Conference Office

The Conference Office is located at 101 Collis, Dartmouth College, Hanover, New Hampshire 03755-3552, 603-646-2485 (ask for Clocks Conference Registration Desk).

On-Site Registration

The scientific registration fee includes entrance to the symposia and poster sessions and admittance to the opening reception and banquet.

Non-scientist family members and guests of registrants may register for a fee of $60. The guest registration fee includes admittance to the Opening Reception and Banquet only. Guest registrants may not attend scientific sessions.

Registration – 101 Collis:

Hours:
Saturday, July 8 . . . . . . 2:00 PM–9:00 PM
Sunday, July 9 . . . . . . 8:00 AM–5:30 PM
Monday, July 10 . . . . . . 8:30 AM–5:30 PM
Tuesday, July 11 . . . . . . 8:30 AM–5:30 PM
Wednesday, July 12 . . . 8:30 AM–2:30 PM

Fees:
APS Member . . . . . . . . . . . . . . . . . . . $250
Retired Member . . . . . . . . . . . . . . . . . . $150
Nonmember . . . . . . . . . . . . . . . . . . . . . . $300
Student . . . . . . . . . . . . . . . . . . . . . . . . $150
Guest . . . . . . . . . . . . . . . . . . . . . . . . . $60
(nonscientist-family members of registrants)

Press

Press badges will be issued in the Conference Office only to members of the working press and freelance writers bearing a letter of assignment from an editor. Representatives of allied fields (public relations, public information, public affairs, etc.) may register as nonmembers in the registration area.

Publications

The Program/Abstract Volume (the June issue of The Physiologist) was mailed to all APS members and will be given to registrants on-site. Replacement copies may be purchased for $25.00 in the Conference Office.

Message Center

There will be a message board near the Registration Desk at 101 Collis. Registrants should check for messages daily. Please suggest that callers who wish to reach you during the day leave a message at the Registration Desk during the registration hours.

Airline Travel

Delta Connection and USAir Express provide air service to nearby Lebanon Airport (about 5 miles from Hanover). Transportation is available from Lebanon Airport to Hanover. Shuttle service is available for those arriving into Boston's Logan or Manchester Airports. Contact the ground transportation desk at the appropriate airport for pricing and to reserve transportation.

Driving Directions

Two interstate highways pass within a few miles of Hanover and make driving from Boston, New York or Montreal area an easy trip.

From Boston: Take I-93 to I-89. Take I-89 to exit 18 and follow signs for Hanover (about four miles).

From New York City, Connecticut and Montreal: I-91 to Exit 13 and follow the signs to Hanover (about one mile).

Parking

Permit parking is available on Dartmouth campus. Your vehicle must display a parking pass at all times while on campus or you car will be towed. Those advance registrants who requested a parking pass may pick it up at the Registration Desk, 101 Collis. Replacement passes may be purchased for $20.00.

Car Rental

Avis has been designated as the official car rental company for the conference. Group rates are available by calling the Avis Reservation Desk at 1-800-331-1600; refer to the Avis Worldwide Discount (AWD) number: D657201.

Social Program

Opening Reception — The Opening Reception will be held at the Top of the Hop located adjacent to the Hanover Inn on East Weelock between College and Crosby Streets. A variety of hors d'oeuvres and cash bar will be featured 8:00-10:00 PM.

Conference Banquet and Lecture — All registrants are invited to attend the Wednesday evening banquet in Alumni Hall located on East Weelock between Crosby and South Park Streets. A cash bar reception is scheduled from 6:30 to 7:00 PM followed by the meal and lecture by J. Woodland Hastings. Each registrant will receive a coupon in the registration packet which MUST be exchanged for a dinner ticket before 10:00 AM on Tuesday, July 11.
**1995 APS Conference**

**Understanding the Biological Clock: From Genetics to Physiology**

**July 8-12, Dartmouth College, Hanover, New Hampshire**

| Saturday  
July 8, 1995 | Sunday  
July 9, 1995 | Monday  
July 10, 1995 | Tuesday  
July 11, 1995 | Wednesday  
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<td><strong>Note</strong></td>
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<td><strong>Summary Reports of Study Groups on Genetic and Physiological Analyses of Circadian Clocks</strong></td>
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<td>Each day will focus on one topic as indicated by the headings</td>
<td>Molecular Analyses of Circadian Oscillators and their Output</td>
<td>Analyses of Circadian Clocks at the Level of Cells and Tissues</td>
<td>Circadian and Circannual Rhythms in Organisms</td>
<td><strong>2:00-9:00 PM—101 Collis Registration</strong></td>
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| 8:30-10:30 AM—Cook Auditorium  
Molecular Basis of the Circadian Oscillator  
Participants:  
Arnold Eskin, U Houston  
Takao Kondo, Natl Inst Basic Biol, Japan  
Jae Dunlop, Dartmouth Med Sch  
Joe Takahashi, Northwestern U  
Amila Selgai, U Pennsylvania | 8:30-10:30 AM—Cook Auditorium  
Cellular Analysis of Circadian Oscillators  
Participants:  
Michael Hastings, Cambridge U  
Martin Zatz, NIMH  
Gene Block, U Virginia  
Till Roenneberg, U Munich | 8:30-10:30 AM—Cook Auditorium  
Circadian Rhythms, Physiology and Behavior  
Participants:  
Theresa Lee, U Michigan  
Fred Karch, U Michigan  
Stephane Reulx, U Munkton  
Bruce Goldman, U Connecticut | 8:30 AM-1:00 PM—Cook Auditorium  
Group Reports  
Carl Johnson, Vanderbilt U  
Terry Page, Vanderbilt U  
Rae Silver, Bamard Col  
C.P. Kyriacou, U Leicester  
J. Woodland Hastings, Harvard U  
Patricia DeCoursey, U South Carolina  
Eberhard Gwinner, Max-Planck Inst, Basel | |
| 7:30-8:00 PM—Cook Auditorium  
Welcome Session:  
Meeting Overview and Organizational Information | 10:00 AM-12:30 PM—Cook Auditorium  
Molecular Biology of the Circadian Clock and its Output  
Participants:  
C. Rob McClung, Dartmouth Coll  
Ueli Schibler, U Geneva, Switzerland  
Jennifer Lorus, Dartmouth Med Sch  
William Schwartz, U Massachusetts  
Mary Pierce, SUNY Ithl Sci Ctr | 10:30 AM-12:30 PM—Cook Auditorium  
Circadian Systems: Input, the Pacemaker, and Output in Multicellular Systems  
Participants:  
Kathy Siwicki, Swarthmore Col  
Steven Reppert, Mass Gen Hosp  
Rebecca Prosser, U Tennessee  
Russell Foster, U Virginia  
Tony van den Pol, Yale U | 8:30-9:00 PM—Alumni Hall  
Banquet and Lecture  
Circadian Clocks, Past, Present, and Future  
Speaker:  
J. Woodland Hastings, Harvard U |
| 8:00-10:00 PM—Top of the Hop  
Opening Reception | 4:00-5:30 PM—Collis Common Ground  
Poster Sessions  
Authors in Attendance | 4:00-5:30 PM—Collis Common Ground  
Poster Sessions  
Authors in Attendance | 4:00-5:30 PM—Collis Common Ground  
Poster Sessions  
Authors in Attendance | 4:30 PM—1:00 PM—Cook Auditorium  
Circadian Organization in the Vertebrates  
Speaker:  
Robert Moore, U Pittsburgh | 4:30 PM—1:00 PM—Cook Auditorium  
Circadian Organization in the Vertebrates: New Directions  
Speaker:  
Michael Menaker, U Virginia |
Invited Session Abstracts

Saturday

2.0 Molecular Basis of the Circadian Oscillator ........................................ A-11
3.0 Molecular Biology of the Circadian Clock and its Output ........................ A-12

Sunday

7.0 Cellular Analysis of Circadian Oscillators ........................................... A-13
8.0 Circadian Systems: Input, the Pacemaker, and Output ............................ A-13
   in Multicellular Systems

Tuesday

10.0 Circadian Rhythms, Physiology and Behavior ................................ A-14
11.0 Human Circadian Control, Physiology and Clinical Applications ........... A-15
12.0 Plenary Lecture – Circadian Organization in the Vertebrates: .......... A-16
     New Directions

Poster Sessions

Sunday/Monday

5.0 Biochemistry, Cell, and Molecular Biology of the Clock ........................ A-17
6.0 Circadian Systems – Neural Substrates and Functional Organization .... A-21

Tuesday/Wednesday

13.0 Behavior and Ecological Relevance of Circadian Rhythmicity ................. A-25
14.0 Clocks, Photoperiodism, and Circannual Rhythms ............................... A-26
15.0 Circadian Clocks and Human Biology: Basic Physiology/Clinical Aspects A-29

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By introduction of a bacterial luciferase genes into cyanobacteria (Synechococcus sp. PCC7942), we developed a transformed strain expressing luciferase as a bioluminescent reporter of the circadian clock. From chemically mutagenized cells, a diverse set of mutants was isolated. Periods of most mutants ranged between 13 and 60 h and many mutants were arrhythmic. Some isolates that could complement these mutations, we constructed genomic DNA libraries which were introduced into each mutant cells, and screened complemented clones that showed normal phenotypes. After an extensive screening, we have isolated each complemented clones from 15 mutants. We are recovering each complementing gene and try to determine whether recover genes are complementary to mutagenized cells.

Bacterial luciferase genes were randomly inserted into the genome to identify genes that are controlled by the circadian clock. Inserted luciferase genes report activity of upstream promoters by bioluminescence. By monitoring the time course of 800 luminescent clones, we found that the bioluminescence expression patterns of almost all colonies manifested clear circadian rhythmicity. These rhythms exhibited a variety of waveforms and phase relationships. This result indicates that genes controlled by circadian clocks may be more widespread than previously expected. This method was then applied to various arrhythmic mutants to examine whether arrhythmic phenotypes are caused by disruption of the central oscillators or of the output pathways. As all bioluminescent clones obtained were arrhythmic, these arrhythmic mutants are probably mutations of the central oscillator.

2.3 The frequency locus encodes a central component of the circadian clock: the level of which is rapidly reset by light. Jay C. Dunlap. Department of Biochemistry, Dartmouth Medical School, Hanover, NH 03755-3844

Based on its genetics, the frequency (frq) locus has been proposed as a key component in the cellular oscillator generating circadian rhythmicity (Dunlap, 1993). Specifically, several testable predictions have been made regarding the regulation of genuine frequency transcripts (including state variables) of the clock, and frq encodes a factor satisfying all of these criteria (Aronson et al. 1994). frq encodes a central component of a molecular feedback loop in which the product of frq depresses the level of its own transcripts, resulting in a daily oscillation in the level of this frq transcript. Rhythmic frq mRNA expression is essential for overt circadian rhythmicity: constitutively elevated expression of frq encoding RNA in a frq background results in arrhythmicity, and no level of constitutive expression is capable of rescuing normal rhythmicity in frq loss-of-function mutants. Step reductions in frq transcript levels at any time in such constitutively elevated strains set the clock to a unique and predicted phase. Recent data (Merrow and Dunlap, 1994) also show phyleggetic conservation of frq structure and function. Finally, it is clear that light acts rapidly (within 2 min) to increase (4-25 fold) the level of transcript(s) arising from frq, consistent with a model in which elevation of the level of frq transcript(s) in the cell is the initial clock-specific event involved in resynchronization of the clock by light (Dunlap, 1993). This means, thresholds, kinetics, and magnitude of the light-induced increase in frq expression are consistent with the salient molecular predictions of Pittendrigh’s model for nonparametric entrainment of circadian oscillators by discrete pulses of light. These data support a model where the Neurospora circadian clock consists of a negative feedback loop where the product of the frq gene regulates the level of the transcript(s) of the frq gene, and that entrainment of this oscillator by light is via rapid step-wise changes in the state variable encoded by frq.

REFERENCES:


2.4 Genetic dissection of the circadian oscillator in the mouse. Jay C. Dunlap.

In order to identify genes that regulate circadian rhythms in mammals, we used a behavioral screen of first-generation progeny of N-ethyl-N-nitrosourea mutagenized mice to attempt to isolate "clock" mutations in the mouse. We identified a recessive, autosomal mutation called Clock that lengthens circadian period by one to two hours in heterozygotes and by four hours in homozygotes. In addition, Clock homozygotes lose circadian rhythmicity after a few days to weeks in constant darkness. Clock heterozygotes, the stability of the period is decreased and the amplitude of the phase response curve to light pulses is increased relative to wild-type mice. Thus, at least three circadian clock properties are altered by the Clock mutation: the steady-state period, the sustained expression of rhythmicity and the phase-shifting response to light. We have initiated genetic mapping of Clock as a first step to the molecular characterization of the gene by the method of positional cloning. Linkage and haplotype analysis has allowed us to place Clock on the midportion of chromosome 4 in a region of conserved symmetry with human chromosome 4. (Supported by grants from the NSF Center for Biological Timing, the MacArthur Foundation, and NIH)

REFERENCES:


3.6 Role of timeless in the Drosophila circadian clock: Amita Sehgal, Dept. of Neurosci., Univ. of Pennsylvania Medical Center, Philadelphia, PA 19104

Genetic analysis of circadian rhythms in Drosophila led to the identification of the period (per) gene, which appears to be a component of the central pacemaker. The recently identified mutation, timeless (tim), renders flies arrhythmic in all behavioral assays of circadian rhythms. In addition, tim eliminates the oscillations in levels of per mRNA and the nuclear expression of per protein. More recent studies have demonstrated that per protein does not cycle in tim flies and is expressed at very low levels. Since the cycling of per mRNA depends on feedback inhibition by per protein, the lack of cycling in tim flies is probably due to the absence of functional per protein. Thus tim appears to affect the post-transcriptional regulation of per protein. The tim locus was mapped to a previously unidentified locus in the Drosophila genome. Recent data on the molecular characterization of the tim gene and on its interaction with per will be presented.

