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.

Nonscientist 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 (nonscientist–family members of registrants)	$ 60

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 Reigstration 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 July 12, 1995
Note ☛ Each day will focus on one topic as indicated by the headings	**Focus** Molecular Analyses of Circadian Oscillators and their Output	**Focus** Analyses of Circadian Clocks at the Level of Cells and Tissues	**Focus** Circadian and Circannual Rhythms in Organisms	**Summary Reports of Study Groups** on Genetic and Physiological Analyses of Circadian Clocks
2:00-9:00 PM–101 Collis *Registration*	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 **Jay Dunlap**, Dartmouth Med Sch **Joe Takahashi**, Northwestern U **Amita Sehgal**, 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 Karsch**, U Michigan **Stéphan Reebs**, U Moncton **Bruce Goldman**, U Connecticut	8:30 AM-1:00 PM–Cook Auditorium *Group Reports* **Carl Johnson**, Vanderbilt U **Terry Page**, Vanderbilt U **Rae Silver**, Barnard Col **C.P. Kyriacou**, U Leicester **J. Woodland Hastings**, Harvard U **Patricia DeCoursey**, U South Carolina **Eberhard Gwinner**, Max-Planck Inst, **Derk-Jan Dijk**, Harvard Med Sch **Anna Wirz-Justice**, Psych Univ, Basel
7:30-8:00 PM–Cook Auditorium *Welcome Session:* Meeting Overview and Organizational Information	10:30 AM-12:30 PM–Cook Auditorium **Molecular Biology of the Circadian Clock and its Output** *Participants:* **C. Rob McClung**, Dartmouth Col **Ueli Schibler**, U Geneva, Switzerland **Jennifer Loros**, Dartmouth Med Sch **William Schwartz**, U Massachusetts **Mary Pierce**, SUNY Hlth 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	10:30 AM-12:30 PM–Cook Auditorium **Human Circadian Control, Physiology and Clinical Applications** *Participants:* **Ken Ichi Honma**, Hokkaido U **Charles Czeisler**, Harvard Med Sch **Josephine Arendt**, U Surrey **Thomas Wehr**, NIMH **Björn Lemmer**, JW Goethe 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**	4:00-5:30 PM–Collis Common Ground *Poster Sessions* **Authors in Attendance**
	7:30-8:30 PM – Cook Auditorium **Molecular Analyses of Circadian Oscillators and Their Output: Model Systems and Molecules** Speaker: **Michael Robash**, Brandeis U	7:30-8:30 PM–Cook Auditorium **The Circadian System in Vertebrates** *Speaker:* **Robert Moore**, U Pittsburgh	7:30-8:30 PM–Cook Auditorium **Circadian Organization in the Vertebrates: New Directions** *Speaker:* **Michael Menaker**, U Virginia	7:00-9:00 PM–Alumni Hall *Banquet and Lecture* **Circadian Clocks, Past, Present, and Future** *Speaker:* **J. Woodland Hastings**, Harvard U

Invited Session Abstracts

Saturday

Sunday

Tuesday

Poster Sessions

Sunday/Monday

Tuesday/Wednesday

2.2

Circadian Clock of Cyanobacteria: Genes In and Out of the Oscillator. T. Kondo[1], S. S. Golden[2], C. H. Johnson[3] and M. Ishiura[1]: [1]National Institute for Basic Biology, Okazaki 444, Japan [2]Dept. of Biology, Texas A&M University, College Station, TX 77843, [3]Dept. of Biology, Vanderbilt University, Nashville, TN, 37235

By introduction of a bacterial luciferase genes into cyanobacterium (*Synechococcus* sp. PCC7942), we developed a transformed strain that expresses luciferase as a bioluminescent reporter of the circadian clock. From chemically mutagenized cells, a diverse set of mutants was isolated. Periods of mutants ranged between 15 and 60 h and many mutants were arhythmic. To isolate genes that can 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 trying to determine whether recovered genes are different by gene transfer into mutant 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 arhythmic mutants to examine whether arhythmic phenotypes are caused by disruption of the central oscillators or of the output pathways. As all bioluminescent clones obtained were arhythmic, these arhythmic mutants are probably mutations of the central oscillator.

REFERENCES:

1. Kondo T., Strayer C.A, Kulkarni R.D., Taylor W., Ishiura M, Golden S.S. and Johnson C.H. (1993) Circadian rhythms in prokaryotes: luciferase as a reporter of circadian gene expression in cyanobacteria. Proc.Natl Acad.Sci., **90**, 5672-5676.

