mean field of the VR output. The free running period was set near 24 h.
Results: (i) In constant illumination, an arrhythmicity in global output was observed. (ii) in constant dark the rhythm free ran, (ii) in a 12/12 LD cycle the VR and DR synchronized with different acrophases, (iii) during the course of a 10/10 LD cycle, the VR synchronized to light and the DR free ran, (iv) an increased light period reduced the coupling in the VR increasing the duration of the global activity, (v) the increased light period increased the duration of the oscillators' individual activity in the DR.
Conclusion: This model of spatiotemporal organization of the SCN was efficient to reproduce the main experimental results already described in many studies and can serve as a basis for the construction of new experiments to help unravel the effects of photoperiod in the coupling.
Support: CNPq, FAPERN.

0098
USE OF MICROCONTROLLERS IN AUTOMATION AND CONTROL OF EXPERIMENTAL PROCEDURES IN CHRONOBIOLOGY

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Introduction: This project was developed in the embedded project discipline of electrical engineering course of the Rio Grande do Norte Federal University in partner with the laboratory of chronobiology for the development of a data acquisition circuit for reduce costs with the use of a specific purpose processor while other solutions using general purpose processors that are more expensive, it was developed in 18F4550 PIC microcontroller of the MICROCHIP™ company.
Methods: The embedded program in the microcontroller was developed in C and ASSEMBLY code, and the control program installed on the personal computer to send data to controller can

to be development in JAVA, C# or LabVIEW® code.
Results: The project contains two parts. In the first part, the embedded system would have to do a light control and timing ON/OFF of the time ranging between one second and some days, adjusted by user using the control program, with registered output. Other feature of the embedded system is that would have to send a log if the lamp doesn't light and identify the status of the lamp if ON or OFF, also, would have to do the control of the light turn on, and turn off, independent between them. This definition of the time is user defined using a control program that send data for the circuit. In the project, the number of the lamps controlled by software was fifteen, because each lamp was adjusted to four cages, thus, was possible to do the control of the light of sixty cages. The other part of the project was the movement registered with multiple sensors, where the sensors would have to do the register for each two millisecond. The number of cages was the maximum possible to the hardware of the PIC, and the counting of the movement in the cages would have to be limited for a time defined by user by software. All these data will be sent the PIC to the personal computer and the personal computer to the PIC using the USB communication.
Conclusion: we get more capacity and lower cost with the use of the 18F4550 PIC microcontroller because this device is cheaper that general purpose device and the embedded system developed is more efficient for data acquisition.

0099
USE OF MICROCONTROLLER AND CONTROL CIRCUIT IN AN UPGRADE OF DATA ACQUISITION SYSTEM DEVELOPED IN LABVIEW™ THAT MONITORS THE ACTIVITY OF ANIMALS (*Rattus novergicus* AND *Callithrix jacchus*) BY MOTION SENSORS
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Introduction: The programmable, application-specific embedded systems appear to be an attractive solution in terms of easy of programming, design cost, power, portability and time-to-market. Currently, biological data processing relies on the high-end systems equipped with expensive, general propose processors.
Methods: The future generation of bioinformatics requires the more flexible and cost-effective computing platforms to meet its rapidly growing market. The proposed study was the result of a project of discipline Special Topics in Microcontrollers Course of Electrical Engineering, Federal University of Rio Grande do Norte with the Laboratory of Chronobiology (LabCrono) of that university. This was an upgrade to the system developed in LabVIEW™ that monitors the activity of animals (*Rattus* novergicus and *Callithrix jacchus*) by motion sensors.
Results: The system was in use did not have an automated control both the timing of lights on the drive. This was done by timers in the rooms of the experiment and always had to be reprogrammed when the lack of energy. The upgrade has enabled improvements in the routines of the system than the previous addition of new routines interacting with the LabVIEW™ drive and control circuit of the lamp (DCCL) composed of a microcontroller (PIC16F877A - Microchip™). As a final result of the system improved, there are signs of the sensors from the cages of animals being received by terminal National Instruments™ BC50 leading to the plate of a NI PCI6025 Desktop. The LabVIEW™ controls the signals received by said plate and in accordance with definitions parameterized by researchers write data to a file in ASCII format. These data will be available for the generation of actogramas to be used in evaluating the results of different research projects. In parallel, the DCCL also monitors the status of lamps and inform the state of them in the monitoring system.
Conclusion: We conclude therefore that the use of microcontrollers is useful in troubleshooting of automation and control of experimental procedures in chronobiology to facilitate researchers in the field, more efficient control of experimental procedures.
Support: CNPq.