MOL LiGULLAR BASIS OF THE CIRCADIAN OSCILLATOR

REFERENCES:

MOL LiGULLAR BASIS OF THE CIRCADIAN CLOCK AND ITS OUTPUT

3.3 Output and Input: Transduction of Time and Entrainment in Neurospora Jennifer J Loros
Department of Biochemistry, Dartmouth Medical School, Hanover, NH 03755

A circadian biological clock controls several aspects of growth and development in the ascomycete fungus Neurospora. This property has allowed genetic and biochemical techniques to be used to study the circadian clock itself (Dunlap, Trends in Genetics 6, 159-169, 1990). One approach has been to examine the pathways whereby clocks act to control cellular metabolism and behavior. Initial efforts targeted the isolation of genes whose expression depends on circadian rhythms. This was first achieved using the feedback inhibition by light and circadian rhythms of the circadian clock-controlled gene, ccg-2, encoding a fungal hydrophobin required for formation of the conidial rosette layer. Genetic and Molecular Genetics in press, 1995.

REFERENCES:
Jennifer J. Loros
The Molecular Basis of the Neurospora Clock.
Seminars in the Neurosciences in press for April 1995

3.4 LIGHT INDUCTION OF IMMEDIATE-EARLY GENES IN THE SUPRACHIASMATIC NUCLEUS (SCN): William J. Schwartz & Neil Aronin
Deyo-Neurology, Medicine & Cell Biology, Univ. Massachusetts Medical School, Worcester, MA 01602.

It has been about five years since the discovery that environmental light regulates the expression of transcriptional regulatory proteins in the SCN, including c-fos and related AP-1 (Jun) proteins. These findings have provided a new tool for the investigation of SCN function, and over 20 laboratories have now published reports using SCN c-fos activity as a cellular marker for the neural effects of light and as a way to resolve anatomical pathways and pharmacological actions. We and others are also studying the regulation of SCN c-fos at the molecular level to determine the precise relationship between transcriptional mechanisms and the circadian expression of circadian rhythms. Recent data suggest that photic regulation of the AP-1 transcription factor in the SCN occurs not only by increases in the amount of AP-1 DNA-binding activity but also by alterations in the composition of the component proteins - some constitutive (Fos, Jun D), some variable (c-Fos, Jun B) - that form the AP-1 complex. An important feature of the regulation of these genes in the SCN is that their induction by light is clock-controlled, this circadian phase-dependent "gate" may form a loop that connects with output pathways from the circadian pacemaker with an input pathway to it. Our current work is focusing on the trans-acting complexes that interact with cis-acting regulatory elements on the c-fos promoter, including the CRE-binding proteins CREB and CREM. Elucidating the roles of these factors may provide a unique opportunity to trace events backward along one of the pacemaker's output pathways to the circadian oscillatory machinery itself.

REFERENCES:
CALCIN AND PHASE SHIFTS IN THE CHICK PINAIL: Martin Zatz
SBP, LCB, National Institute of Mental Health, Bethesda, MD 20892

Chick pineal cells in dispersed cell culture display a persistent, photo-
responsive, circadian rhythm of melatonin output and release. Light
pulses have at least two distinguishable effects on these cells: acute
suppression of melatonin output and phase shifts (entrainment) of the
underlying pacemaker. Previous results linked calcium influx through
the plasma membrane to acute regulation of melatonin synthesis but denied a
role for such influx in entrainment. Those experiments did not, however,
address the role of intracellular calcium flux. We therefore tested the
effects of pulses of caffeine, thapsigargin, and EGTA on the melatonin
rhythm, and their interactions with the effects of light pulses. Caffeine
had two distinguishable effects on these cells: acute enhancement of
melatonin output (attributable to phosphodiesterase inhibition) and phase
shifts of the circadian pacemaker with a light-like pattern (attributable
to release of intracellular calcium). Thapsigargin (which specifically
blocks the pump that replenishes intracellular calcium stores) thereby
increasing cytoplasmic calcium and depleting those stores) had no phase
shifting effects by itself, but reduced the size of phase advances induced by
caffeine or light. EGTA (which specifically chelates calcium, thereby
lowering cytoplasmic calcium and depleting intracellular stores) also
reduced the size of phase advances induced by caffeine or light without
inducing a phase shift by itself at that phase. Taken together, these results
point toward a role for intracellular calcium fluxes in entrainment of
the circadian pacemaker. Elevations of cytoplasmic calcium per se, do not
appear to be sufficient. Rather, it is speculated that induced changes in
calcium oscillations or distribution may mediate photoentrainment.

REFERENCES:
M. Zatz & D.A. Mullen
Does calcium influx regulate melatonin production through the deciduous
ciradian pacemaker in chick pineal cells? Effects of
ntrisulfipire, Bay K 8644, Co++, and low external Ca++.
T. Pozzan, R. Rizzuto, P. Volpe & J. Melonei
Molecular and cellular physiology of intracellular calcium stores.
C. Fewtrell
Ca++ oscillations in non-excitable cells.

FEED-BACK LOOPS IN THE CIRCADIAN SYSTEM OF GONTAULAI
POLYDEDA - LIGHT, FOOD, AND BEHAVIOR. Till Roenneberg
University of Munich, Medical School, D-80336 Munich, Germany

One of the experimental prerequisites to show the endogenous
nature of circadian rhythms is to keep experimental conditions 'constant'.
We have shown that in the amphibian G. polyedra, the relative abundance of
the caudal and the posterior regions of the body is important in
entraining the circadian rhythms of the animals. In order to investigate
these rhythms, we have developed a new microscopic technique that
permit us to study the circadian rhythms of individual G. polyedra.

REFERENCES:
M.R. Ralph and N. Mrosovsky
Behavioral inhibition of circadian responses to light.
E. Gwinner
Tagzperiodische Schwankungen der Vorzugsrichtungen bei

Although in German, is worthwhile to have a friend translate this
paper, because it is one of the few to show circadian changes in
self-selection of light intensity.

CIRCADIAN SYSTEMS: INPUT, THE PACemaker, AND OUTPUT IN MULTICELLULAR SYSTEMS

A POTTPOURRI OF CIRCADIAN STUDIES. Steven M.
Reppert, Lab of Developmental Chronobiology, Mass General
Hosp & Harvard Med School, Boston, MA 02114

This laboratory has made recent advances in three areas.
First, we have cloned a family of G protein-coupled receptors for
the pineal hormone melatonin. Our principal studies with a
high affinity receptor from mammals show that it is expressed
in the hypophysial pars tuberalis and suprachiasmatic nucleus
(SCN). This receptor likely mediates the reproductive and
circadian actions of melatonin in mammals. Second, by
culturing cells from neonatal rats on fixed microelectrode
arrays, we have recorded spontaneous action potentials from
individual SCN neurons for days or weeks, revealing prominent
circadian rhythms in firing rate. Despite abundant functional
synapses, neurons in the same culture express circadian rhythms
of different phases and periods. These data provide strong
evidence that single SCN neurons are circadian clocks. Third,
we have cloned a structural and functional homolog of the
chick circadian clock gene in Antheraea pernyi.

REFERENCES:
1. S.M. Reppert, D.R. Weaver, T. Ebisawa
Cloning and characterization of a mammalian
melatonin receptor that mediates reproductive and
2. D.K. Welsh, D.E. Logothetis, M. Meister, S.M.
Reppert
Individual neurons dissociated from rat
suprachiasmatic nucleus express independently
phased circadian firing rhythms. Neuron in press
3. S. M. Reppert, T. Tsai, A.L. Roca, I. Sauman
Cloning of a structural and functional homolog of the
circadian clock gene period from the giant silkworm
PHASE SHIFTING THE SCN CIRCADIAN CLOCK IN VITRO
Rebecca A. Provenzi
Dept. of Zoology, University of Tennessee, Knoxville TN 37996.

The production of new 24-h rhythms in behavior and physiology is the absence of known synchronizing stimuli appears to be a characteristic shared by mammals, if not all, organisms. The primary circadian pacemaker in the suprachiasmatic nucleus (SCN). One piece of evidence for this conclusion is the SCN's ability to produce new circadian rhythms in isolation from the light-dark cycle. In particular, the SCN produce a robust 24-h rhythm in free-running conditions that lasts for at least 3 cycles in vitro. This rhythm is typically observed by synchronizing the free-running rhythms of individual animals for long periods followed by a period of isolation in a brain slice preparation. In general, the SCN produce a robust 24-h rhythm when isolated from the light-dark cycle.

These data suggest that the SCN is the pacemaker for several nocturnal rodents. (Supported by NIH grant MH49089)

A-14 CIRCADIAN SYSTEMS: INPUT, THE PACEMAKER, AND OUTPUT IN MULTICELLULAR SYSTEMS MONDAY

PHASE SHIFITNG THE SCN CIRCADIAN CLOCK

8.3

IN VITRO

REFERENCES:
1. M.U. Gillette
2. J.D. Miller
Summarizes early SCN slice control and phase shifting studies.

8.4

THE REGULATION OF VERTEBRATE CIRCADIAN RHYTHMS BY LIGHT
Russell G. Foster*, Sharleen Argamaso-Herman, Susan Douglas*, Allan Froehlich†, Jennifer Provenzi, Robby Scott†, Department of Biology & NSF Center for Biological Timing, University of Virginia, Charlottesville, VA 22903. *New address: Department of Biology, Imperial College, Prince Consort Road, London SW7 2B.

In mammals, known ocular photoreceptors entrain circadian rhythms in the light-dark cycle. In an attempt to identify these photoreceptors we have used various mammals that lack specific retinal elements, and determined the effect of these on circadian responses to light. C57BL/6J and C3H/He retinal degenerate (rd/d + rd) mice, which lack rod photoreceptors beyond 80 days of age, show unaltered circadian responses to light. Recent results in male and female mice suggest that photoreceptors other than cones or by cone-like opsins in some unidentified retinal cell remains to be determined. In male mice show increased circadian responses to light, affecting both sensitivity and dynamic range. The different response kinetics of transgenic, rd/d+ mice could result from a reorganization of the entrainment pathway, and responses are larger in transgenic mice because rods are affected earlier in postnatal development. On the basis of our action spectrum analysis of extraretinal photopigments in non-mammalian vertebrates.

REFERENCES:
3. Most recent transgenic results
5. Most recent transgenic models
7. Most recent results

CIRCADIAN RHYTHMS, PHYSIOLOGY AND BEHAVIOR

10.1

SEX DIFFERENCES IN FORMAL CIRCADIAN PROPERTIES OF O. DEGUS
Theresa M. Lee* and Tammy-Jo James* Michigan State University, East Lansing, MI 48824-1000.

Sex and 1 (in mice) found that female degus housed alone phase advanced significantly slower after a 6-h shift than male, while there was no sex difference in reentrainment rate after 6-h delays. Because the phase angle of activity onsets and temperature minimums of entrained males and females (L112/D12) do not differ, we hypothesized that the sex difference in reentrainment rate was due to differences in free-running rhythms (τ) and/or phase response curves (PRC). Tau of intact and ovariectomized (OVX) females did not differ in 0 lux (Labyak & Lee, in press), free-running rhythms (T) and/or phase response curves (PRC). Tau of intact and ovariectomized (OVX) females did not differ in 0 lux (Labyak & Lee, 1996).

These phase shifts are not blocked by 10mM Mg2+ in the extracellular medium. Currently my lab is beginning to investigate possible interactions between this afferent system and the other two primary SCN input: a direct retinal projection via the intergeniculate leaflet of the lateral geniculate nucleus, with neurotransmitter V and GABA in its known neurotransmitters. These studies should help us understand how the SCN clock is modulated in intact animals.

REFERENCES:
1. M.U. Gillette
2. J.D. Miller
Summarizes early SCN slice control and phase shifting studies.

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2. J.D. Miller
Summarizes early SCN slice control and phase shifting studies.

10.2
REGULATION AND EXPRESSION OF A CIRCANNUAL RHYTHM
Fred J. Karsch. University of Michigan, Ann Arbor, MI 48109-0404

Seasonal reproductive cycles of many long-lived species are maintained by an endogenous rhythm of neuroendocrine activity. Photoperiod entrains this rhythm via the circadian rhythm of melatonin secretion. Studies in sheep have revealed that changes in duration of the nocturnal increase in melatonin secretion entrain the reproductive rhythm by regulating pulsatile oscillations of gonadotropin-releasing hormone output from the hypothalamus. Further, only a portion of the annual photoperiodic cycle is needed to entrain the circannual reproductive rhythm; in this regard, photoperiodic input during spring and summer is especially crucial. Thyroid hormones are essential for the expression of the circannual reproductive rhythm of sheep. If thyroid hormones are absent during a restricted "window" of time around the end of the reproductive period in winter, the seasonal reduction of pulsatile oscillations of gonadotropin-releasing hormone secretion fails to occur and the breeding season persists. A hierarchy of biological periodicities and multiple hormonal components of the hypothalamic-pituitary axis thus contribute to the regulation and expression of the circannual reproductive rhythm.