   This paper describes a development of cyanobacteria that reports circadian clock by bacterial luciferase reporter.

2. Kondo T. and M. Ishiura. (1994) Circadian rhythms of cyanobacteria: Monitoring the biological clock of individual colonies by bioluminescence. J. Bacteriol., **176**, 1881-1885. Monitoring of the bioluminescence rhythm from colonies of transformed cyanobacteria on agar plate.

   Monitoring bioluminescence rhythm from individual colony on agar plate was reported.

3. Kondo T., N.F.Tsinoremas, S. S. Golden, C. H. Johnson, S. Kutsuna and M. Ishiura (1994) Circadian clock mutants of cyanobacteria. Science, **266** 1233-1236

   This paper describe clock mutants of cyanobacteria and cloning of mutant gene by complementation.

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 be made regarding the regulation of genuine components (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 transcript, 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 sets the clock to a unique and predicted phase. Recent data (Merrow and Dunlap, 1994) also show phylogenetic conservation of *frq* structure and function. Finally, it is now clear that light acts rapidly (within 2 minutes) 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 resetting of the clock by light (Crosthwaite, Loros and Dunlap, submitted). The fluence threshold, 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 in which 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:

Jay C. Dunlap
Genetic Analysis of Circadian Clocks
Annual Review of Physiology
55, 683 - 728, 1993.

Merrow, M. and Jay C. Dunlap
Intergeneric Complementation of a Circadian Rhythmicity Defect: Phylogenetic Conservation of the 989 amino acid Open Reading Frame in the Clock Gene Frequency
EMBO Journal
13, 2257 - 2266, 1994.

Aronson, B., Johnson, K., J. Loros, and Jay C. Dunlap
Negative Feedback Defining a Circadian Clock: Autoregulation of the Clock Gene *frequency*
Science
263, 1578 - 1584, 1994.

2.4

GENETIC DISSECTION OF THE CIRCADIAN OSCILLATOR IN THE MOUSE. Joseph S. Takahashi, M. H. Vitaterna, D. P. King, A. M. Chang, P.L. Lowrey, W. F. Dove*, F. W. Turek, L.H. Pinto. NSF Center for Biological Timing, Department of Neurobiology and Physiology, Northwestern University, Evanston, IL 60208; *McArdle Laboratory for Cancer Research and Laboratory of Genetics, University of Wisconsin Medical School, Madison, WI.

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. In our first screen we identified a semidominant, autosomal mutation called *Clock* that lengthens circadian period by one hour in heterozygotes and by four hours in homozygotes. In addition, *Clock* homozygotes lose circadian rhythmicity after a few days to weeks in constant darkness. In *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 5 in a region with conserved synteny with human chromosome 4. (Supported by grants from the NSF Center for Biological Timing, the MacArthur Foundation, and NIH)

REFERENCES:

1. Vitaterna, M.H., D.P. King, A.-M. Chang, J.M. Kornhauser, P.L. Lowrey, J.D. McDonald, W.F. Dove, L.H. Pinto. F.W. Turek and J.S. Takahashi. 1994. Mutagenesis and mapping of a mouse gene, *Clock*, essential for circadian behavior. *Science* 264:719-725.

2. Takahashi, J.S., L.H. Pinto and M.H. Vitaterna. 1994. Forward and reverse genetic approaches to behavior in the mouse. *Science* 264:1724-1733.

3. Takahashi, J.S. 1995. Molecular neurobiology and genetics of circadian rhythms in mammals. *Annu. Rev. Neurosci.* 18:531-553.

2.5

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.

REFERENCES:

1. Sehgal, A., Price, J.L., Man, B. and Young, M.W
Loss of circadian behavioral rhythms and per RNA oscillations in the Drosophila mutation, timeless
Science
263 (1994), 1603-1606
This paper describes the isolation and initial characterization of timeless.

2. Vosshall, L.B., Price, J.L., Sehgal, A., Saez, L and Young, M.W.
Block in nuclear localization of period protein by a second clock mutation, timeless
Science
263 (1994), 1606-1609
This paper describes the effect of timeless on the subcellular distribution of per protein.