H – Circadian Timing System

0100
5-HT$_{1A}$ AND 5-HT$_{1B}$ RECEPTORS IN THE SUPRACHIASMATIC NUCLEUS OF THE COMMON MARMOSET (*Callithrix jacchus*)
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Introduction: The core nucleus of the mammalian circadian timing system (CTS) is the hypothalamic suprachiasmatic nucleus (SCN) which receives a strong serotonergic projection from the raphe. Serotonin is involved in the fine adjustments at several brain centers and in the SCN these actions are reported as been primarily mediated by the receptor type 5-HT$_1$. In rodents, there is a consensus that the general 5-HT projection pattern arrives at the ventral portion of the SCN, mismatching the arriving fibers of the retinohypothalamic tract. Our objective was to locate of 5-HT$_{1A}$ and 5-HT$_{1B}$ within the SCN, and comparing their location with the retinal and serotonergic projection in the marmoset SCN.
Methods: Four marmosets were deeply anesthetized to receive a unilateral intraocular injection of CTb. After 5-7 post-injection days, the marmosets were reanesthetized and perfused transcardially with phosphate-buffered saline, followed by paraformaldehyde. The brains were removed from the skull and postfixed for 2-4 h. Brain blocks containing the SCN were frozen and sectioned in the coronal plane (30 µm). Sections were sequentially collected into six separated compartments. To each animal, one in each six series was used to reveal immunoreactivity for CTb, and three others were used for the immunohistochemical detection of the 5-HT, 5-HT$_{1A}$, and 5-HT$_{1B}$. The two remaining series were employed to perform double labeling experiments combining serotonin receptors label and calbindin (CB) label. The sections were processed immunohistochemically against CTb, 5-HT$_{1A}$, 5-HT$_{1B}$. For the double labeling, the tissue was simultaneously incubated with an antibody against one of the two 5-HT subtype receptors joint with mouse-anti-CB. The sections were examined under brightfield illumination and epifluorescence.
Results: In a New World primate species, *Callithrix jacchus*, the occurrence of the positive labeling for the subtypes 5-HT$_{1A}$ and 5-HT$_{1B}$ are mostly at the dorsal SCN. We also confirm the dualistic nature of the placement of the 5-HT-positive fibers in the SCN of *Callithrix*, with higher density in their dorsal and ventral contours, with a core devoid of any labeling.
Conclusion: We believe that such display is highly suggestive that the exclusively dorsal distribution of these SCN arriving projections observed in this species holds a distinct serotonergic characteristic, performed by the 5-HT$_1$ family subtype receptors. These data confirm that *Callithrix jacchus* is an important experimental subject in the study of the serotonergic circuitry involved in the generation and modulation of circadian rhythms.
Support: CNPq, CAPES, FINEP, PROPESQ-UFRN.

0101
THE SUPRACHIASMATIC NUCLEUS AND THE INTERGENICULATE LEAFLET IN THE ROCK CAVY (*Kerodon rupestris*): RETINAL PROJECTIONS
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Introduction: A wide variety of behavioural and physiological processes show circadian rhythms which are generated by a time-keeping system, also called circadian timing system. This system in mammals is built by two principal brain centers: a known central pacemaker, the hypothalamic suprachiasmatic nucleus (SCN), and a secondary component, the thalamic intergeniculate leaflet (IGL). The aim of this work was to characterize the retinal projections in the SCN and the IGL in a Brazilian rodent species, the rock cavy (*Kerodon rupestris*).
Methods: Six animals were deeply anesthetized and subjected to unilateral intraocular injections of cholera toxin subunit B (CTb). Following a 5 to 7 days survival time, the animals were again anesthetized and perfused transcardially with buffered saline and 4% paraformaldehyde. Brains were coronally and sagittally sectioned at 30µm on a freezing microtome. Sections through the SCN and the IGL were submitted to immunohistochemistry (avidin-biotin-peroxidase protocol) to reveal the anterogradely transported CTb.
Results: In coronal sections from animals intraocularly injected with CTb, both SCNs, with a contralateral predominance, were filled with CTb-immunoreactive (IR) terminals, forming a dense plexus in the ventral region, denser in the ventrolateral portion and spreading sparsely to the dorsal portion. Retinal afferents to the IGL were shown by anterograde transport of intraocularly injected CTb. CTb-IR terminals were observed in the IGL and the entire lateral geniculate complex of the rock cavy with a strong predominance on the side contralateral to the injected eye. The sections in the sagittal plane allowed seen whole extension of the retinal projections inside the studied nuclei.
Conclusion: These results show that the SCN and IGL of *Kerodon rupestris* display similarities in the pattern of distribution of retinal projections profile in relation to other studied species.
Support: CNPq, CAPES, PROPESQ-UFRN-Brazil.