Supported by NSF IRN 90-26510 and NIH HD 07689.

REFERENCES:

CIRCADIAN CLOCKS AND TIME PLACE LEARNING IN A CYPRINID FISH. Stephan G. Reebs. Univ. de Moncton, Moncton, NB. E1A 3E9, Canada

This paper presents an ecologically relevant example of circadian clock use by fish. In temperate lakes, golden shiners, Notemigonus crysoleucas, are known to feed in open waters at dawn, near the littoral at noon, and back in open waters at dusk. In this time place association, is time discrimination based on a circadian clock, or is it simply a response to environmental cues such as light or food? In the lab under an artificial 12-h photoperiod, groups of 8 shiners were trained to feed on one side of their tank in the morning, on the other side at midday, and back to the first side in the evening. After at most 3 weeks, the fish had learned to be on the correct side at the correct time of day, even when no food was delivered. This pattern persisted after a 6-h phase advance of the light-dark cycle, and took 3 days to phase-shift. Ongoing experiments are testing whether phase-delaying lights-off on one day leads to a delay in the timing of switches from side to side on the next day. Up to now, we have good evidence that circadian endogenous mechanisms are involved in time-place learning. Circadian clocks free the fish from dependence on unstable light signals, and allow them to anticipate food arrival in specific places and efficiently compete for it.

REFERENCES:

HUMAN CIRCADIAN CONTROL, PHYSIOLOGY AND CLINICAL APPLICATIONS

ADAPTING TO PHASE SHIFT: EFFECTS OF MELATONIN ON HUMAN CIRCADIAN RHYTHMS. Josephine Arendt and Stephen Deacon. University of Surrey, Guildford, GU2 7XH, UK

The pineal hormone melatonin appears to serve similar functions in all vertebrates. By its pattern of secretion it conveys information about phase, duration and strength of the daily photoperiod for the organisation of seasonal and circadian physiology. In humans melatonin, suitably timed, will phase shift the endogenous melatonin rhythm and core body temperature, validated markers of the endogenous melatonin rhythm. However, in over 500 individuals who were forced to phase shift melatonin and bright light are likely to provide optimum conditions for adapting to phase shift. Melatonin may, as a coordinator of biological rhythms, help to maintain structural rhythmicity and thereby influence many aspects of health.
NEW APPROACHES TO THE STUDY OF CIRCADIAN ORGANIZATION. Michael Menaker, Dept Biol & NSF Ctr Biol Timing, Univ Virginia, Charlottesville, VA 22903, USA

By circadian organization, I mean both the way in which the entire circadian system above the cellular level is put together physically, and the principles and rules that determine the ways in which its component parts interact to produce overt rhythms of physiology and behavior. It is my contention that understanding this organization is as important and as interesting a task as any before us, and at the moment is severely neglected. Are there really "circadian systems" in the sense of integrated ensembles of tissues and organs that interact to produce overt rhythms with complex but definable properties? The answer is clearly "yes"—I will illustrate this briefly with examples from vertebrates, but such systems can be found in many invertebrates as well. Can we hope to understand their organization in detail? The answer here is also "yes"—we already understand a good deal about some of them. Their level of complexity is perhaps a bit above that of other systems whose organization we understand reasonably well (e.g., the hypothalamic-pituitary-adrenal axis) but it is clearly not hypercomplex and presently out of reach (e.g., "the brain"). However, it is my belief that we need new model systems specifically chosen to provide direct experimental access to questions of circadian organization. I will discuss two "new" models that may be useful in this endeavor and the progress that has been and might be made in using them. Viewed as a specific and limited lesion in the circadian system, the tau mutation in the golden hamster has enabled us to approach questions of circadian organization that would be otherwise difficult or impossible. I will briefly review the conclusions from the published work, the interpretation of some unpublished results, and where they may lead. The utility of this model suggests strongly that other circadian mutations should be studied in this way to the maximum degree possible. We are developing a new reptile model using the green iguana (Iguana iguana). This robust and readily available animal is ideal for studying circadian photoreception: furthermore, it is possible to record as many as five different circadian rhythms simultaneously and automatically from a single individual, and we have evidence that suggests strongly that under some circumstances, the rhythms of body temperature and activity free run with different periods. I will explore the challenges and opportunities that are likely to arise in working with this model.
5.1
MATHEMATICAL MODEL OF A CIRCADIAN OSCILLATOR WITH POSITIVE POST-TRANSCRIPTIONAL AUTOREGULATION: C. D. Thrun. Dartmouth Medical School, Hanover, NH 03755.


$$\frac{dR}{dt} = \frac{p_1}{p_2 + F} - p_R, \quad \frac{dF}{dt} = \frac{p_4 + p_F^2}{p_3 + p_F^2} - p_R,$$

$p_1 = 7.82, p_2 = 1.42, p_3 = 6.84, p_4 = 321, p_5 = 95.2, p_6 = 32.1, p_7 = 2$.

The model would imply that FRQ or PER is positively post-transcriptionally regulated. This model therefore suggests that an important part of the circadian oscillator oscillation is positive feedback.

5.3

prd-2 is a recessive clock mutation that lengthens the period of the circadian condensation rhythm from the wildtype value of 21.5 hours to about 25.5 hours at 25°C. Genetic mapping of prd-2 localized the gene to the right arm of linkage group V between fis-2 and a.m. A chromosome walk in the Volmer-Yaunosky cosmid library in the genetically determined region of prd-2 yielded a set of cosmids spaning about 170kb. These cosmids were tested in a transformation assay for the ability to compliment prd-2 and restore wildtype rhythmicity. Some of the cosmids showed partial rescue of the mutant phenotype, shortening the period by up to 2 hours in approximately 20% of the primary transformants.

Analysis of homoeotyzic microcosmid isolates showed that the suppressed phenotype is stable and not an effect of heterokaryosis. Analysis of transformants from overlapping cosmids localized the suppressing DNA to a 4kb region.

5.4
COORDINATE REGULATION OF THE GONIAULAX CIRCADIAN CLOCK BY PROTEIN KINASES AND PHOSPHOPROTEIN PHOSPHATASES: J. Hastings, N. Recht, and J. Woodland. Hastings, Harvard University, Department of Molecular and Cellular Biology, Cambridge, MA 02138.

Protein phosphorylation is crucial in regulating many eukaryotic cellular processes, and there are now numerous instances demonstrating its relevance to the circadian mechanism. Our goal is to identify kinases or phosphatases which participate in the regulation of the circadian clock. We have approached the screen inhibitors of these enzymes for their effects on the bioluminescence rhythms of the dinoflagellate Gonyaulax polyedra, thus attempting to characterize transcriptional/translationally (translational) sites of action. In this manner, we have shown that staurosporine, an inhibitor of serine/threonine protein kinases, induces period lengthening of the bioluminescent glow rhythm at nanomolar concentrations. Serine/threonine-sensitive kinases identified in Gonyaulax extracts may be important in controlling circadian rhythmicity. The specific serine/threonine phosphatase inhibitors okadaic acid, calyculin A, and cantharidin also alter the progression of the circadian clock. These drugs inhibit dephosphorylation of Gonyaulax proteins in vivo and block phosphatase activity in vitro. Thus a serine/threonine phosphatase, possibly a protein phosphatase 1-type (PP1) enzyme, may be responsible for dephosphorylation crucial to the function of the circadian clock. A Gonyaulax PP1 has been isolated from a cDNA library and the predicted polypeptide sequence closely resembles that of PP1 enzymes from other organisms. Since several substrates of PP1 have been identified, it may be possible to determine which one is important to the circadian mechanism.

5.5
Structure/Function Analysis of the Circadian Oscillator Component FRQ Suggests Action in the Nucleus: Martha Merrow, Norman Leidner, Chenghua Luo, Susan Crosthwaite, and Jay C. Dunlap. Dartmouth Medical School, Hanover, NH 03755-3844.

frq encodes a central component of a molecular feedback loop in which the product of frq depresses the level of its own transcript, resulting in a daily oscillation in the level of this frq transcript (Ammoson, Johnson, Lorton, and Dunlap, SCIENCE 263: 1578, 1994), and FRQ is a nuclear protein. Rhythmical frq mRNA expression is essential for overt circadian rhythmicity: Constitutively elevated expression of FRQ-encoding RNA in a frq background results in arrhythmia, and no level of constitutive expression is capable of reversing normal rhythmicity in frq loss-of-function mutants. Step reductions in frq transcript levels at any time in such constitutively elevated strains sets the clock to a unique and predicted phase. frq is also phylogenetically conserved (Metrow et al., Science 263: 1578, 1994).

Ag data suggest that the frq/FPR feedback loop is a part of the clock which resides partly within the nucleus, as the nuclear localization of FRQ is not affected by the light-induced repression of frq mRNA levels. However, the authors suggest that some of the kinetic constraints governing the feedback cycle. Additional evidence for nuclear action is that expression of the FRQ protein in a heterologous (baculovirus) system results in nuclear localization, as predicted from sequence data.

Supported by NICNG grant GM43988.

5.6
LIGHT-INDUCED RESSETTING OF A CIRCADIAN CLOCK IS MEDIATED BY A RAPID INCREASE IN FREQUENCY TRANSCRIPT: Susan K. Crosthwaite, Jennifer J. Loros, and Jay C. Dunlap. Department of Biochemistry, Dartmouth Medical School, Hanover, New Hampshire 03755.

One important property of circadian oscillators is that they can be entrained to the daily light-dark cycle. An understanding of the clock must therefore include knowledge about the action of the light signal. We have looked at the effect of light on frequency (frq), a gene known to encode a component of the clock in Neurospora crassa. Cycling of frq mRNA abundance is essential for overt rhythmicity and is regulated via feedback inhibition of frq by FRQ (Ammoson et al., SCIENCE 263: 1578, 1994). Two minutes of light pulses given at different circadian times cause a rapid increase in the level of frq transcript. This increase can be detected within 5 minutes: levels peak between 15 and 30 minutes after the light pulse and then fall to control levels. frq mRNA levels in the loss of function mutant frq-2, and in a strain carrying an artificially-inducible copy of frq have shown that the light-induced accumulation of frq mRNA is the result of induction rather than simply release from feedback inhibition by FRQ. The magnitude of the light-induced increase in frq mRNA and the extent of clock resetting are correlated, with a threshold for each response of 8 m moles photons/2s. This threshold along with the speed and magnitude of the light-induced increase in frq transcript suggests that this is an early clock-specific event involved in resetting of the clock by light. Additionally, these data indicate the saai me molecular mechanisms for the model for nonparasite entrainment (entrainment to discrete pulses) of the clock, and thus suggest that this may be a general pattern by which circadian oscillators are entrained.

Supported by NLM grant GM43988 to JCD and AFOSR grant 94-01510.
5.7
CELLULAR DYNAMICS OF THE DROSOPHILA PER PROTEIN.
Kathleen S. Siwicki, Bill Bug, Mary Grace Folwell and Erik Horvath.
Swarthmore Colleage.

From the earliest studies of PER expression in Drosophila, we have observed differences between photoreceptors and per expressing lateral neurons (LNs) in their localization and circadian cycling. The LNs are likely to be pacemaker cells that control the fly's activity rhythms. These neurons are a small proportion of the per-expressing cells, and thus contribute only a small fraction to biochemical measurements of the protein in fly head homogenates. We have exploited recent advances in fluorescence imaging technology to develop a quantitative in situ assay for PER protein, which we have used to study the dynamics of circadian oscillations in per expressing specific cell types. We have examined per+ flies in 12 hr LD cycles, and confirmed earlier observations of diurnal rhythms of PER. In photoreceptors, nuclear PER cycles with a trough around "lights-off", and a peak during the night, around ZT 20. Significant levels of cytoplasmic PER in per+ photoreceptors (relative to a different background fluorescence in per−/−) were detected only at a single ZT in the middle of the night. In LNs, PER immunofluorescence is in phase with the photoreceptors, but falls after "lights-on", several hours later than the falling phase of photoreceptor PER. PER is readily detectable in both nuclei and cytoplasm of some LNs, although it is predominantly nuclear in the majority of the PER containing cells in the circasimplicefly is exceedingly rare. One implication of our results is that photoreceptors and LNs may differ in the timing of PER degradation; thus, some aspects of PER cycling may be unique to the pacemaker cells.

5.8
ANALYSIS OF CIS-REGULATORY SEQUENCES IN THE DROSOPHILA MELANOGASTER PERIOD GENE PROMOTER.
Harriet Hsu and Paul E. Hardin. Department of Biology, Brown University, Providence, RI 02912.

The period (per) gene is involved in regulating the circadian rhythms of D. melanogaster. The abundance of per mRNA and protein undergoes circadian oscillations, which comprise a feedback loop that appears to be required for behavioral rhythms when expressed in certain brain neurons and glia cells. We are investigating the role of cis-regulatory elements in the promoter region in order to understand the mechanism underlying the oscillation of per mRNA and their effects on per feedback loop and behavior. Transgenic flies bearing transgenes containing different promoter versions of per promoters fused to an E. coli lacZ or per upstream sequences fused to either a Drosophila histone H3 donor or a transposable basal promoter-lacZ fusion. LacZ mRNA abundance from these fusion genes has been measured to determine the expression pattern for these transgenes has been followed via histochemical staining. Preliminary results show that sequences sufficient for mRNA cycling can activate heterologous promoters; and suggest that cycling elements are present in sequences from -603bp to -341bp and an element capable of driving expression in the eye is present in sequences from -341 to -156bp relative to the transcription start site. Current studies are directed towards delineating the minimal elements sufficient for circadian RNA cycling, characterizing the number and position of the cycling elements, determining whether these elements act as transcriptional enhancers and identifying tissue specific spatial elements.