MOLECULAR BIOLOGY 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 crassa*, including the timing of the initiation of conidiogenesis. This property has allowed genetic and molecular techniques to be used to study the circadian clock itself from converging angles (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 transcript levels are controlled by the clock. The first two genes isolated, designated *clock controlled genes-1 &2* (*ccg*-1, *ccg*-2), identified by a subtractive hybridization procedure have both been shown by nuclear run-on analysis to have clock regulated transcription as the primary point of regulation, clearly indicating cis-acting sequences involved in the clock regulation. One of these genes, *ccg*-2, encoding a fungal hydrophobin, is positively regulated by light, and transcripts accumulate during asexual development. To sort out the basis of this complex regulation, deletion analysis of the *ccg* 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 nucleotide 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 *ccg*-2 gene. We are currently using this element as a probe in gel-mobility shift assays to identify *trans*-acting clock factors.

Another approach to understanding the *Neurospora* clock system is to identify the genetic and molecular components of clock entrainment. In every organism examined to date, the predominant agent involved in resetting the clock is visible light. Although no single line of effort has yet identified any of the individual components involved in the light signal transduction pathway to the clock mechanism, it is known that *frq* is a clock component, and we have shown that the level of *frq* responds to light. Examination of the light inducibility of the *frq* large transcript in two photo-blind strains, wc-*1* and wc-2 shows the *wc-1* but not the *wc-2* defect to block the*frq* increase. These experiments now allow us to directly examine the effects of light on a known component of the clock, and to begin to understand the molecular basis of the signal transduction pathway and of clock resetting.

Supported by NIH grants GM34985 and MH44651, AFOSR grant F49620-94-1-0260, and NSF MCB-9307299

REFERENCES:

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

Bell-Pedersen, D., J. C. Dunlap and J. J. Loros,
The *Neurospora* circadian clock-controlled gene, *ccg-2*, is allelic to *eas* and encodes a fungal hydrophobin required for formation of the conidial rodlet layer.
Genes & Development
6, 2382-2394, 1992.

Arpaia, G., Loros, Jennifer J., Dunlap, Jay C., Morelli, G. and G. Macino
Light Induction of the Clock-Controlled Gene ccg-1 is not Transduced through the Circadian Clock.
Molecular and General Genetics
in press, 1995

3.4

LIGHT INDUCTION OF IMMEDIATE-EARLY GENES IN THE SUPRACHIASMATIC NUCLEUS (SCN). William J. Schwartz & Neil Aronin. Depts. Neurology, Medicine & Cell Biology, Univ. Massachusetts Medical School, Worcester, MA 01655.

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 photic entrainment 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 B, 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 an output pathway 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:

1. Morgan J.I. & Curran T.
Stimulus-transcription coupling in the nervous system: involvement of the inducible proto-oncogenes *fos* and *jun*.
Ann. Rev. Neurosci. 14 (1991) 421-451.

Reviews immediate-early genes in a neural context

2. Schwartz W.J., Aronin N., Takeuchi J., Bennett M.R. & Peters R.V.
Towards a molecular biology of the suprachiasmatic nucleus: photic and temporal regulation of c-*fos* gene expression.
Seminars in the Neurosciences 7 (1995) 53-60.

Reviews immediate-early gene expression in the SCN

3. Ginty D.D., Kornhauser J.M., Thompson M.A., Bading H., Mayo K.E., Takahashi J.S. & Greenberg M.E.
Regulation of CREB phosphorylation in the suprachiasmatic nucleus by light and a circadian clock.
Science 260 (1993) 238-241.

Photic regulation of phosphorylated CREB in the SCN

7.2

CALCIUM AND PHASE SHIFTS IN THE CHICK PINEAL. Martin Zatz, SBP, LCB, National Institute of Mental Health, Bethesda, MD 20892

Chick pineal cells in dispersed cell culture display a persistent, photosensitive, circadian rhythm of melatonin production 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 circadian pacemaker in chick pineal cells? Effects of nitrendipine, Bay K 8644, Co^{++}, and low external Ca^{++}.
Brain Res.
463 (1988): 305-316.

Initial paper on Ca^{++} and chick pineal melatonin rhythm

T. Pozzan, R. Rizzuto, P. Volpe & J. Meldolesi
Molecular and cellular physiology of intracellular calcium stores.
Physiol. Revs.
74 (1994): 595-636.

Review.

C. Fewtrell
Ca^{++} oscillations in non-excitable cells.
Ann. Rev. Physiol.
55 (1993) 427:454.

Review.