0102
PRESENCE OF NeuN IN THE INTERGENICULATE LEAFLET AND SUPRACHIASMATIC NUCLEUS: A COMPARISON BETWEEN THREE RODENTS SPECIES
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Introduction: The circadian timing system (CTS) contains as major centers the hypothalamic suprachiasmatic nucleus (SCN) and the thalamic intergeniculate leaflet (IGL). Both play a pivotal role in generation and modulation of circadian rhythms. The SCN receives environmental inputs, such the light-dark information, through direct retinal projections. The IGL acts as a non-photic mediator and center of a second photic entrainment pathway of the SCN. The aim of this work was to verify, by using immunohistochemical techniques, the NeunN distribution within SCN and IGL, comparing three rodents species: Wistar rat (*Ratus norvegicus*), mice (*Mus musculus*) and rock cavy (*Kerodon rupestris*). The last one is a rodent originating from Brazil northeast, wich one shows a activity pattern predominant crepuscular.
Methods: The animals were previously anesthetized and perfused transcardially with saline containing heparine, following by 4% paraformaldehyde solution in phosphate buffer 0,1M, pH 7,4. The brains were removed, frozen and sectioned on a sliding microtome in coronal plane (30 µm). The sections were collected in six series. Then, sections of a series were submitted to Nissl method of staining (Thionin) for cytoarchitectonic delimitation. Another series were processed immunohistochemically (ABC protocol) to reveal the presence of NeuN.
Results: Comparing the species, we can say that exist a scarce NeuN labeling in the SCN of mice and rock cavy, in wich we founded a predominant labeling in the ventral part. In the rat we found the strongest labeling comparing to other species studied, although also in the ventral portion of the SCN. About IGL, was observed a evident cellular labeling in the three species studied.
Conclusion: Considering the CTS in the three studied rodent species, the NeuN-immunoreactivity is a good marker for all IGL, but only for the ventral SCN.
Support: CNPq, CAPES, FINEP, PROPESQ-UFRN.

0103

SEROTONIN AND THE CIRCADIAN TIMING SYSTEM IN THE ROCK CAVY (*Kerodon rupestris*)

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Introduction: The biological rhythms are expressed in all organic structures even in the absence of external stimuli. In mammals the circadian cycle is governed by a central pacemaker, which has been pointed out in the course of decades of research as being the suprachiasmatic nucleus (SCN) of the hypothalamus. The SCN receives photic information from the retina by the retinohypothalamic tract, and non-photic inputs from other pathways, to adjust the behavior to the light-dark cycle. The thalamic intergeniculate leaflet (IGL) originates one of these pathways that acts on the SCN modulating its function. Both SCN and IGL, as detected in several mammal species, are known to be influenced by pathways originated in the midbrain raphe, which utilizes serotonin (5-HT) as neurotransmitter. The objective of this study was to identify the serotonergic groups in the brain stem and the distribution of serotonergic terminals on the SCN and the IGL of the rock cavy (*Kerodon rupetris*), a rodent native of the northeastern Brazil, whose locomotor activity is mostly crepuscular.
Methods: Two animals were used, which after pre-anesthesia with atropine sulfate and tramadol, were anesthetized with a mixture of ketamine, xylazine and diazepan and followed by transcardiac perfusion. After that, the fixed brains were removed from the skull to be sectioned one in sagittal and the other in coronal sections on sliding freezing microtome. The sections were collected in six compartments, one of the which was stained by Nissl method with thionin and another was submitted to 5-HT immunohistochemistry. 5-HT-IR perykaria were identified forming several cluster cells along the midline of the brainstem, recognized as raphe nuclei. A dense 5-HT-IR terminal plexus distribution of 5-HT terminals was seen in the ventral portion of the SCN along its entire rostrocaudal extent and filling all IGL.
Conclusion: Because 5-HT is a neurotransmitter present in large quantity in the raphe nuclei, it is believed that the SCN and the IGL receive 5-HT modulatory influence from those nuclei. However additional studies with neuronal tracers are need to demonstrate such connections, as well as to establish the source of the projection from raphe nuclei to SCN and IGL.
Support: CNPq, CAPES, FINEP, PROPESQ-UFRN.