This work is supported by NINDS grant # R03-NS11214.

We have demonstrated that the chlorophyllogenic ZC mutant of Euphotocyclus exhibits a circadian rhythm of mitosis. To determine how the circadian cell cycle couples to the cell division cycle (CDC), we have monitored cell-cycle oscillations in the levels (western blotting) and activity (histone 3 kinase assay) of the mitotic kinase, cyclin-dependent kinase (CDK). The CDK is activated by the G1/S- and G2/M-phase specific cyclin (CC) (both reduced level in the latter), but circadian changes in electrophoretic mobility were detected, which persisted during stationary phase. In contrast, histone H1 kinase activity oscillated with a peak during mitosis in dividing cells and disappeared in nondividing cells. Cyclin D levels fluctuated with a peak just preceding the peak of the mitotic checkpoint in the cell cycle. A surge of the CDK 2 into the cytoplasm, not only in circadian timekeeping, but also in signal transduction between clocks. Supported by NSF grant DDC-9105752.

5.14 RHYTHMIC SYNTHESIS OF RUBISCO IN GONYAULAX. Patrick Salom, Paul Markovic, J.W. Hastings* and D. Morse. University of Montreal, Montreal, Canada H3X 1Z8 and ~ Harvard Biological Laboratories, Cambridge, MA 02138.

Ribulose-1,5-bisphosphate carboxylase/oxygenase (rubisco), which catalyzes the first step in carbon fixation, is normally formed from a mixture of large (55 kDa) and small (14 kDa) subunits. The abundant 55 kDa protein in Gonyaulax seen by Coomassie blue staining of two D gels was confirmed to be the large subunit by sequence analysis. Northern analysis indicates that the transcript level is constant throughout the circadian cycle, indicating that rubisco expression is under circadian regulation. A synthesis rate was found to be highest during the period between CT 11 and CT 13 which requires only large subunits for activity. The synthesis rate of the major PCP (55 kDa protein) is more than ten-fold over a 24 hour period in LL or LD. The synthesis rate during this period is high between CT 1 and CT 11 suggesting that protein is being synthesized in time for the daily photosynthesis. We have found that this new synthesis does not contribute sufficiently to the levels of protein already present and so measurable changes in the levels of protein are not observed. The synthesis rate changes observed are consistent with the case for other clock regulated protein that is not present in the photosynthetic rhythm, as for example when by mRNA translation of extramourina and Northern blot analysis.

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5.16 CLONING AND EXPRESSION OF LUCIFERASE IN GONYAULAX POLYEDRA. Liming Li, Maria Mittag and Woodland Hastings. The Biological Laboratories, 16 Divinity Avenue, Harvard University, Cambridge, MA 02138.

The bioluminescence rhythm in the unicellular dinoflagellate, Gonyaulax polyedra, is regulated by the circadian clock. At least three luciferase genes, luciferin (the substrate) and a luciferin binding protein (LBP) which are involved in the bioluminescence process undergo changes in their amounts and activities during the circadian cycle. To understand the mechanism of the circadian regulation of the luciferase, we have isolated a 4.0 kb cDNA clone, which is confirmed to be lcl gene by both sequence analysis and the luciferase activity of its protein product. Three repeats, each about 800 nt long, have been identified in the 1cl coding region. One such repeat unit is sufficient for luciferase activity. The mRNA levels of 1cl at different circadian times were also examined by Northern analysis and the overall pattern found to be consistent throughout the circadian cycle, indicating that transcriptional control is involved in the circadian regulation, as with 1RP.

5.17 SYNTHESIS OF GONYAULAX POLYEDRA GLYCERALDEHYDE 3-PHOSPHATE DEHYDROGENASE IS UNREGULATED UNDER CIRCADIAN CONTROL. Thomas F. Fagan*, James C. Complin*, David W. Morse* and J. Woodland Hastings. Dept. of Cellular and Molecular Biology, Harvard University, Cambridge, MA 02138.

In the marine dinoflagellate Gonyaulax polyedra there are numerous processes that are under circadian regulation, including bioluminescence, cell mitosis, photosynthesis, and cell division. The bioluminescence rhythm, which is best understood, has been shown to correlate with the daily rhythm of transcription and translation of the luciferase and the luciferin-binding protein (LBP) genes. Various aspects of clock regulation of cellular metabolism. In the case of luciferase, the periodic cycle of luciferase activity is not under circadian control. Western analysis of the protein indicates CCG-1 to be present in the undifferentiated hyphae in a time-of-day-specific manner. Immunocytochemical localization shows that expression and localization are not related to the cell division cycle.

5.18 Analysis of Clock Controlled Genes in Neurospora. Shinnosuke Kohara, Deborah Ball, Patricia Kristin M. Landgraf, Mihai Shibahara, Hsiu-Hsun Chen, Rox Doudall and Jennifer J. Loras. Department of Biochemistry, Dartmouth Medical School, Hanover, NH 03755.

A circadian clock controls many cellular processes such as the expression of genes and the activity of enzymes. This property has allowed genetic and molecular techniques to be used to elucidate the pathways whereby clocks control cellular metabolism and rhythmicity. This work was focused on the per gene which is one of the molecular components of the circadian clock. The per gene is required for robust rhythmicity, the maintenance of circadian entrainment, and the control of several molecular processes. It is possible to study the relationship between molecular processes and transcriptional changes.

Additionally, eight new clock-controlled genes have been recently isolated by differential screening between a set of time-specific cDNAs libraries. Sequence analysis shows that the Neurospora glucokinase, glyceraldehyde-3-phosphate dehydrogenase and clg 12 encode the Neurospora copper chaperonine genes. The cloning and analysis of a wide spectrum of genes whose transcript abundance is clock regulated is presenting a picture of extremely diverse aspects of clock regulation of cellular metabolism.
involved in mediating light and, since the shape of the action spectrum indicated that opsin may be the reproductive system of the gypsy moth males. Preliminary results indicate that the timing of sperm release when exposed to light at circadian time 16, causes a 5 h delay of sperm release on the following day. To obtain an action spectrum, gypsy moth males were exposed to a monochromatic light, between 420 and 600 nm, and the timing of sperm release was measured. In our effort to define components of this circadian system we explored the input pathway to the clock, light, and transcripts accumulate during asexual development. To sort out the basis of this complex regulation, deletion analysis of the cgg-2 promoter was carried out to localize the cis-acting elements mediating clock light and developmental control. A distinct positive clock element was localized to within a 45 nt region, just upstream of the TATA box. Using an unregulated promoter/reporter system we show that this element is necessary and sufficient for conferring clock regulation on the cgg-2 gene. We are currently using this element as a probe in gel mobility shift assays to identify trans-acting clock factors.

The timing of changes in the expression of the transcriptional regulator C-fos in response to light is similar to those in three tissues which modulate circadian rhythms and neuroendocrine function: the retina, the hypothalamus, and the pineal gland. Adult Djungarian hamsters were either left in darkness or were exposed to a pulse of bright light sufficient to abolish pineal melatonin synthesis beginning 3.5 h after lights off. One cohort of animals was returned to darkness after 20 min. Pinoloids and retinas were taken from hamsters killed in darkness at 3 h and after lights off (D) and from light pulsed hamsters killed at 3.75 h after lights off (LP). At 5.5 h after lights off, pineal glands and retinas were taken from two groups of hamsters killed in darkness: 1) those that remained in the dark (D); and 2) those that were returned to darkness following a light pulse (LP-D). Total RNA was extracted from the pineal glands and retinas. The RNA was electrophoresed in a formaldehyde agarose gel and transferred to a nylon membrane. A Djungarian hamster derived c-fos probe, which had been generated from RNA extracted from light-pulse treated retina, was transcribed to produce a 32P-labeled RNA probe for Northern analysis. At 3.5 h after lights off, c-fos expression was detectable in both the pineal gland and the retina. However, no c-fos signal was detectable in the pineal glands of light pulse treated hamsters, in contrast to an increase in c-fos transcript level in the retina of LP hamsters. At 5.5 h after lights off, hamsters that have remained in darkness continued to show detectable c-fos expression in the pineal, while the c-fos signal remained in the retina even after recovery from the light pulse.

The N. crassa cgg-2 gene, encoding a fungal hydrophobin, is transcriptionally regulated by the circadian clock. In addition, cgg-2 is positively regulated by light, and transcripts accumulate during assexual development. To sort out the basis of this complex regulation, deletion analysis of the cgg-2 promoter was carried out to localize the cis-acting elements mediating clock light and developmental control. A distinct positive clock element was localized to within a 45 nt region, just upstream of the TATA box. Using an unregulated promoter/reporter system we show that this element is necessary and sufficient for conferring clock regulation on the cgg-2 gene. We are currently using this element as a probe in gel mobility shift assays to identify trans-acting clock factors.

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5.25
DOPAMINE ACTS AS AN EFFECTOR OF THE CIRCADIAN CLOCK IN GOLDFISH RETINA. Stuart C. Mange* and Yu Wang*. University of Alabama School of Medicine, Birmingham, AL.

In the fish retina, cone horizontal cells, a type of second order cell, receive synaptic contact exclusively from cones. A circadian clock regulates cone horizontal cell (HC) light responses so that cone input predominates during the subjective day and rod input predominates during the subjective night (Mangel and Wang, 1994). To determine whether dopamine acts as an effector of the circadian clock, the effects of dopamine and various dopamine agonists and antagonists on the light responses of L-type cone HCs were studied during the subjective day and night. Following 14 days of a 12/12 hr light/dark cycle, goldfish were maintained in constant darkness for 3-4 hrs. Surgery was performed under dim red or infrared light. Retinae were superfused in darkness for 90 min, following which a HC was impaled without the aid of any light flashes. Application of dopamine (1 μM) or quinpirole (1 μM), a D2-like receptor agonist, during the subjective night increased cone input and diminished rod input. Quinpirole was more effective than dopamine (10 μM), a D1 antagonist, or forskolin (10 μM), an activator of adenylate cyclase, during the subjective day reduced cone input and increased rod input. Enclopirole (50 μM), a D2 antagonist, and SCH23390 (10 μM), a D1 antagonist, were without effect. Because D4 receptors are found on cones, but not on HCs (Cohen et al., 1992), these results suggest that a circadian clock regulates rod and cone input to cone HCs by modulating rod-cone coupling. The clock increases dopamine levels during the day so that D4 receptors on cones are activated. This in turn decreases rod-cone coupling via a decrease in cAMP. Supported by grants from the NIH and NSF.

5.27
EVIDENCE FOR A CIRCADIAN RHYTHM IN CARTILAGE. Debora L. Nickle* & Josh Wallman*. Biology Department, City College of New York, New York, N.Y. 10031.

In the growing chick, elongation of the eye is rhythmic, increasing during the day and decreasing at night. Experimentally induced changes in the rate of ocular elongation are associated with changes in the rate of synthesis of matrix proteoglycans (PGs) by chondrocytes in the sclera. Perhaps related to this rhythmicity in ocular growth, we report that the synthesis of PGs in isolated pieces of sclera in vitro is rhythmic and persists for several cycles.

Six mm punches of sclera from chick eyes were cultured in individual chambers, in a temperature-controlled flow through perfusion system. A defined medium (N) containing labeled sodium sulfate was continuously replenished via a multi-channel pump at 2 ml/hr and samples of the "conditioned medium" collected for biochemical analysis at 2 hr intervals for 72 hrs. Cultures were perfused at 33°C, a temperature near the midpoint of the circadian cycle. We find that the uptake of sulfate into PGs shows a rhythm of approximately 24 hrs, which persists for at least 2 cycles in vitro. Reversing the lighting in the cage or inducing a strong phase-shift of the rhythm. Analyses by enzymatic digestion and size-exclusion columns show the labeled molecule to be similar to aggrecan, the major cartilage proteoglycan.

In conclusion, we show that the synthesis of an extracellular matrix molecule by chondrocytes may be under control of a circadian oscillator. To our knowledge, this is the first evidence for the existence of circadian rhythms in vertebrate tissue of non-nerve origin.

CIRCADIAN SYSTEMS—NEURAL SUBSTRATES AND FUNCTIONAL ORGANIZATION

6.1

Antibodies raised against S-antigen (arrestin), a component of the phototransduction cascade, were injected directly into the brain of the adult blow fly, Calliphora vicina, via the compound eye. In a proportion of flies so treated, the free-running period (Y) was lengthened, resembling a circadian clock. In about 20% of flies, the free-running period (Y) was lengthened, resembling a circadian clock. These results focus attention on four groups of S-antigen positive neurons in the mid-brain as likely candidates for the auto-optic ("deep brain") photoreceptors in this insect.

5.26
TEMPERATURE MODULATES PACEMAKER AMPLITUDE IN CHICK PINEAL CELLS. K. Kevin Barrett* and Joseph S. Takahashi*. NSF Center for Biological Timing, Department of Neurobiology and Physiology, Northwestern University, Evanston, IL 60208.