7.4

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

One of the important experimental prerequisites to show the endogenous nature of circadian rhythms is to keep experimental conditions 'constant'. We now have ample proof that circadian oscillations are endogenously generated, but the question remains of how constant the environment can be when many systemic functions, from enzyme activity to behavior, oscillate throughout the circadian day. Efferent processes of the circadian system are bound to feed back onto the pacemaker in many indirect ways, the simplest example would be the closing of eyelids during sleep.

We have investigated such circadian feed-back loops in the marine unicellular alga G. polyedra and found that these can be far more direct than just acting via behavior and can even have a rhythmic influence on the 'constant' conditions. The circadian control of the swimming behavior leads to a phase specific self-selection of light intensities, which feeds back both on the circadian light input and on photosynthesis. Chemical inhibition of photosynthesis, which itself is under circadian control, changes period and phase of the pacemaker. The circadian light input itself also appears under circadian control with a drastically increased sensitivity for blue light during the subjective night. Finally, the clock-controlled nitrate metabolism (both NO_3-uptake and the activity of nitrate reductase are circadian) constitutes yet another feed-back loop, since changes of the medium's nitrate concentration influence the period and can induce phase shifts of more than 7 hours.

These results indicate that the circadian system is not just a clock, shielded from environmental influences (except those which entrain it to the 24-hour day), but that it is a highly adaptive timing system, which actively samples the environment for the availability of important resources.

REFERENCES:

M.R. Ralph and N. Mrosovsky
Behavioral inhibition of circadian responses to light.
Journal of Biological Rhythms
7 (1992): 353-360.

Shows the interaction of non-photic (activity feed-back) and photic stimuli on the circadian system.

B. D. Aronson, , K. A. Johnson, J. J. Loros, J. C. Dunlap.
Negative feedback defining a circadian clock: autoregulation of the clock gene frequency
Science
263 (1994): 1578-1584

Describes feed-back in the circadian oscillator itself and sets up experimental criteria for components of the central circadian loop.

E. Gwinner
Tagesperiodische Schwankungen der Vorzugshelligkeit bei Vögeln.
Z. vergl. Physiol.
52 (1966): 370-379.

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

8.2

A POTPOURRI 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 hypophyseal 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 circadian clock gene *period* from silkmoths. Silkmoths provide an advantageous system for cellular analysis of clock neurons in insect brain.

REFERENCES:

1. S.M. Reppert, D.R. Weaver, T. Ebisawa
Cloning and characterization of a mammalian melatonin receptor that mediates reproductive and circadian responses.
Neuron
13 (1994):1177-1185

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 silkmoth *Antheraea pernyi*.
Neuron
13 (1994):1167-1176

8.3

PHASE-SHIFTING THE SCN CIRCADIAN CLOCK IN VITRO Rebecca A. Prosser. Dept. of Zoology, University of Tennessee, Knoxville TN 37996.

The production of near-24 hr rhythms in behavior and physiology in the absence of known synchronizing stimuli appears to be a characteristic shared by most, if not all, organisms. The primary clock controlling these rhythms in mammals resides in the suprachiasmatic nuclei (SCN). One piece of evidence in support of this conclusion is that the SCN continue to produce 24 hr rhythms after isolation in a brain slice preparation. In particular, the SCN produce a robust 24 hr rhythm in spontaneous neuronal activity that lasts for at least 3 cycles *in vitro*. This rhythm typically is assessed by sampling the spontaneous firing rates of many individual neurons for brief periods, and then averaging these firing rates over 1 to 2 hrs according to the times they were recorded. The time of peak activity for this rhythm is extremely stable, showing little variability either between experiments or across cycles within a single experiment. This time-of-peak can therefore be used as a marker for the phase of the underlying circadian clock, and manipulations that induce stable shifts in the time of peak activity can be assumed to have altered the phase of the SCN clock.