0104

EFFECT OF THE CHRONIC DEFICIENCY OF POLYUNSATURATED FATTY ACIDS ON SYNCHRONIZATION OF THE CIRCADIAN RHYTHMS IN WISTAR RATS

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Introduction: A wide variety of behavioural and physiological processes display circadian rhythms which are generated by a time-keeping system, called circadian timing system. Two principal brain centers compose this system in mammals: a known central pacemaker, the hypothalamic suprachiasmatic nucleus (SCN), and a secondary component, the thalamic intergeniculate leaflet (IGL). The lipids are essential components to maintain the body metabolic balance. In addition, we know that polyunsatured fatty acids (PUFA) are essential for the development of the central nervous system and its lack in the diet, especially omega-3 and omega-6, important components of cellular membrane, allows changes in structure and functioning of the brain. The aim of this work was to evaluate the effects of a PUFA deficient diet on the expression of the circadian rhythms in rats Wistar.
Methods: A group of animals was submitted to a PUFA lacking diet since gestational period until adulthood (experimental group) and another group to a normal diet (control group). Both groups were first exposed to a LD 12h-light (150 Lux)-12h-dark (1 Lux) cycle. After 2 weeks in this regimen, the time of lights-on was delayed by 6 hours; 3 weeks later, it was advanced by 6 hours, and 4 weeks later, the animal were placed in constant dark conditions. After 4 weeks the animals were then returned to LD 12:12 for 3 more weeks and then again to constant darkness for 4 weeks. The locomotor activity of the animals was measured using an infrared motion sensor above the cage. Output from the sensors was integrated with an computer running data acquisition software. Analysis of thythm characteristics and graphical output, actograms, were undertaken using the El Temps computer program (Diez-Noguera, Barcelon, Spain).]
Results: The analysis of the actograms shows that the experimental animals spend more time to attain the synchronization (mean 10,3 cycles) compared to control group animals (mean 5,3 cycles).
Conclusion: These results allow us to conclude that a PUFA lacking diet affects the circadian rhythms regulation.
Support: CNPq, CAPES, FINEP, FAPEAL-AL, PROPESQ-UFRN.

0105

EFFECT OF THE CHRONIC DEFICIENCY OF POLYUNSATURATED FATTY ACIDS ON GFAP IMMUNOREACTIVITY IN THE THALAMIC INTERGENICULATE LEAFLET OF WISTAR RATS

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Introduction: Determinative factors for the development of the nervous system, particularly during the gestation and lactation periods, promote significant changes in neuronal phenomena and affect its functioning. Animals submitted to deficient essential polyunsatured fatty acids (PUFA) diet show morphological and neurochemical alterations in cerebral regions involved with cognition and memory. In this study, we analyze whether the chronic deficiency of PUFA, as the acid linoleic (or omega-6) and the acid α-linolenic (or omega-3) influence the expression of glial fibrillary acidic protein (GFAP) in the thalamic intergeniculate leaflet (IGL) of Wistar rats. The IGL is a known circadian center involved in the modulation of the suprachiasmatic nucleus, considered to be the main circadian pacemaker in mammals.
Methods: A group of animals was submitted to a poor omega-3 and omega-6 diet (experimental group) since the gestation and lactation until the adult phase and another group to a normal diet (control group). After this period the rats were perfused transcardially using 4% paraformaldehyde in a 0.1 M phosphate buffer. The brains were removed, frozen on dry ice and then cut in coronal sections with thickness of 30 µm, being submitted to the immunohistochemical reaction for GFAP.
Results: The results showed that, in the animals submitted to the lack of essential fatty acids, the corresponding region to the IGL evidenced astrocytes presenting immunoreactive reduction of the expression of GFAP in relation to the control animals. The quantification of the GFAP-immunoreactivity was carried out by measuring of the optic density and the percentage of pixels in binarized images of the region in study for posterior statistic analysis of the data.
Conclusion: These results suggest that the expression of the GFAP in the IGL can be influenced by neurochemical processes involving PUFA in some phase of the animal, especially during the development and maintenance of the neuronal phenomena in determinative phases for neurogenesis and gliogenesis, differentiation and maintenance of the neural tissue.
Support: CNPq, CAPES, FINEP, FAPEAL-AL, PROPESQ-UFRN.

0106

THE SUPRACHIASMATIC NUCLEUS AND THE INTERGENICULATE LEAFLET IN THE ROCK CAVY (*Kerodon rupestris*): NEUROCHEMICAL ORGANIZATION