Temperature has various effects on the chick pineal clock: temperature pulses shift the phase of the melatonin rhythm, a temperature cycle entrains the clock in vitro, and higher temperatures slightly lengthen the period (temperature compensation) (Barrett and Takahashi, 1993; Zait et al., 1994; Barrett and Takahashi, in press). In addition to these effects, temperature modulates the amplitude of the pacemaker. There are several mechanisms for the effects of temperature on the clock. Temperature affects the production of S-antigen antibody interferes with circadian entrainment at 40°C than at 37°C. (1) The phase response curve to subthreshold stimulation (10 μA/cm²) is shifted to longer durations at 37°C than at 40°C. (2) The phase shift is smaller at 37°C than at 40°C. (3) The phase shift is larger at 40°C than at 37°C. Using a limit-cycle model and assuming the light pulses causing rhythm disruption drive the system near the singularity, we can infer that the pacemaker amplitude is larger at 37°C than at 37°C.

Supported by NIH 1 F32 MH 10369 to R.K.D. and by NIH RG R37 MH 25992 to J.S.T.
6.3 A NON-NEURAL ENDOCRINE PACEMAKER IN INSECT DEVELOPMENT.

Xanthine Valpoure and Colin U.H. Steel.
Department of Biology, York University, North York, Ontario M3J 1P3

The cellular responses comprising insect development are elicited by steroid moulding hormones (ecdysteroids) synthesized by the prothoracic glands (PGs). We recently showed ecdysteroid synthesis by PGs (measured by RIA) is rhythmic and under circadian control in vivo. PGs are stimulated rhythmically in vivo by a peptide hormone from the brain. Peptide release is also under circadian control. However, this rhythm of ecdysteroid synthesis is not a "slave" driven by the brain peptide. We report that PGs in vitro are directly photosensitive and contain their own circadian clock. Arrhythmic PGs (from animals reared in LL) were maintained in vitro for 12 h. A "lights off" cue given at any time in vitro promptly elicits a free-running rhythm of ecdysteroid synthesis, with peaks in the subjective night. Therefore, PGs qualify as pacemakers. These steroid-synthesizing glands are the first known endocrine pacemakers that are not derived from nervous tissue.

6.5 CIRCADIAN PHASE DIFFERENCES IN A RETINAL PACEMAKER NEURON (P-DH) OF ORTHOPTERA: DETERMINANTS TUNED BY A PHASE-SHIFTING NEUROTRANSMITTER, SEROTONIN. Jon V. Jalkanen and Steven Barnes.
Department of Biology, SUNYA, Albany, 12222 and Dept. Medical Physiol., Univ. Calgary, Alberta, Canada, T2N 1N4.

Pacemaker neurons of the A1PN eye express a robust circadian rhythm of neuronal impulse activity. We dissociated the retina into primary culture and made whole cell patch recordings from a subset of basal retinal pacemaker neurons. These neurons had resting potentials near -40 mV and, if recorded in the presence of spontaneous action potentials, >60 mV amplitude. Under voltage clamp 3 ionic currents were characterized, including a fast Na current, a Ca current, a delayed rectifier K current, and a hyperpolarising activated Cl current. Serotonin, which phase shifts the circadian rhythm of the intact eye, enhanced the delayed rectifier K current by 50%. The magnitude of the delayed rectifier K current exhibited phase differences, being larger during the predawn phase. These results show that many pacemaker neuron circadian properties are retained in dissociated neurons and identify ionic currents involved in the expression and perhaps the mechanism of circadian clock.

6.6 GANGLION CELLS OF THE RETINOHYPOTHALAMIC TRACT IN MAMMALS. H.M. Cooper, J. Negroni and A. Attar.
Cerebellar Vision, I.N.S.E.R.M. U-371, 99675, FRANCE.

We have used retrograde tracers and viral tract tracing methods to examine the morphology and distribution of retinal ganglion cells (RGCs) which project to the suprachiasmatic nucleus (SCN) in diurnal and nocturnal mammals (sheep, primate, gerbil, mole-rat). In all species RGC morphology is similar and resembles the ganglion cell of the compound photoreceptor cell. The cell soma is small to medium sized (9-15 m diameter) and has 2-3 sparsely branched primary dendrites with an asymmetrical spatial organization. Dendrites are long and thin, often extending for more than 250 μm. Retinohypothalamic RGCs constitute a minority (< 1%) of the total ganglion cell population in all species, except for the mole rat in which the majority of cells projects to the SCN. The topographical distribution differs between nocturnal and diurnal species. RGCs are distributed over the entire surface of the retina in nocturnal species, but in the dorsal-nasal region in diurnal species. In all animals RGCs are sparsely and evenly distributed in homologous areas of the retina, resulting in a uniform coverage of the visual field. Single, double, and bilateral injections in the SCN show that the retinohypothalamic tract lacks precise topographic organization. Modeling this organization illustrates how retinal and optical constraints are combined to increase the efficiency of this system for the detection of diffuse, ambient light levels.

6.7 NERVE GROWTH FACTOR (NGF) AND CIRCADIAN RHYTHMS: EFFECTS OF NGF AND A PGFRECEPTOR MUTATION. Diego A. Goltzemberg, Mark W. Hard, Xue-Fen Leu, and Martin R. Ralph.
Department of Psychology, University of Toronto, Toronto, Ontario, M5S 1A1, CANADA.

The cellular responses comprising insect development are elicited by steroid moulding hormones (ecdysteroids) synthesized by the prothoracic glands (PGs). We recently showed ecdysteroid synthesis by PGs (measured by RIA) is rhythmic and under circadian control in vivo. PGs are stimulated rhythmically in vivo by a peptide hormone from the brain. Peptide release is also under circadian control. However, this rhythm of ecdysteroid synthesis is not a "slave" driven by the brain peptide. We report that PGs in vitro are directly photosensitive and contain their own circadian clock. Arrhythmic PGs (from animals reared in LL) were maintained in vitro for 12 h. A "lights off" cue given at any time in vitro promptly elicits a free-running rhythm of ecdysteroid synthesis, with peaks in the subjective night. Therefore, PGs qualify as pacemakers. These steroid-synthesizing glands are the first known endocrine pacemakers that are not derived from nervous tissue.

6.8 CALCINEURIN MODULATES CIRCADIAN RHYTHMS AND CIRCADIAN RESPONSES TO LIGHT. Martin R. Ralph and Diego A. Goltzemberg.
Department of Psychology, University of Toronto, 100 St. George Street, Toronto, Ontario, M5S 1A1, CANADA.

Circadian rhythms in mammals are generated by pacemaker cells in the hypothalamic suprachiasmatic nucleus (SCN) and are entrained to 24 hour environmental cycles by daily phase shifts of the endogenous oscillation. Light is the primary synchronizing agent, and light-induced phase shifts of circadian rhythms require the activation of biochemical pathways that result in the induction of immediate-early genes, particularly AP-1 components. Additionally, phase responses to light indicate that CsA induces phase shifts of circadian rhythms in a phase dependent manner that is similar to non-photic effects on the clock. In addition, one specific phosphatase activity may be a link through which the immune system could influence rhythm generation and entrainment in mammals.
6.9

cGMP-DEPENDENT PROTEIN KINASE INHIBITORS BLOCK LIGHT-INDUCED PHASE ADVANCES OF CIRCADIAN RHYTHMS IN VIVO. Anuradha Mathur, Diego A. Golombek and Martin R. Ralph. Department of Psychology, University of Toronto, Toronto, Ontario M5S 1A1, CANADA.

Biological rhythms in nature and in the laboratory can be synchronized by 24 hour cycles of light and dark. Synchronization is thought to be accomplished primarily through daily phase delays and advances of the endogenous circadian rhythm which in mammals is generated in the hypothalamic suprachiasmatic nucleus (SCN). In the SCN, numerous second messenger pathways may participate in photic signal transduction. In these studies, the involvement of cyclic nucleotide-dependent pathways in hamsters using inhibitors of cAMP-dependent kinase (PKA) and cGMP-dependent kinase (PKG). In a constant dark, axiogenic environment, selective and non-selective inhibitors of PKG injected near the SCN of hamsters, had no effect on phase delays produced by light pulses given in the early subjective night (early in the animals' active period), but significantly attenuated phase advances induced in the subjective night. A selective inhibitor of PKA had no effect at either time point. In addition, cGMP agonists had no effect on rhythmicity in the absence of light. These results suggest that PKG activity is necessary but not sufficient for normal photic responsiveness and that PKA activity is not required. The phase dependence of the effect of PKG inhibition supports the notion that photic entrainment is influenced by biochemical pathways that differentially regulate sensitivity in a phase-dependent manner.

6.11

DOPAMINERGIC ACTIVATION IS A TRANSIENT MECHANISM FOR ENTRAINMENT IN SYRIAN HAMSTERS. Pauline A. Davis*, Dept. of Biology, Northeastern Univ., Boston, MA 02115.

The circadian pacemaker of mammals is entrained during development by circadian rhythms of the mother. One component of the entrainment mechanism is the suprachiasmatic nucleus (SCN). In rats and hamsters, the SCN contains a lightresponsive retinal projection. In rats, SCN stimulation shifts circadian rhythms in an RL dependency of the rod opponent system (Weaver et al., 1993; Viswanathan et al., 1994). Furthermore, daily entrainment of the SCN, which in the rat is entrained by light pulses given in the early subjective night, is thought to be accomplished by a light responsive retinal projection. In these studies, we investigated the effects of dopamine agonists on the entrainment of hamsters. In vivo, hamsters from receiving 8-OH-DPAT, a 5-HT1A and 5-HT7 receptor agonist, at circadian time 11 (CT 11) increased activity to the 8-OH-DPAT produced shifts. Phase-shifting effects of this drug on the activity of the hamster were observed at circadian time 11 (CT 11). These effects were dependent on the presence of light. When 8-OH-DPAT was administered at CT 6 on all 3 days, mice administered melatonin at CT 10, and 0 (p < 0.0001). Thus, blockade of dopamine receptors by 8-OH-DPAT 10 improves the efficacy of treatment. Supported by a U.S. National Institutes of Health grant (RO1 DA097582).}

6.13

A NOVEL DOGOS REGIMEN ENHANCES THE FACILITATION OF REENTRAINMENT BY MELATONIN IN MICE. S. Benloucif' and M. L. Dubocovich*, Departments of Molecular Pharmacology and Biological Chemistry, Northwestern University Medical School, Chicago, IL 60611.

Administration of melatonin to C57BL/6 mice free running in DD shifts the phase of circadian activity rhythms according to a phase response curve, i.e., phase advances at CT 10 and phase delays at CT 2. In a recent model, administration of melatonin for 3 days at the new dark onset facilitates the rate of reentrainment following an acceleration of the 1.0 hr period. Previous studies demonstrated that whether melatonin administration 2 hr after onset at CT 10 (CT 0, 8, 6) would further enhance the facilitation of reentrainment by melatonin. C57BL/6 mice were entrained to a 12:12 LD cycle, which was photostimulated with either melatonin (0.1 mg/kg) or vehicle (1% ethanol/saline) for 3 days at either the new dark onset (CT 0) or at CT 10 on days 0, CT 0 on day 1, and CT 6 on day 2. As found previously, reentrainment was observed 3 days at CT 6 facilitated the rate of reentrainment (p < 0.02), measured by daily advances in running wheel activity after pre-shift baseline. When administered according to the above regimen only at CT 6, melatonin facilitated the rate of reentrainment compared to those mice treated at a lower dose of melatonin at CT 0, 8, 12. In addition, treatment with melatonin at CT 6 resulted in a significantly greater rate of reentrainment (p < 0.001). Thus, administration of melatonin at CT 10 improves the efficacy of treatment. Supported by a U.S. National Institutes of Health grant (RO1 DA097582).
5.16  A SEROTONIN AGONIST PHASE SHIFTS THE MELATONIN RHYTHM IN RATS.
Sally A. Ferguson, Shawn A. Rowe, and David J. Kennaway.
Circadian Physiology Group, Department of Obstetrics & Gynaecology, University of Adelaide Medical School, Adelaide, Australia 5005.

The suprachiasmatic nucleus (SCN) is entrained to the light-dark cycle via the retino-hypothalamic tract and to a lesser extent via the retina-geniculate pathway. The serotonergic projection from the raphé nuclei to the SCN has also been shown to mediate circadian entrainment. The retinohypothalamic tract is less clear; however, there is evidence that serotonin in the SCN may interact with light input to the SCN via the geniculohypothalamic tract.

6.18  NEURAL CONNECTIONS BETWEEN ANTERIOR HYPOTHALAMIC GLUCOCORTICOID RECEPTORS AND THE SONOI MADUCER OF HAMSTER SUPERACHASMATIC NUCLEUS (SCN).-immune histochemistry and immunostaining for Fos protein to assess the co-localisation of Fos and glucocorticoid receptor (GR) immunoreactivity in the hamster (BAR). This is the first demonstration of a direct connection between the SCN and the anterior hypothalamus and provides a potential mechanism for the regulation of glucocorticoid-mediated activities in the SCN.

6.19  DISTRIBUTION OF NADPH-DIAPHORASE ACTIVITY AND LIGHT-STIMULATED FOS IMMUNOREACTIVITY IN THE RABBIT SUPERACHASMATIC NUCLEUS. Shimon Amir*, Barry Robinson*.

The rabbit suprachiasmatic nucleus (SCN) exhibits a high density of Fos-immunoreactive neurons at both rest and in response to light stimulation. The distribution of Fos expression in the SCN is consistent with a role for the SCN in the modulation of circadian rhythms and is consistent with the concept of a circadian clock in the rabbit.