I am interested in the cellular mechanisms underlying clock functioning and clock control, and the approach I have used is to investigate the mechanisms underlying afferent modulation of the SCN clock in rats. Of the SCN's three main afferents, I have focused primarily on the serotonergic (5-HTergic) input from the raphe. Our results suggest that 5-HT advances the SCN clock in the subjective day via activation of 5-HT_7 receptors, increases in cAMP, activation of PK-A, and opening of Ca^{2+}-dependent K^+ channels. Any increases in intracellular Ca^{2+} involved in this process may be through release of intracellular Ca^{2+}, since these phase shifts are not blocked by 10mM Mg^{2+} in the extracellular medium. Currently my lab is beginning to investigate possible interactions between this afferent system and the other two primary SCN inputs: a direct retinal projection, which utilizes an excitatory amino acid as its neurotransmitter, and an indirect retinal projection via the intergeniculate leaflet of the lateral geniculate nuclei, with neuropeptide Y and GABA as its known neurotransmitters. These studies should help us understand how the SCN clock is modulated in intact animals.

REFERENCES:

1. M.U. Gillette
SCN electrophysiology in vitro: rhythmic activity and endogenous clock properties. In Suprachiasmatic Nucleus: The Mind's Clock D.C. Klein, R.Y. Moore, and S.M. Reppert, Eds. Oxford Univ. Press, 1991, pg 125-143.
Summarizes early SCN slice control and phase shifting studies.

2. J.D. Miller
On the nature of the circadian clock in mammals.
American Journal of Physiology
264 (1993): R821-R832

Recent review of experiments related to SCN clock functioning and control.

3. R.A. Prosser, H.C. Heller, and J.D. Miller
Serotonergic phase advances of the mammmalian circadian clock involve protein kinase A and K^+ channel opening.
Brain Research
644 (1994): 67-73
Most recent experiments investigating mechanisms of serotonergic phase shifting.

8.4

THE REGULATION OF VERTEBRATE CIRCADIAN RHYTHMS BY LIGHT. Russell G. Foster*, Sharleen Argamaso-Hernan, Susan Doyle*, Allan Froehlich*, Ignacio Provencio, Bobby Soni*. 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 2BB.

In mammals, unknown ocular photoreceptors entrain circadian rhythms to the light-dark cycle. In an attempt to identify these photoreceptors we have used mammals which lack specific retinal elements, and determined the effect of these defects on circadian responses to light. C57/BL and C3H/He *retinal degenerate* (*rd/rd*) mice, which lack rod photoreceptors beyond 80 days of age, show unattenuated circadian responses to light[1]. Recent results in rodless transgenic mice (Tm) have confirmed that rod photoreceptors are not required for circadian responses to light, but responses differ from *rd/rd* mice. Tm animals show increased circadian responses to light, affecting both sensitivity and dynamic range. The different response kinetics of transgenic, *rd/rd* and +/+ mice could result from a reorganization of the entrainment pathway, and responses are larger in the transgenic mice because rods are affected earlier in postnatal development[2]. On the basis of our action spectrum (*rd*) and molecular studies of the degenerate mouse retina (*rd* & Tm), M-cones (511nm) & UV-cones (359nm) are strong candidates for photoentrainment. Transgenic mice which lack cones are currently under investigation. The blind mole rat has subcutaneous eyes and lacks visual but not circadian responses to light. To date, we find a single opsin with high similarity to the mouse and human green cone opsins[3]. Whether circadian responses to light are mediated by cones or by cone-like opsins in some unidentified retinal cell remains to be determined. These results in mammals will be compared with our molecular analysis of extraretinal photopigments in non-mammalian vertebrates.

REFERENCES:

1. Provencio I, Wong S, Lederman A, Argamaso SM, Foster RG
Visual and circadian responses to light in aged retinally degenerate mice.
Vision Research Volume 34 (1994) pp: 1799-1806.

2. Foster RG, Frochlich A, Argamaso-Hernan SM, McCall M.
Rodless transgenic mice show increased circadian responses light.
Investigative Ophthalmology & Visual Science Volume 36 (1995) Abstract: 2505.

Most recent transgenic results

3. Argamaso SM, Froehlich AC, McCall MA, Nevo E. Provencio I, Foster RG
Photopigments and circadian systems of vertebrates.
Biophysical Chemistry (1995) (in press)

Recent review.

10.1

SEX DIFFERENCES IN FORMAL CIRCADIAN PROPERTIES OF *O. DEGUS*. Theresa M. Lee* and Tammy-Jo Jones*. Univ. of Michigan, Ann Arbor, MI, 48104-1687.