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Introduction: The circadian timing system is constituted by two principal brain centers: a known central pacemaker, the hypothalamic suprachiasmatic nucleus (SCN), and a secondary component, the thalamic intergeniculate leaflet (IGL). The aim of this study was to identify and characterize the circadian system of the rock cavy using immunohistochemical techniques, in order to establish a regional rodent model for circadian research.
Methods: The SCN and the IGL were evaluated as the presence of neuroactive substances and neuronal or glial markers. Six animals were deeply anesthetized and perfused transcardially with buffered saline and 4% paraformaldehyde. Brains were coronally sectioned at 30µm on a freezing microtome. Sections through the SCN and the IGL were submitted to immunohistochemistry (avidin-biotin-peroxidase protocol) to reveal some endogenous substances, such as vasoactive intestinal polypeptide (VIP), vasopressin (VP), neuropeptide Y (NPY), serotonin (5-HT), enkephalin (ENK) and the astrocyte marker glial fibrillary acidic protein (GFAP).
Results: VP-IR neurons were observed in the SCN, predominantly concentrated in the dorsal portion only at rostral levels. Some VIP-IR neurons immersed in a dense neuropil were found in a ventromedial position in the SCN. The SCN also displays NPY- 5-HT- and ENK-terminals. GFAP expression is also detected in the SCN, although without great contrast with surrounding areas. The rock cavy IGL is marked by NPY-neurons and ENK-terminals. The IGL is also marked by intense expression of GFAP.
Conclusion: These results show that the SCN and IGL of *Kerodon rupestris* display similarities in the pattern of distribution of retinal projections and neurochemical profile in relation to other studied species.
Support: CNPq, CAPES, FINEP, PROPESQ-UFRN.

0107

PRESENCE OF PROTEIN NUCLEAR SPECIFIC NEURONAL (NeuN) IN CIRCADIAN TIMMING SYSTEM OF CAPUCHIN MONKEY (*Cebus apella*)

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Introduction: The circadian timing system (CTS) it is composed by a group of neural structures specialized that establish a temporal organization of the physiologic processes and behaviors. Their central components are the suprachiasmatic nucleus (SCN) of hypothalamus and the intergeniculate leaflet (IGL) of thalamus. The objective of this work was to verify, through the immunohistochemical technique, the presence of the protein nuclear specific neuronal (NeuN) in CTS of the capuchin monkey (*Cebus apella*), a primate of the New World.
Methods: The animals were previously anesthetized and perfused transcardially with saline containing heparine, followed by 4% paraformaldehyde solution in phosphate buffer 0,1M, pH 7,4. The brains were removed, frozen and sectioned on a sliding

microtome in coronal plane (30 µm). The sections were collected in six series. Then, sections of a series were submmited to Nissl method of staining (Thionin) for cytoarchitectonic delimitation. Another series were processed immunohistochemically (ABC protocol) to reveal the presence of NeuN.
Results: NeuN positives neurons were observed in the central structures of CTS of the Capuchin Monkey, SCN and Pre-Geniculate nuclei (PGN). The PGN is the equivalent structure to IGL of the rodents.
Conclusion: The neurons that compose the central structures of CTS in this primate species, express the protein NeuN.
Support: CNPq, CAPES, PROPESQ-UFRN.

0108
CHARACTERIZATION OF CIRCADIAN TIMING SYSTEM OF THE COMMON MARMOSET (*Callithrix jacchus*) BY NeuN AND GFAP
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Introduction: The circadian timing system (CTS) is composed by a set of structures that shows endogenous oscillation, responsible for the maintenance of some biological rhythms, despite of constant environmental conditions. The CTS of primates is comprised by the suprachiasmatic nucleus (SCN) of the hypothalamus, which is the central pacemaker, and the pregeniculate nucleus (PGN) of the thalamus, a component homologous to the intergeniculate leaflet of rodents, that acts as a second photic pathway entrainment to the SCN and also participate of non-photic synchronization mechanisms. NeuN is a nuclear neuron specific marker, widely employed to distinguish neurons and glia cells. The glia cells are identified by glial fibrilary acidic protein (GFAP). In this way, our objective was to charaterize some components of CTS of the common marmoset (*Callithrix jacchus*), a New World primate, utilizing NeuN and GFAP immunoreactivity.
Methods: Adult male marmosets were previously anesthetized and them submitted to transcardiac perfusion. Then, the brains were removed from the skull, frozen and sectioned on a sliding microtome in coronal sections of 30 µm. The sections were processed immunohistochemically against NeuN and GFAP. Afterwards the sections were mounted on glass slides and observeds by optic microscopy.
Results: We observed that the PGN was densely marked by NeuN and GFAP immunorreactivity, delimiting this region. On the other hand in the SCN, NeuN was strongly founded in ventral portion, with a pale immunoreactivity in the dorsal part. In this region the GFAP immunoreactivity was inverse to the NeuN.
Conclusion: So we conclude that PGN, possibly, have a similar number of neurons and glia cells, and both proteins can be used to delimit this region. The SCN can be divided in two portions through the analysis of NeuN and GFAP.
Support: CNPq, CAPES, FINEP, PROPESQ-UFRN.