6.20  EFFERENTS OF THE SUPERACHASMATIC NUCLEUS FORM CLOSE APPositions WITH BOTH ESTROGEN RECEPTOR AND GABAa RECEPTORS IN HAMSTERS. Horacio O. de la Iglesia and Eric L. Bitman.

The SCN forms close appositions with both estrogen receptor (ER) and GABAa receptors in the hamster. This suggests a potential role for the SCN in the regulation of estrogenic and GABAergic functions, which may be important in the regulation of circadian rhythms and sleep-wake cycles.
Rhythms. (Research supported by a Focused Giving

vasopressin plays a modulatory role in endogenously

well-defined circadian rhythmicity and never lost

gland) and the lateral eyes are components of the circadian system of nonmammalian

Biology, University of Virginia, Charlottesville, VA 22903.

demonstration that isolated pineals of some birds, lizards and fishes, when cultured in

conditions were utilized: 1) ad-lib feeding during

the 24h cycle and 2) two separated lh feeding

sections does not affect the eyes' ability to synthesize or secrete

outputs., The hypothesis that melatonin is the homr>nal output involved

secretion by both the pineal and eyes: The pineal contributes two-thirds of the blood melatonin and the eyes the remaining one-third. An

intracocular clock drives the ocular melatonin rhythm. The eyes play a major role in the circadian system of Japanese quail because bilateral eye removal raises the body temperature and activity rhythms of female quail to damp into arrhythmicity faster in DD, although rhythmicity is less robust, and 25% are rendered arrhythmic. Optic nerve section does not affect eye and intraocular clock drives the ocular melatonin.

results suggest that a pacemaker in the eye acts to maintain rhythmicity of central oscillators (in 3X) and the ocular pacemaker is coupled to the central system via both neural and hormonal outputs. The hypothesis that melatonin is the hormonal output involved was tested by observing the effects of continuous melatonin administration (via silastic capsules) and cyclic melatonin administration (via the drinking water). Continuous melatonin administration caused arrhythmicity or period changes in pinealectomized birds in 11 and melatonin entrained normal birds when administered daily. The hypothesis that melatonin is importantly involved in linking pacemakers in the eye to the rest of the circadian system.

INTERACTIVE EFFECTS OF LIGHT INTENSITY AND CLONIDINE TREATMENT ON FREE-RUNNING CIRCADIAN RHYTHMIC IN RATS

Alan M. Hogenrasswasser, Suzanne Uweyer, and Ashley Tranaki, University of Maine, Orono, ME. 04469.

Previous research from this laboratory has shown that chronic administration of the alpha-2 adrenergic agonist, clonidine, causes a free-running period of circadian activity rhythms in rats maintained in running-wheel cages under constant light. In contrast, shortening of free-running period has not been observed consistently in similar studies conducted in constant darkness. The hypothesis that clonidine decreases the free-running period even under light intensities producing asymptotically long periods, indicating that this treatment does not produce a simple reduction in the effective light intensity. In this study, we tested this hypothesis by examining the effects of clonidine on the activity rhythms of rats maintained under a series of different light intensities, including light intensities of 100 and 500 lux, while the rats were maintained in constant darkness. The results of this study indicate that clonidine decreases the free-running period of circadian activity rhythms in rats maintained under light intensities of 100 and 500 lux, and that these decreases are proportional to the light intensity. The results also suggest that the decrease in free-running period is not due to a simple reduction in the effective light intensity.
6.27
SEPARATION OF HOMEOSTATIC AND CIRCADIAN CONTROL OF BODY TEMPERATURE IN THE GOLDEN HAMSTER. Roberto Refinetti. Department of Psychology, College of William & Mary, Williamsburg, VA 23187.

The body temperature of hamsters is homeostatically controlled, in the sense that it actively resists variations in environmental temperature. Body temperature is also under circadian control, in the sense that it oscillates with a period of approximately 24 h in the absence of oscillations in the environment. Although it has been assumed that the circadian control of body temperature is subsumed by the homeostatic control, this assumption has rarely been put to test. If there is integration of the circadian and homeostatic control of body temperature then activation of thermoregulatory responses should defend the circadian oscillation in body temperature. However, the present studies in golden hamsters show that the boundary between the thermodistance responses of ambient temperature selection and cold-induced thermogenesis opposes (rather than defends) the circadian rhythm of body temperature. Hamsters tested in a temperature gradient selected higher ambient temperatures during the low (rather than the high) phase of their body temperature rhythm. Similarly, hamsters maintained at 24°C and exposed to -10°C for an hour displayed stronger cold-induced thermogenesis during the low phase of the body temperature rhythm. It is concluded that there is separation rather than integration of the homeostatic and circadian control of body temperature in golden hamsters. The available data on rats are consistent with the same interpretation for this species.

6.29
THE EFFECTS OF EXERCISE AT THE DIFFERENT TIME OF A DAY UPON CIRCADIAN RHYTHMS OF BLDLD ELECTROLYTE AND ACTIVITY MEASUREMENT IN RATS.

1. Liang
Hunan Teacher College,Beijing city,Beijing, P.R. of China

64 Wistar rats in 2 groups-Experimental(EG) and Experimental(CG)were employed: the different 4 time-fixed points of a day: a rats in 8, after taking the treadmill:run(6km/h-20min) for 30 minutes, were put into water(28°C-29°C) for swimming until they exhausted and sunk to the bottom of the pool, and then being picked out and cut heads for sampled blood immediately. The sampling processes for CG were just same as that of EG, but the rats in CG did not take the exercise. Beckman 42 Biochemistry analyzer was used to assay the concentrations of the indexes. All the rhythmic characteristics were represented by Minnesota Consultant. The results showed that the chronotropic exercise made amplitude of the male dihydroepiandrosterone decrease (P<0.05), while that of the blood glucose was increase (P<0.05). Rats, the arophases for both indexes advanced. The finding indicated that the high intensity chronovercircise could influence the enzyme activity related to aerobic metabolism and the level of blood glucose and make the arophase advance. The patterns of these kind of the physiologic oscillations may be related to the patterns of the exercise at the different time.

13.1
CIRCADIAN RHYTHMICITY FOR LATENCY TO MATING IN DROSOPHILA. Bernard Possidente*, Helmut V. D. Hiarch and Debra K. Possidente*, Skidmore College, Biology Dept., Saratoga Springs, NY 12866.

Virgin flies were tested for copulation latency hourly in 12:12LD at the time of re-eclosion. Mean latency was shortest at about six hours after lights-on, and longest in the dark. The frequency of mating in the top quartile ("fast flies") peaked at about the same time. Mated pairs 180° out of phase followed the female’s rhythm. Wild type and per- mutants, noted inter se, were tested after four days in 12:12LD or DD. Wild types showed a peak for fast flies at about six hours after lights-on and lowest in the dark. None of these groups showed a peak for mean latency when all flies were included. Per- had a longer mean latency than wild-type. Latency was longer in DD than LD for wild-type but not per-.

We conclude that there is evidence for circadian regulation of latency to copulation, and for determination of circadian timing by the phase of the female partner. An effect of light deprivation on latency was strain-dependent.

13.2
POSSIBLE CIRCADIAN PERIOD MUTATION IN THE ALBINO DEERMOUSE, P. maniculatus. Patricia J. DeCourcey* and Sharon Lynn.* University of South Carolina, Columbia, SC 29208.

The activity patterns of an aged albino P. maniculatus with erratic activity patterns was studied by continuous time-lapse videotaping for 5 days in an LD 16:8 hr light schedule. The records indicated approximately 6-hr cycles with alternating short peaks of high level activity and intervening intervals of torpor-like rest. Attempts were made to breed the female with a wild type male for genetic analysis of a possible spontaneous circadian period mutation. Breeding was unsuccessful, and the death of the variant female precluded further activity recording in constant conditions. Two close relatives were successfully crossed, producing 9 viable offspring. The wheel-running activity of these mice was collected as actograms in order to screen for abnormal free runs or entrainment patterns. Aberrant activity patterns centered around arrhythmia in LL and very long circadian periods (25.5 hr) in constant dark. Because the unusual periodicity of the variant female may have been related to age, gender or albino condition, activity was also monitored for control groups including both young and aged individuals of both sexes for wild type as well as for albino mutant P. maniculatus. The control mice showed normal entrained nocturnal activity rhythms and free-running periods in constant darkness. The research has been supported by an NSF grant to the senior author.
nocturnal pattern of wheel running, with diurnal species (McElhinny, unpublished hours after the lights went out, and was Arvicanthus niloticus is a murid rodent with ----

Katona, Department of Psychology, Michigan no nocturnal female was paired with a male and cycle. One female, however, exhibited a activity that regularly continued for 7 relative infrequent during the day. This nocturnal female was paired with male and produced 14 offspring in 4 litters. Of these animals, 13 exhibited nocturnal wheel running patterns like the mother, and 53% exhibited diurnal patterns. None exhibited intermediate patterns. Thus, the mechanisms determining the distribution of activity relative to the light-dark cycle can be dramatically influenced by selective breeding in this species.

Individual variation in the circadian vs. ultradian control of running wheel activity in meadow voles.

13.6 Modifications of ultradian and circadian CO2 oscillations by several environmental changes. M. Stuufel, A. Perra-

13.5 INDIVIDUAL VARIATION IN THE CIRCADIAN VS. ULTRADIAN CONTROL OF RUNNING WHEEL ACTIVITY IN MEADOW VOLES.

Marie Kerbeshian* and F.H. Bronson*. Department of Zoology, University of Texas, Austin, TX 78712

Two populations of meadow voles (Microtus pennsylvanicus), one born in the laboratory and one captured in the field, showed continuous variation in the degree to which their running wheel activity was under circadian control. Many individuals were primarily or completely nocturnal in their use of a running wheel, other individuals displayed a purely ultradian rhythm of activity, and a few of the latter even ran more in the daytime than they did at night. Most but not all individuals' daily patterns of activity were stable over a three month period of time, but a one generation selection experiment involving the two extreme phenotypes yielded no evidence of a genetic basis for this variation. Further experimentation showed that the variation in activity patterns was not the result of a general disruption of the circadian system. Voles from the two extreme phenotypes did not differ in the timing of their daily rhythms of pineal melatonin content or circulating levels of corticosterone. Thus, the individual variation in running wheel activity patterns in meadow voles is most likely the result of an experiential influence that acts specifically to partially or completely uncouple non foraging locomotion from circadian control while allowing or promoting ultradian control. The nature of the experience that yields this variation is unknown.

Supported by NIH grant HD-30423 and an NSF Graduate Research Fellowship

14.1 ANNUAL FAT CYCLE OF THE VIRGINIA OPOSSUM, Didelphys virginiana: a circadian rhythm synchronized by prevailing temperature. West Virginia School of Osteopathic Medicine, 400 N. Lee St., Lewisburg, WV 24901

Over a two year period, three male and two female opossums were kept in captivity. Food and water were available ad libitum and the daily light-dark cycle was identical to those of the prevailing ambient photoperiod, and ambient temperature was maintained at 22°C. Body weight and food consumption were measured on several days each week. For males, the annual period of rapid weight gain (2.16 ± 0.26 kg, at a rate of 0.02 ± 0.01 kg/day) was initiated between mid-August and mid-September, it lasted for 67 to 142 days, and ended between 1 November and 1 January. For females, the first period of rapid weight gain in captivity (2.00 ± 0.21 kg, at a rate of 0.023 ± 0.001 kg/day) was comparable to those measured for males, but the second one had a reduced magnitude and rate of gain (0.88 ± 0.32 kg, at a rate of 0.016 ± 0.007 kg/day). Timing of the females' period of rapid weight gain was similar to that of the males'. If the Virginia opossum possesses an endogenous circannual rhythm of fattening, these data suggest that seasonal changes in photoperiod may serve to synchronize it with prevailing environmental conditions. This study was supported by a grant from the West Virginia School of Osteopathic Medicine.

14.2 CIRCADIAN RHYTHMS OF PINEAL MELATONIN PRODUCTION IN FEMALE RATS ARE ABOLISHED BY GROWTH OF MALIGNANT TUMORS. H Bartsch*, C Bartsch*, J Debroecker*, D Makke*, T Lippert*. Section of Clinical Pharmacology, University Women's Hospital, and Institute of Physiological Chemistry, D-72076 Tübingen, Germany; *Central Institute for Laboratory Animal Breeding, D-30455 Hannover, Germany

The pineal hormone melatonin shows a pronounced diurnal rhythm which peaks during the dark period. Growth of malignant tumors decreases the CR amplitude more than those of GR and reduces the CR phase advance in rats. Melatonin suppression by xenograft transplantation of urinary bladder carcinomas was due to a decrease in the duration of the nocturnal peak. Xenograft transplantation of urinary bladder carcinomas was due to a decrease in the duration of the nocturnal peak. Xenograft transplantation of urinary bladder carcinomas was due to a decrease in the duration of the nocturnal peak.