Goel and Lee (in press) found that female degus housed alone phase advance significantly slower after a 6 h shift than do males, 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 (L12: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), therefore OVX females were used to eliminate the problems of estrus-induced changes in circadian rhythms. Six intact males and six OVX females were housed in constant lighting conditions of 0, 580 and 5800 lux for 2-6 months to determine τ. PRC data were gathered by housing animals in isolation chambers in 0 lux, and administering 20 min light pulses across the circadian day (N=112 ♂ pulses, N=78 ♀ pulses). Males had significantly faster τ's than females in 0 lux (23.2 $\pm$ .1 h vs 23.7 $\pm$ .1 h). The τ's of both sexes increased significantly at 580 and 5800 lux, however the τ's of males increased significantly more than those of females (e.g. ♂ increase .63 $\pm$ .09 h from 0 to 580 lux, while ♀ increase .28 $\pm$ .09 h). The PRC's of males and females differed in several respects: the phase delay zone began earlier and was shorter in duration for males (CT 0-7) than females (CT 2-14), while the phase advance zone was earlier and longer for males (CT 14-23) than for females (CT 17-24). However, females have significantly larger delays and advances than males during the peak delay (CT3-6) and advance (CT20-22) zones. These data suggest that the formal circadian properties of this diurnal rodent are altered by either adult or early steroid exposure as previously described for several nocturnal rodents. (Supported by NIH grant MH49089)

REFERENCES:

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 generated 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 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 hypothalamo-pituitary axis thus contribute to the regulation and expression of the circannual reproductive rhythm.
Supported by NSF-IBN-9206510 and NIH-HD-07689.

REFERENCES:

1. E. Gwinner
Circannual Rhythms
Springer-Verlag, 1986.

Book on existence, characteristics and regulation of circannual rhythms.

2. C.J.I. Woodfill, N.L. Wayne, S.M. Moenter
Photoperiodic synchronization of a circannual reproductive rhythm in sheep: identification of season-specific time cues.
Biol. Reprod. 50 (1994): 965 976.

Identification of circadian melatonin patterns that are effective or ineffective in entraining the circannual reproductive rhythm.

3. F.J. Karsch, G.E. Dahl
Role of the thyroid gland in seasonal reproduction in the ewe.
In Perspectives in Comparative Endocrinology (1994): 594–601.
Eds. K.G. Davey, R.E. Peter and S.S. Tobe
Medical Research Council of Canada, Ottawa.

Overview describing involvement of thyroid hormones on seasonal reproductive rhythm.

10.3

CIRCADIAN CLOCKS AND TIME-PLACE LEARNING IN A CYPRINID FISH. Stéphan 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 unreliable light signals, and allow fish to anticipate food arrival in specific places and efficiently compete for it.

REFERENCES:

H. Biebach, H. Falk, and J.R. Krebs.
The effect of costant light and phase shifts on a learned time-place association in garden warblers (Sylvia borin): hourglass or circadian clock?
Journal of Biological Rhythms.
6, 1991, 353-365.
The Biebach group brought renewed interest to time-place learning with their work on various bird species.

R.E. Mitslberger.
Circadian food-anticipatory activity: Formal models and physiological mechanisms.
Neuroscience and Biobehavioral Reviews.
18, 1994, 171-195.
An insightful review, including time-place association.

S.G. Reebs.
The anticipation of night by fry-retrieving convict cichlids.
Animal Behaviour.
48, 1994, 89-95.
Another example of ecologically relevant behaviour underlined by circadian mechanisms in fish.

HUMAN CIRCADIAN CONTROL, PHYSIOLOGY AND CLINICAL APPLICATIONS

11.3

ADAPTING TO PHASE SHIFT: EFFECTS OF MELATONIN ON HUMAN CIRCADIAN RHYTHMS Josephine Arendt and Stephen Deacon, University of Surrey, Guildford, GU2 5XH, 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 biological clock, together with sleep timing, cortisol and prolactin. Two phase response curves have been published and these to some extent mirror the PRC to light. Melatonin acutely suppresses core body temperature. When it is timed to phase advance, the degree of temperature suppression is closely related to the magnitude of induced phase shift and changes in temperature may be an integral part of the phase-shifting mechanism. Evidence for complete entrainment of free-running rhythms by melatonin is not substantial and largely based on entrainment of sleep-wake cycles in some blind subjects. However when used in such a way as to reinforce ambient time cues such as in adaptation to simulated or actual time zone change or shift work it is clearly able to enhance the rate of adaptation of many behavioural and hormonal circadian rhythms. Adaptation of sleep, mood and performance can precede resynchronisation of endogenous melatonin. Acute effects of melatonin include transient sleepiness and/or loss of alertness. It is likely that its ability to improve sleep, mood and performance after forced shift of ambient time cues involves both acute effects and circadian phase shift. In both controlled and uncontrolled studies of this nature (N>500) no significant incidence of serious side effects has been found to date. Suitably timed 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 structured rhythmicity and thereby influence many aspects of health.