0109
CYTOARCHITECTURAL AND NEUROCHEMICAL CHARACTERIZATION OF THE PARAVENTRICULAR NUCLEUS OF THE THALAMUS: A COMPARATIVE STUDY BETWEEN THE ROCK CAVY (*Kerodon rupestris*) AND THE PRIMATE COMMON MARMOSET (*Callithrix jacchus*)
Sousa TB, Duarte RB, Nascimento Jr ES, Silva SF, Freitas LM, Soares JG, Engelberth RCGJ, Cavalcante JS, Cavalcante JC, Costa MSMO.*
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Introduction: The paraventricular nucleus of the thalamus (PVT) is the most dorsal and widest component of the midline thalamic group. It plays a role in the integration of multiple sensorymotor, somatovisceral and motivational-emotional systems, besides to be involved in the circadian timing system regulation. The PVT, particularly its anterior portion, is the largest extrahypothalamic target of the efferent connections of the suprachiasmatic nucleus (SCN). Besides the SCN, the PVT also receives afferent terminals from the thalamic intergeniculate leaflet, another circadian center related to synchronization of the circadian rhythms. By considering these connections, the PVT is usually conceived as an output pathway of the circadian timing system. The PVT is derived from the epithalamus together with the pineal gland, responsible by melatonin production, which is essential to control of seasonal behaviors and activity-rest phase signal.
Methods: The aim of this work was to describe the cytoarchitecture and neurochemical profile of the PVT of two native species of the Brazilian Northeast region, the rodent rock cavy (*Kerodon rupestris*), and the primate common marmoset (*Callithrix jacchus*). The pattern of the retinal projections to the midline thalamus of both species was also compared. Cytoarchitectonic and immunohistochemical techniques and the cholera toxin subunit B (CTb) as an anterograde tracer were used in this study.
Results: Enkephalin (ENK)-, neuropeptide Y (NPY)-, and parvalbumin (PV)-immunoreactive fibers and terminals and calbindin (CB)- and calretinin (CR)-immunoreactive perikarya were detected in the rock cavy PVT. ENK-, NPY- and CB-immunoreactive fibers and terminals and CR-immunoreactive perikarya, fibers and terminals were observed in the common marmoset PVT, in which no PV-immunoreactivity was detected. CTb-immunoreactive fibers and terminals, indicating a retinal input, were observed in the caudal rock cavy PVT. In the common marmoset, besides the PVT, CTb-labeled terminals extend to other midline thalamic nuclei, such as rhomboid, interanteromedial, and reuniens.
Conclusion: This work allows to establish a comparative description between two typical Brazilian species, defining the cytoarchitectonic and neurochemical profile of the PVT and the pattern of the retinal connection with the midline thalamic nuclei.
Support: CNPq, CAPES, FINEP, PROPESQ-UFRN.