MELATONIN (MEL), a hormone of the pineal gland, mediates photoperiod-induced changes in reproduction and thermoregulation in a number of rodent species. The nocturnal secretory pattern of MEL is controlled by the suprachiasmatic nucleus (SCN). Recent studies in our laboratory and the laboratories of others have revealed that MEL can also modulate circadian clock function. In the Djungarian hamster, a number of studies indicate such an effect. 1) Daily MEL injections given 3 hrs before dark onset in long (LD 16:8) animals cause a strongly positive phase angle of wheel-running activity. 2) Daily subcutaneous MEL injections given 3 hrs before onset of wheel-running activity in short day-insensitive individuals (characterized by a robust negative phase angle and a compressed and inverse circadian organization of wheel running activity and an associated sensitivity to a short photoperiod (resulting in gonadal regression, etc). 3) Sensitivity of in vitro SCN neurons to MEL injection about 3 hrs prior to projected light off. 4) Daily MEL injections at the new light on asynchrony on entrainment of wheel running activity following a 6 hr phase advance in the light:dark cycle. Given these multiple effects, it remains unclear why MEL should affect clock function during subjective day, a time when this hormone is not normally present. (NIH MHS2546)


The golden spiny mouse Acomys russatus exists in hot and arid environments. We have observed that in the laboratory it displays circannual activity patterns in the field and in the laboratory but is driven into diurnal activity when it coexists with the common spiny mouse Acomys cahirinus. The aim of the present study was to investigate whether the social cues released from A. cahirinus affect the circadian rhythmicity of A. russatus and its response to a change in photoperiod; b) does the presence of the pineal gland affect the responses to the social cues. The daily rhythms in activity and body temperature Tb in A. cahirinus or its odor. In addition, these responses were also studied in sham-operated and pinealectomized mice. Indicated that the daily rhythm of Tb and activity of A. russatus respond to the change in photoperiod but these responses differ in the absence and presence of A. cahirinus or its odor. In these experiments, these responses were eliminated in pinealectomized animals. These data indicate a role for the pineal in seasonal adaptation in the presence of social cues. This research was supported by the BSF.

SEASONAL BREEDING, MELATONIN INFUSIONS, AND THE TINY PINCAL GLAND OF A TROPICAL BAT, ANOURA GLYAENOTY on September 9, 2003 at 21:47:15 by Mark Binkley.


Mammals in the deep tropics apparently do not (and perhaps cannot) use photoperiod to regulate seasonal breeding. It has been hypothesized that these deep tropical mammals might, therefore, have a reduction in both the size of their pineal gland and the secretion of melatonin. A population of the tropical bat Anoura glaucopterys on the Caribbean island of Trinidad lacks reproductive responses to photoperiod even though breeding is highly seasonal. Births occur only in November or December, and this seasonal breeding must be enforced using a non-photoperiodic cue. Consistent with the hypothesis, Anoura glaucopterys have a minute, thin, and rod-like pineal gland (type ABY). However, despite having a very small pineal gland, this species produced a far higher pineal melatonin pattern. Serum melatonin levels in most individuals were undetectable during the light period and rose to a peak averaging 100 pg/ml in the last third of the dark period. Our results provide some weak support for the hypothesis, but suggest that melatonin levels may not be closely related to pineal gland size.
14.9

CIRCADIAN RHYTHMS OF INDOLE METABOLISM IN GONY-
AULAX POLYEDRA. Rüdiger Hardeland*, Birgit Fürlberg*, Gudrun
Behmann*, Susanne Buckland*, Burkhard Pögeker* and Ivonne Balzer*.

The dinoflagellate G. polyedra exhibits a circadian rhythm of melatonin characterized by a sudden increase shortly after the onset of darkness. A temperature step from 20 to 15 °C leads to a manyfold augmentation of this carboxylase is not rate-limiting for indoleamine formation. The rhythm of tryptophan hydroxylase, shows a pronounced circadian rhythm, which is almost antiphase to that of melatonin. Aromatic amino acid de-
carboxylase is not involved in the induction of this rhythm. The rhythm of melatonin is not explained by the pannum of hydroxyindole O-methyltrans-
ferase activity. This enzyme may contribute to the nocturnal maximum of 5-
methoxytryptamine, another cys-tyrindole indoleamine, which is, however,
profoundly formed via deacetylation of melatonin by aryl acyiamidase
and which attains its maximum in the second half of the night. Our data
suggest that the rise of melatonin is mainly caused by an increase of N-acetyl-
yntransferase, an enzyme which is unstable in preparations from GonouaIux
and the rhythm of which is not known with certainty, and that the decline of
melatonin observed in the second half of the night results from aryl acy-
aimidae, presumably in connection with a decline of N-acetyltransferase.

14.10

EFFECT OF A SHORT PHOTOPERIOD ON ESTROGEN
RECEPTOR IMMUNEACTIVITY IN SYRIAN HAMSTER BRAIN.
R. Mangels* and J.B. Powers* Neuroscience and Behavior Program,
University of Massachusetts, Amherst, MA. 01003

Syrian hamsters are seasonal breeders. In this species, reproduction is inhibited by prolonged exposure to a short photoperiod (SP). One effect of SP is to alter neural sensitivity to the effects of gonadal steroids. In female hamsters, SP exposure enhances the negative feedback effects of estrogen (E) on gonadotropin secretion and inhibits the facilitatory effects of estrogen and progesterone (P) on luteinization. This study examined photoperiodic influences on luteinization and estrogen receptor immunoreactivity (ERIR). Ovariectomized Syrian hamsters were housed in either a long photoperiod (LP; 16L:8D) or SP (16L:8D) for 10 weeks. Some of these then received E+P and were tested for luteinization. The remainder were sacrificed, and immunocytochemistry was performed using the H222 ER-antibody. Computerized image analysis was used to assess ERIR. SP females exhibited significant impairments in behavior. Initial results indicate that SP does not affect the number of ER-containing cells in either the ventromedial or ventralateral hypothalamus. Analysis of additional estrogen responsive areas will be presented.

14.11

EFFECT OF TIMED DAILY INJECTIONS OF 5-HYDROXYTRYPTOPHAN AND L-DIHYDROXYPHENYLALANINE IN YOUNG MALE SYRIAN HAMSTERS.
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Physiology, West Virginia School of Osteopathic Medicine, 400 North
Lee Street, Lewisburg, WV 24901.

Temporal expression of neurotransmitter precursor.
5-hydroxytryptophan (5-HTP) and L-dihydroxyphenylalanine (L-DOPA), have been proposed to regulate seasonal physiological changes in vertebrates, such as Syrian hamsters. We tested whether rapidly growing, 1-week-old, male Syrian hamsters (Mesocricetus auratus) would show changes in body, testis, or epididymal fat pad weights after 14 daily intraperitoneal injections of 5-HTP and L-DOPA in either a 0-hr, 8-hr, or 16-hr rotation. Some groups received only 5-HTP, L-DOPA, or saline. There were also several non-injected controls. Injections were given at 0000, 1400, or 2200 hrs. The hamsters were kept on continuous light for 1 week before the experiment and during the 2 week period of injections. Each group of 9 hamsters receiving injections had subgroups of 3 hamsters that received injections on 3 different schedules allowed by hours of injection to test for any time-of-day effects. Body weight changed from about 67 g to about 100 g. The 0-hr rotation group had depressed (p < 0.05) testis weights (1.40 ± 0.17 g) as compared to the 8-hr group (2.00 ± 0.18 g) or the 16-hr group (2.00 ± 0.17 g) and slightly depressed epididymal fat pad weights. Effects of temporal relations suggested by colleagues were supported. Funded by the Gans Research Fund and WVSOM.

14.12

EVIDENCE FOR SEPARATE SITES OF CONTROL OF THE PHOTOPERIOD AND DAILY RHYTHMS IN FETAL PROLACTIN IN THE SHEEP
C. McMillen, L. Small, C. Young and D.G. Moonlight
Department of Physiology, The University of Adelaide, Adelaide, SA and
Monash University, Clayton, Vic, Australia.

We have investigated the effect of surgical disconnection of the fetal hypothalamus and pituitary (HPD) on the relationship between length of photoperiod and testis prostate and epididymid weights and on the daily rhythm in fetal melatonin (MT) and prolactin (PRL).

Fetal HPD or a sham operation was carried out around 110h gestation. Fuses carrying either LPD fetal sheep (n=10) or intact fetal sheep (n=12) were then exposed to long (LL:16L:8D) or short (SL:8L:16D) photoperiods in the laboratory. Some of these then received E+P and were tested for lordosis. The remainder were sacrificed, and immunocytochemistry was performed using the H222 ER-antibody. Computerized image analysis was used to assess ERIR. SP females exhibited significant impairments in behavior. Initial results indicate that SP does not affect the number of ER-containing cells in either the ventromedial or ventralateral hypothalamus. Analysis of additional estrogen responsive areas will be presented.

14.13

PHOTOPERIODIC REGULATION OF LH, FSH, GH, α-MSH AND β-ENDORPHIN, BUT NOT PROLACTIN, REQUIRES AN INTACT HYPOTHALAMO-PITUITARY SYSTEM IN RAMS.
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We have previously shown that the photoperiodic regulation of the secretion of prolactin persists in hypothalamo-pituitary disconnected (HPD) rams providing evidence that melatonin acts, at least in part, in the pituitary gland to mediate effects of photoperiod (I Neuroendocrinol. 6: 251-260, 1994). To establish whether this pituitary effect is restricted to the control of prolactin we have now measured the long-term changes in the blood concentrations of LH, FSH, GH, α-MSH and β-endorphin (β-END) in the same groups of HPD (n=8) and control (n=8) rams. The animals were blood sampled twice weekly while exposed to alternating 16-week periods of long (16L:8D) and short (8L:16D) days for 73 weeks. In the control rams there was a significant (p<0.01) effect of photoperiod on the plasma concentrations of LH (2.39 ± 0.23 vs 0.80 ± 0.09 ng NIH-S23H/ml, mean ± SEM long vs short days respectively, FSH (152 ± 5.3 ± 53 vs 10.64 ± 1.62 ng NIH-FSH-S14ml, α-MSH (174 ± 20.9 ± 23 vs 42.5 ± 4.3 pg/ml) and β-END (156.9 ± 66.7 vs 159.1 ± 20.1 pg/ml). In the HPD rams there was no significant photoperiodic modulation of any of the pituitary hormones: LH (0.34 ± 0.05 vs 0.38 ± 0.05 ng/ml), FSH (2.31 ± 0.33 vs 1.54 ± 0.33 ng/ml, GH (11.3 ± 0.13 vs 1.34 ± 0.18 ng/ml), α-MSH (170.6 ± 41.0 vs 144.6 ± 26.5 pg/ml) and β-END (490.7 ± 28.8 vs 499.7 ± 26.5 pg/ml). The results show that the HPD operation caused a permanent decrease in the secretion of LH, FSH and GH, and an increase in the secretion of α-MSH and β-END consistent with the postovulative rise in the hypothalamus. The disruption of the photoperiodic responses in the HPD animals indicates that an intact hypothalamo-pituitary system is required for the photoperiodo-melanocortin relay to affect the secretion activity of gonadotrophs (LH and FSH) and melanocortins (α MSH and β-END).
15.1

DAILY VARIATIONS OF ADENOSINE METABOLISM IN HUMAN BLOOD.

Victoria Changara de Sáéchez, Orlando Hernández-Montes, Mario Sáéchez, Rosario Valerio,
*Lucas Rosenthal, *Federico Fernández-Castro and *Rani Debbane-Cole, Department of
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Daily variations of adenosine and its metabolites in several tissues of the rat, could be
involved in the fasting and feeding metabolic pattern, as well as the sleep-wake cycle
of the rat, mainly through the modulation of energy homeostasis of the cell, as well
as changing the membrane structure and function. These findings lead us to explore
similar rhythm in humans investigating if adenosine and its metabolism, including
the activity of its metabolizing enzymes in the blood presented daily varations.
Young healthy males were adapted for two days to the room conditions where the
experiment was performed. Awakening period was from 06:00 to 23:00 h, and the
blood sampling was done every hour from a heparinized catheter placed in the
tibial vein. The results showed that adenosine and its metabolites (inosine, hypoxanthine and xanthine acid) adenosine synthetizing (5'-adenylylaminohexose hydrolase and 5-nucleotidase) degrading (adenosine kinase) enzymes, as well as adenosine nucleotides (ATP, ADP and AMP) presented statistically significant fluctuations analyzed by ANOVA. The energy
charge and the phosphate levels do not present significant change. When the cosinor
method was applied to the observed changes, most of the studied parameters presented
oscillatory components close to 24 h, except adenosine and lactate which showed only
ultradays fluctuations. The acrophase of the parameter that presented rhythm pattern
was observed during the dark or light period. The results suggest that adenosine and
its metabolism also might play an important role in the rhythm pattern of the humans.

15.3

IDENTIFICATION OF PHOTIC RESPONSIVENESS IN THE
SCN OF NEWBORN PRIMATES. S.A. Bivies, Riley Hosp.,
Indiana Univ. Sch. of Med., Indianapolis, IN 46202.

Little is known about how the developing circadian system is
regulated in primates. To provide insights developing primate
clock, baboons (Papio sp.) were studied. Histologic studies
showed that the baboon SCN appeared similar in appearance
and function to the SCN of humans. The baboon SCN also expressed 5'-I
melatonin and 5'-IJSCH-23982 binding indicating the presence of
melatonin and D1 dopamine receptors. To test for photic
responsiveness, the 2-deoxy-2-'CJglucose (DG) method was used
to study animals at the end of gestation. After these animals
birthday, animals were injected with DG at either mid-day, mid-night, or
after 2000 lux of light-at-night. Autoradiographic images showed
increased metabolic activity in the SCN during the day, but not at
night. Following light at night, SCN metabolic activity increased
dramatically. Light-at-night also induced c-Fos mRNA expression
in the SCN indicating light-respondiveness. These data provide
the first direct evidence that the primate SCN are responsive to
light at birth.