REFERENCES:

1. Wetterberg, L. (ed)
Light and Biological Rhythms in Man
Wenner-Gren International Series, Pergamon, 1994

See Chapters by Lewy and others for overviews and state of the art articles

2. J. Arendt
Melatonin and the Mammalian Pineal Gland
Chapman Hall, 1994

Latest comprehensive textbook on melatonin

3. A. Samel, H.M.Wegman, M.Vejvoda, H. Maas
Influence of melatonin treatment on human circadian rhythmicity before and after a simulated 9 hour time shift.
J Biol Rhythms 6:235-248, 1991.

Important article looking at many circadian rhythms

12.0

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.

REFERENCES:

5.1

MATHEMATICAL MODEL OF A CIRCADIAN OSCILLATOR WITH POSITIVE POST-TRANSCRIPTIONAL AUTOREGULATION. C. D. Thron. Dartmouth Medical School, Hanover, NH 03755

The circadian clock components *frq* of *Neurospora crassa* and *per* of *Drospohila* have gene products which inhibit transcription of their own gene, producing negative feedback (Aronson, et al., Science **263**: 1578, 1994; Zeng et al., EMBO J. **13**: 3590, 1994). Negative feedback might cause circadian oscillations, but the kinetic requirements for biochemical feedback oscillations are rather stringent, and simple negative-feedback loops do not appear to be common causes of biological oscillations. The best known biochemical oscillators work by positive feedback or autocatalysis (A. Goldbeter, in Biological Kinetics, L. A. Segel, Ed., Cambridge University Press, Cambridge, 1991, pp. 107-154). The autocatalytic circadian oscillator model of Pavlidis and Kauzmann (Arch. biochem. Biophys. **132**: 338, 1969) is here modified to include negative feedback by inhibition of transcription by the gene product. For R = transcript and F = gene product,

$$\frac{dR}{dt} = \frac{p_1}{p_2 + F} - p_3 R; \qquad \frac{dF}{dt} = \left[p_4 + \frac{p_5 F^2}{p_9 + F^2}\right] R - p_7 F,$$

($p_1 = 7.82$, $p_2 = .143$, $p_3 = 6.84$, $p_4 = .321$, $p_5 = 95.2$, $p_7 = 32.1$, $p_9 = 2$). The model would imply that FRQ or PER is positively post-transcriptionally autoregulated. The autocatalytic process could be either translation or a post-translational process. This model therefore suggests that an important part of the circadian oscillator may lie among the most ancient reactions of cell biochemistry.

5.2

COMPARATIVE PHYLOGENETIC AND FUNCTIONAL ANALYSIS OF *frequency (frq)* HOMOLOGS. SUPPORT FOR A TRANSCRIPTIONAL REGULATORY ROLE. M.T. Lewis, L. Morgan, and J.F. Feldman. University of California, Santa Cruz 95064.

The putative amino acid sequence of the *Neurospora crassa* frq protein contains sequences suggesting it is a nuclear transcription factor. Using PCR, we have cloned *frq* homologs from other filamentous fungi including *Chromocrea spinulosa*, *Leptosphaeria australiensis*, *Podospora anserina* and four additional *Neurospora* species. Alignment of the *Chromocrea* and *Leptosphaeria* sequences with those previously published for *Neurospora* and *Sordaria* shows that the former are about 50% identical to the latter and to each other. There are both regions of near total divergence and highly conserved regions including a segment with predicted helix-turn-helix structure that may act as a DNA-binding domain. Amino acids at positions altered in the *frq* mutants are conserved among all species. Sequences consistent with frq being a transcription factor are generally conserved; most predicted post-translational modification sites are not. Transformation of the *Neurospora frq9* mutant with the *Chromocrea* homolog rescued the pigmentation defect of the mutant but not the circadian defect; transformation with the *Leptosphaeria* homolog failed to rescue either phenotype.