0110
THE EFFECTS OF CAFFEINE ON MEMORY IN COMMON MARMOSET (*Callithrix jacchus*)
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Introduction: We evaluated the effects of caffeine on memory in *Callithrix jacchus*, through the conditioned place preference task, and locomotor activity.
Methods: We used 10 females and 10 males, between 3 and 9 years old, from Primate Center of UFRN (IBAMA no 1/24/92/0039-0). This experiment comprised five phases: (1) Pre-exposure – 2 days; (2) Training, in which they were conditioned to discriminate between a non-rewarded context (NRC) and a rewarded context (RC) for 8 days; (3) Oral administration of caffeine or placebo (10 mg/kg - both substances) for 8 days, approximately 1,5 h before sleep, when the marmosets were distributed in groups: placebo (PL), acute (AC), repeated (RP), abstinence (AB); (4) One day of retraining, followed by administration of caffeine or placebo; (5) Test, without food. Each session had duration of 8 minutes, and began at 6:15 am for pre-exposure, training and test. The retraining began at 3:15 pm, because it should be a sleep-dependent task. For behavioral data, frequency of contacts, latency (seconds) and percent time spent in RC and NRC were registered. For locomotion, we registered the general activity, and locomotion in contact with apparatus and outside of it.
Results: In training, the animals enter and stay more in RC versus NRC (percent time, frequency and latency; Wilcoxon test, p<0,001), and moved more in contact with apparatus than outside of it (test *t*, p<0,001). On test, marmosets under influence of caffeine percurred more quadrants than placebo group (*PL:* 21,8 ± 0,9; *AC:* 30,2 ± 1,3; *RP:* 30,4 ± 1,3; AB: 32,4 ± 3,6). Moreover, the repeated and abstinence groups had a bad performance in task in relation to placebo and acute groups (percent time RC- PL: 53,4 ± 9,9%; AC: 78,3 ± 4,9%; RP: 10,3 ± 2,8%; AB: 20 ± 8,9%. Frequency RC- PL: 1,8 ± 0,3; AC: 2,2 ± 0,3; RP: 0,6 ± 0,2; AB: 0,8 ± 0,3. Latency RC- PL: 211,8 ± 49 s; AC: 141,4 ± 21 s; RP: 362,8 ± 38,3 s; AB: 396 ± 40,2 s). Additionally, the marmosets that ingested caffeine presented an increase in locomotor activity when compared with the placebo group.
Conclusions: We suggest that prolonged administration of caffeine near to sleep, which is responsible for offline processing of memories, disrupts the memory for this specific task to marmosets in caffeine abstinence and taking repeated doses. Additionally, the results corroborate works that show caffeine increase the locomotion.
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0111
PROLACTIN, A POTENTIAL REGULATOR OF THE REM SLEEP RESPONSE TO STRESS
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Introduction: The present study was undertaken to investigate the link between hormonal stress response mechanisms and the changes in sleep associated with psychogenic stressors. Both REM sleep and prolactin levels are increased in rodents following restraint stress. This suggests prolactin may be involved in the ability of restraint stress to increase REM sleep amount.
Methods: In the current study, we examined sleep following restraint stress in cabergoline treated mice. Cabergoline is a dopamine D2 agonist that is in clinical use as a prolactin release inhibitor. Male C57BL/6J mice (3-4 months of age) were implanted with EEG/EMG recording electrodes and placed in 12:12 LD. After acclimation to the sleep recording apparatus, mice received an i.p. injection of either 0.25 mg/kg cabergoline or vehicle prior to 1-hr of restraint stress during the light phase (ZT 5-6) and allowed an 18-hr recovery opportunity. Polysomnograms were hand scored for REM sleep and data were analyzed using ANOVA to detect between and within factor differences. Restraint stress elicited a significantly smaller increase in REM sleep amount when animals were pretreated with cabergoline.
Results: REM sleep amount in restrained mice pretreated with cabergoline (44.0 min) was less than ($p < .05$;dark phase ZT 12-24) restrained mice pretreated with vehicle (72.3 min). Cabergoline also decreased REM sleep amount during the light phase and early part of the dark phase in non-restrained animals. This sleep loss resulted in a REM sleep rebound in the latter half of the dark phase. Our data demonstrate that the inhibition of prolactin secretion reduces the REM sleep amount increase induced by restraint. Our results also indicate that baseline REM sleep amount during the rest phase is partially dependent on prolactin This is inferred from the ability of cabergoline to reduce light phase REM sleep in non-restrained animals.
Conclusion: Collectively, these data demonstrate the importance of prolactin as a sleep regulator, and as a mediator of the ability of stress to alter REM sleep. In addition, prolactin levels exhibit a sexual dimorphism in which adult females have higher basal prolactin levels. Thus these data also support the hypothesis that sex differences in the ability of restraint stress to alter sleep are dependent on prolactin.
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0112
PSYCHOCHRONONEUROIMMUNO TRANSDUCTIONOLOGY: ENDOTOXIN AND CYTOKINE EFFECTS ON CIRCADIAN BEHAVIOR
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Introduction: Diverse lines of research have linked the circadian system to immune function, from daily regulation of immune cells to the effect of immune challenges on the output of circadian rhythms. This bidirectional interaction between both systems has gone under intensive study in recent years and is proving to be an interesting field of clinical research, Moreover, it is becoming increasingly important as circadian disturbances are being related to cancer processes, as risk factors or even as prognosis markers. According to previous research from our lab and other reports, the circadian rhythm of activity can be affected by immune acute or chronic stimuli, which can act directly on the SCN in mammals. In this direction, we have performed several experiments to assess what kind of molecules can result in circadian and whether these effects are SCN mediated and how.
Results: We had previously reported that LPS injection at CT15 induce photic-like phase delays in wheel-running rhythms, and we now show that such responses are partially inhibited in TLR4 (toll-like receptor 4) null mice. Also, LPS- induced reduction of activity (masking) is impaired in mutant mice. LPS effects appear to be mediated by central proinflammatory cytokine activity. Reduction of activity has also been reported in hamsters after chronic infusion of TGF-α via an osmotic pump. We tried to reproduce these results in mice, and we obtained a severe fall in daily activity in both control and treated groups, but with a stronger effect in TGF-α treated animals. However, we found no significant changes in per-luc expression in SCN transgenic slices when treating with TGF-α or other cytokines. Since we have previously reported that SCN glia is a immune-circadian interface, we also analyzed SCN transfected astrocytes in culture and found a significant reduction of per1-luc expression after TNF-α treatment.
Conclusion: Here we show that endotoxin and cytokine-mediated circadian effects are the result of a complex network of pathways which in some cases affect clock gene expression and might be relevant for specific patophysiological mechanisms.