15.5

MELATONIN-INDUCED TEMPERATURE SUPPRESSION AND ITS
ACUTE PHASE-SHIFTING EFFECTS CORRELATE IN A DOSE-
DEPENDENT MANNER IN HUMANS. Stephen Deacon* and Josephine
Arendt. Chronobiology Laboratory, Endocrinology and Metabolism Group,
Indiana Univ. Sch. of Med., Indianapolis, IN 46202.

Melatonin is able to phase shift the endogenous circadian clock and can
induce acute temperature suppression. It is possible that there is a direct
relationship between these phenomena. In a double-blind, placebo-controlled
crossover study, 6 healthy volunteers maintained a regular sleep/wake cycle in
a normal environment. From dusk until 7400 h on days (D)1-4 subjects
remained in dim artificial lighting (< 50 lux) and darkness (< 1 lux) from
2400 h. At 1700 h on D3 either melatonin (0.05mg, 0.5mg or 3mg) or
placebo was administered. Melatonin treatment induced acute, dose-dependent
temperature suppression and decrements in alertness and performance
efficiency. On the night of D3, earlier sleep onset, shorter sleep duration and
decrements in the alertness and performance response. A significant dose-dependent
phase advance in the plasma melatonin onset time and temperature nadir (D4-5) was observed with a trend for the alertness
and performance efficiency. On the night of D3, earlier sleep onset, shorter sleep duration and
decrements in the alertness and performance response. A significant dose-dependent
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and performance response.


EFFECTS OF HIGH DENSITY LiO3 ON DIURNAL BLOOD PRESSURE IN WISTAR-KYOTO (WKY) AND SPONTANEOUSLY HYPERTENSIVE RATS (SHR)

DA Calabrese, S-T Zhu, M Wyss, and S Oparil, Hypertension Program, University of Alabama at Birmingham, Birmingham, AL.

We have previously demonstrated that high (8%) dietary NaCl exposure significantly increases daytime mean arterial pressure (MAP) in SHR, but not in normotensive WKY controls. In the following study, radio-frequency transducers (DataSciences, Inc.) were implanted into 8-9 week old male SHR and WKY allowing for sampling of mean aortic pressure every 2-3 min. After 10 days of recovery, animals were fed basal (1%) or high (8%) NaCl diet for 7 weeks. A rhythm analysis was applied by means of the nonlinear least-squares fitting program PHAROSFIT. Plate of bone loss in MAP were

MAP

Clock Hour

WKY% 8

A B C D

Scapula 15.17±10.50 15.02±10.38 15.38±10.72

Lau 23.43±10.56 23.02±10.32 22.33±10.40

Sleepless 0.24±0.09 0.52±0.26 0.36±0.25

Activity 17.65±5.2 7.87±3.4 11.18±5.7

Light 0.84±0.1 0.76±0.7 0.55±0.4

Amplitude 0.30±0.07 0.47±0.28 0.78±0.43

sig. diff. (p<0.05) from A & C.

These data demonstrate that high dietary NaCl significantly increases 24-hour MAP in SHR, through increases in both daytime and nighttime MAP. In WKY, high dietary NaCl increases nighttime MAP, but decreases daytime MAP, with no net effect on 24-hr MAP.

EVIDENCE FOR A THALAMIC ROLE IN HUMAN CIRCADIAN RHYTHM and HUMAN BIOLOGY: BASIC PHYSIOLOGICAL/CLINICAL ASPECTS

15.7

SOME CLOCK: MODELS IN ALZ IN DESCRIBING DEMENTIA SHAPING WITH AGING.

Hazle Hosain, Richard P. Spencer, University of Connecticut Health Center, Farmington, CT 06030.

Even after maturity, bone remodeling continues throughout life. We have utilized models of tubular bone in an effort to describe age-related changes in diameter and mineral. A simple model assumed that, although outer radius (R) grows with age, inner radius (r) might change to keep area constant and s = [(1-Age)/C]2. A second model is based on the fact that bone strength remains constant, then k = - r = k, s = l, and this results in an equation in which bone area (A) is in direct proportion to r. For a constant amount of mineral (M), bone mineral density would fall depending upon the area, with k = M, A for given length. The first model gives:

A = B + (C-x)2

for the second: B = 2x(C-x)2

These models may be useful in comparing the sexes as well as different populations, and in evaluating the long term effects of therapeutic interventions.

15.8

A MATHEMATICAL MODEL FOR THE RHYTHMICITY OF ENDOCRINE SYSTEM. Biological clock genes control many feeding patterns and circadian rhythms. We have previously demonstrated that high (8%) dietary NaCl exposure significantly increases 24-hour MAP in SHR, but not in normotensive WKY controls. In the following study, radio-frequency transducers (DataSciences, Inc.) were implanted into 8-9 week old male SHR and WKY allowing for sampling of mean aortic pressure every 2-3 min. After 10 days of recovery, animals were fed basal (1%) or high (8%) NaCl diet for 7 weeks. A rhythm analysis was applied by means of the nonlinear least-squares fitting program PHAROSFIT. Plate of bone loss in MAP were

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Activity 17.65±5.2 7.87±3.4 11.18±5.7

Light 0.84±0.1 0.76±0.7 0.55±0.4

Amplitude 0.30±0.07 0.47±0.28 0.78±0.43

sig. diff. (p<0.05) from A & C.

These data demonstrate that high dietary NaCl significantly increases 24-hour MAP in SHR, through increases in both daytime and nighttime MAP. In WKY, high dietary NaCl increases nighttime MAP, but decreases daytime MAP, with no net effect on 24-hr MAP.
15.13 THE ROLE OF MELATONIN IN MODULATING THE CIRCADIAN RHYTHM OF SLEEP. Adam CL, Pryor, Cameron J van den Hurk and Derek Duncan, Sleep & Circadian Rhythm Laboratory, University of Adelaide, Dept. of Obstetrics & Gynaecology, The Queen Elizabeth Hospital, Woodville SA 5011, Australia.

Recent experiments indicate that the pineal hormone melatonin may modulate the circadian sleep and wakefulness rhythm via a hypothalamic effect. It decreases core temperature and increases sleep quality, then we hypothesize that suppression of melatonin production should induce sleep disruption and core temperature decrease. To investigate this, melatonin levels of 8 healthy young males aged between 19-23 years were manipulated during a four day protocol. The effects of melatonin suppression on nocturnal sleep between 2300 and 0700 h were studied using polysomnography. On the first two nights subjects completed an adrenalin and a baseline recording night. The following two nights consisted of a melatonin condition and a control condition, to which subjects were randomly assigned. Melatonin suppression was achieved by oral administration of 100 mg at 1900 h and subjects were placed between 2200, 0200 and 0400 h. On the control night, sleep onset by hypothermic effects and are consistent with a model whereby the age-related disturbances of the sleep/wake cycle may reflect abnormal circadian function. Therefore, in this study, we examined whether alterations in the rhythm of the plasma levels of melatonin and changes in the sleep architecture are observed in cirrhotic patients with subclinical hepatic encephalopathy (HE). We studied 24-hour plasma melatonin profiles and sleep recordings by polysomnography in seven non-alcoholic cirrhotics and carefully matched controls. Neuropsychological testing confirmed the presence of subclinical hepatic encephalopathy. Cirrhotic subjects showed markedly elevated melatonin levels during daytime (93.7±20.7 μg/ml vs 15.9±1.06 μg/ml in controls at 8:00 AM, p<0.007) hours, when melatonin is normally absent. In addition, the time of onset of melatonin rise and the peak time of melatonin levels were constant and significantly delayed (Onset at 9:30±30 min vs 7:50±26 min in controls, p<0.013). Peak time at 5:36 AM ±29 min (p<0.001). Although polysomnographic recordings were not different, sleep diaries indicated increased nocturnal awakenings and more frequent daytime naps. Conclusion: Alterations of circadian rhythmicity contribute to the disturbances of the sleep/wake cycle frequently found in cirrhotic patients. This disruption could be a result of metabolic brain disturbances occurring in hepatic encephalopathy.

15.15 MELATONIN: A NEUROENDOCRINE MEDIATOR OF THE CIRCADIAN RHYTHM OF TEMPERATURE & SLEEP. Cameron J van den Hurk and Derek Duncan, Sleep & Circadian Rhythm Laboratory, The University of Adelaide, Dept. of Obstetrics & Gynaecology, The Queen Elizabeth Hospital, Woodville SA 5011, Australia.

With age, the secretion of melatonin by the pineal gland decreases and the nocturnal drop in core temperature (Tc) is reduced. These findings suggest that endogenous melatonin may mediate the circadian rhythm of sleep/wake behaviour by its hypothalamic effect on Tc. To examine this hypothesis, we suppressed subjects' nocturnal melatonin secretion and measured Tc and sleep onset latency (SOL). Ten young healthy males were studied on three consecutive nights, between 2300 and 0700 h. One of three conditions was completed each night: baseline, nocturnal melatonin suppression (MS), or a control condition (AM). Nocturnal melatonin suppression was achieved by oral administration of atenolol (100 mg) at 1900 h. In the AM condition, any effect of atenolol on melatonin suppression was controlled for by the administration of atenolol at 1900 h followed by 1 mg oral doses of melatonin at 2200, 0200, 0400 h. This was in order to restore at least physiological levels of melatonin. Atenolol and melatonin placbos were given at matching times in the baseline condition. Mean 6-sulphatoxy melatonin (6s-aMT) in nightly urines was used as a measure of total melatonin production. 6s-aMT was decreased to a level typical of those observed in the elderly (25% of baseline; p<0.05). Melatonin suppression (MS) also significantly increased mean nocturnal Tc (p<0.05), mean nocturnal SOL to stage 1 (p<0.001) and mean nocturnal SOL to stage 2 (p<0.05). Exogenous melatonin given in the AM condition, reversed the increases in Tc and SOL to values not significantly different from baseline values. These results indicate that melatonin may facilitate sleep onset by hypothalamic effects and are consistent with a model whereby the age-related decline in melatonin secretion and an attenuated drop in nocturnal Tc mediates some of the increases in age-related sleep disturbances. If this is the case, then exogenous administration of melatonin may prove beneficial in alleviating sleep disturbances associated with increased nocturnal Tc.


Patients with liver cirrhosis often complain of the inability to sleep during the night while falling asleep during the day. Disturbances in their sleep/wake cycle may reflect abnormal circadian function. Therefore, in this study, we examined whether alterations in the rhythm of the plasma levels of melatonin and changes in the sleep architecture are observed in cirrhotic patients with subclinical hepatic encephalopathy (HE). We studied 24-hour plasma melatonin profiles and sleep recordings by polysomnography in seven non-alcoholic cirrhotics and carefully matched controls.

15.17 TUMOR-AGE DEPENDENT DEPRESSION OF THE CIRCADIAN AMPLITUDE OF MELATONIN IN PATIENTS WITH UNOPERATED PRIMARY BREAST AND PROSTATE CANCER IS CONNECTED TO DISTURBANCES IN THE SECRETION OF PROLACTIN, TSH AND GROWTH HORMONE. C Hartsh*, H Hartsch*, S H Fluechter*, H Lippert*. Section of Clinical Pharmacology, University Women's Hospital, D-72076 Tubingen, Germany, *Department of Urology, Klinikum Winterberg, D-66119 Saarbrucken, Germany

The circadian profiles of melatonin as well as of central and peripheral hormones were determined in untreated patients with primary breast cancer (BC, n=23), age-matched controls with benign breast diseases (n=15) as well as in untreated patients with primary prostate cancer (PC, n=17) and carefully matched controls with benign prostatic hyperplasia (n=20) prior to operation. The amplitude of melatonin was depressed by approximately 50% in BC and PC compared to controls. A sub-division of cancer patients according to tumor size revealed a progressing decline of melatonin from T1 to T3 which was accompanied by parallel disturbances in the circadian secretion of prolactin, TSH and growth hormone. The circadian profiles of LH, FSH, thyroxine, testosterone and cortisol on the other hand remained relatively unchanged. Inhibition of pineal melatonin secretion by tumor growth may be responsible for the observed disturbances of adrenocorticotropic hormone secretion via central neuroendocrine mechanisms.


In the timing of breast cancer tumor growth within the fertility cycle determines its curability (Lancet 1993;343:494). Natural killer cell activity and interleukin-2 production covary with this cycle of curability (JNCI 1990;82:1232) with cellular immune defenses most robust and surgical curability highest during the early luteal phase. We asked whether breast cancer tumor take and growth vary with the phase of the fertility cycle in mice. 40 cycling 12 wk C3H/HeJs female mice received syngeneic breast cancer cells subcutaneously daily proestrus (P), estrus (E), metestrus (M), or diestrus (D). Time to tumor appearance, tumor size and fertility phase were measured. We found no difference in tumor growth (p<0.01). When average tumor size is measured over time a typical sigmoidal growth pattern is observed. When, however, the same data are organized according to fertility cycle position of measurement, a prominent phase locked rhythm between tumor size and fertility cycle phase is apparent. These results suggest that breast tumors in mice may be waxing and waning with the fertility cycle as was observed by Sir Astley Paston Cooper in 1836 in women with breast cancer (Proc. Prat. Surgery 1836).