0113
IMMUNOMODULATION BY CLASSICAL CONDITIONING IN NZB/W (F1) MICE: LIFESPAN AND CIRCADIAN PHASE MODULATION

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Introduction: Systemic Lupus Eritematosus (SLE) is a systemic inflammatory disease, chronic, recurring and potentially fatal, being considered as an auto-immunological syndrome. The therapeutic basis for treating patients with SLE is the agent cyclophosphamide (CY), known to produce adverse reactions to the organism. Previously, Ader and Cohen have demonstrated an alternative way of administrating this immunossupressor agent, based on pavlovian classical conditioning, in order to reduce the aggression caused by CY. Considering that every organism on earth shows a temporal organization as well as the influence of this organization on learning and memory processes, the purpose of this study is to provide elements for the understanding of the temporal aspects involved in immunomodulation. The primary objective is the detection of a different pattern of response in a conditioned immunomodulation paradigm conducted in two different phases of the light-dark cycle.
Methods: NZB/W (F1) mice were selected, a strain with high predisposition for the spontaneous development of SLE. Divided into two groups, the animals were submitted, in different phases of day, to a classical conditioning immunomodulation protocol, consisted in weekly parings of saccharin solution and CY injections. The success of the paradigm was evaluated by comparing the progression of proteinuria and the lifespan among the groups. Simultaneously, water intake behavior was recorded, in order to evaluate two rhythmic parameters, amplitude and spectral power density of the 24-hour rhythm, with the progression of SLE.
Results: Comparing the development of proteinuria and lifespan of animals of the experimental to the control groups, it was possible to find significant differences. The comparison between the experimental groups revealed that the lifespan of the animals submitted to the CY during the light phase was greater than the dark phase animals. Concerning the rhythmic parameters, there was evidence of association between the stability of the signal from the timing systems and the evolution of SLE, demonstrated by the maintenance of higher levels of amplitude and spectral power density of the 24 hour rhythm in animals exposed to the conditioning paradigm.
Conclusion: In this animal model of SLE, the classical conditioning of immunossupresion is sensitive to the phase of the light-dark cycle. Moreover, the lack of stability of the circadian system seems to be important to the progression of the disease.
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0114
PSYCHOGENIC OR PHYSICAL STRESS PRODUCE A SIMILAR MANNER OF MODULATION ON THE PINEAL GLAND IN RATS

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Introduction: We have previously shown that perfusion of pineal glands with concentration of corticosterone, which mimics activation of the hypothalamic-pituitary-adrenal (HPA) axis, leads to an increase in nocturnal melatonin surge. In cultured pineal glands we observed a dual effect of corticosterone, it inhibits melatonin production when both alfa and beta adrenoceptors are stimulated, while it potentiates melatonin output when only beta-adrenoceptors are activated. Glucocorticoid receptors and inhibition of the nuclear transcription factor kappa B (NFKB) mediate glucocorticoid effect in the pineal gland. Here, our aim was to test whether heightened corticosterone by restraint (psychogenic stress) or cold exposure (physical stress) could change nocturnal melatonin surge in rats.
Methods: Male Wistar rats (12:12 L/D cycle, aged 3-4 months). Pineal content of N-acetylserotonin (NAS) and melatonin (MEL) from rat killed at ZT 18-20 were quantified by HPLC (ng/pineal) into 3 groups (5/group): control with individually housed in cage (CTRL), restraint in an apparatus (REST), and cold in a refrigerator (5.0 ± 1.0 °C) (COLD), for 0.5 or 2h. Plasma level of corticosterone (ng/mL) and TNF (pg/mL) were determined into 3 groups: naïve, non-experimentally manipulated euthanized at ZT11 (N Day) or at ZT14 (N Night), restraint and cold for 2h euthanized at 0, 10, 30, 60 and 120 min after stress. In both studies, gastric ulcers were determined after euthanasia. The regression of the data was tested by two-way ANOVA.
Results: REST and COLD did not induced gastric ulcer neither sudoresis. Pineal NAS did not modify in manipulated groups in relation to control groups. Pineal MEL doubled from REST 0.5 (1.7±0.2) to REST 2.0 (3.3±0.6), and from COLD 0.5 (1.9±0.5) to COLD 2.0 (3.4±0.8), while no regression on time was detected for CTRL animals. No level of TNF was observed, while corticosterone increased more than 2-fold just after 2h of restraint or cold stress, returning naïve values in 30 min (148.8±32.5) following an exponential curve.
Conclusion: Our data clearly show that the increase in corticosterone, due to activation of the HPA axis, leads to an increase in nocturnal melatonin, without changing the production of NAS. Taking into account that we observed no gastric ulcer or other sign of exacerbated sympathetic activation, our data corroborates to the hypothesis that under a normal sympathetic output, enhancement of corticosterone promotes an increase nocturnal melatonin surge in rats.
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