5.3

CLONING AND TRANSFORMANT ANALYSIS OF THE *period-2 (prd-2)* CLOCK GENE OF *Neurospora crassa*. L. Morgan, M.T. Lewis, N. Recht, K. Wymore, and J.F. Feldman. Department of Biology, University of California, Santa Cruz 95064.

prd-2 is a recessive clock mutation that lengthens the period of the circadian conidiation 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 *lys-2* and *a m*. A chromosome walk in the Volmer-Yanofsky cosmid library in the genetically determined region of *prd-2* yielded a set of cosmids spanning about 170kb. These cosmids were tested in a transformation assay for the ability to complement *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 homokaryotic microconidial 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 *GONYAULAX* CIRCADIAN CLOCK BY PROTEIN KINASES AND PHOSPHOPROTEIN PHOSPHATASES. James Comolli, Pankaj Tiwari, 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. Our approach is to screen inhibitors of these enzymes for their effects on the bioluminescence rhythm of the dinoflagellate *Gonyaulax polyedra*, then attempt to characterize their intracellular site(s) 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. Staurosporine-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 within the Nucleus. Martha Merrow, Norman Garceau, 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 (Aronson, Johnson, Loros, and Dunlap, SCIENCE 263, 1578-1584, 1994). 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 sets the clock to a unique and predicted phase. *frq* is also phylogenetically conserved (Merrow and Dunlap, EMBO J. 13, 2257 - 2266, 1994) for both structure and function. As data suggest that the *frq*/FRQ feedback loop is a part of the clock resides partly within the nucleus, we have studied the kinetics of FRQ-induced depression in *frq* RNA levels to determine 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 NIGMS grant GM34985.

5.6

LIGHT-INDUCED RESETTING OF A CIRCADIAN CLOCK IS MEDIATED BY A RAPID INCREASE IN FREQUENCY TRANSCRIPT **Susan K. Crosthwaite, Jennifer J. Loros & 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 of the action of light at the molecular level.

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 (Aronson *et al.*, Science 263, 1578-1584,1994). Two minute 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* RNA levels in the loss of function mutant *frq*^{9}, 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 μmoles photons/m^2/s. 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 confirm the salient molecular predictions for the model for nonparametric 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 NIGMS grant GM34985 to JCD and AFOSR grant F49620-94-1-0260

5.7

CELLULAR DYNAMICS OF THE *DROSOPHILA* PER PROTEIN.
Kathleen K. Siwicki, Bill Bug, Mary Grace Folwell and Erik Hom.
Swarthmore College, Swarthmore, PA 19081

From the earliest studies of PER expression in *Drosophila*, we have observed differences between photoreceptors and *per*-expressing lateral neurons (LNs) in terms of the protein's subcellular 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 gene's products 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 nuclear and cytoplasmic PER within specific cell types. We have examined per^+ flies in 12 h:12 h 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 background fluorescence in per^{01}) were detected only at a single ZT in the middle of the night. In LNs, PER immunofluorescence rises 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 nucleus and cytoplasm of *some* LNs, although it is predominantly nuclear in most. Cells with PER concentrated in the cytoplasm are 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
Haiping Hao* and Paul E. Hardin*. Department of Biology and Institute of Biosciences and Technology, Center for Advanced Invertebrate Molecular Sciences, Texas A & M University, College Station, Tx 77843-3258

The *period* (*per*) gene is involved in regulating the circadian rhythms of *D. melanogaster*. The abundance of *per* mRNA and protein undergo circadian oscillations, which comprise a feedback loop that appears to be required for behavioral rhythms when expressed in certain brain neurons and/or glia cells. We are investigating the *cis*-regulatory elements in the *per* 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 have been generated which carry either truncated versions of *per* promoter regions fused to an *E. coli*-lacZ or *per* upstream sequences fused to either a *Drosophila* hsp70 or P-element transposase basal promoter-lacZ fusion. LacZ mRNA abundance from these fusion genes has been measured by RNase-protection and spatial expression 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 eyes is present in sequences from -341 to -156bp relative to the transcription start site. Current studies are directed towards delimiting 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 # R29-NS31214.

5.9

QUANTITATIVE TRAIT LOCI ANALYSIS OF CIRCADIAN RHYTHMS IN MICE: MODIFIERS OF *CLOCK* ?
Kazuhiro Shimomura*, Martha H. Vitaterna*, David P. King*, Genn Suyeoka*, Anne-Marie Chang*, Lawrence H. Pinto*, Fred W. Turek and Joseph S. Takahashi*. *NSF Center for Biological Timing and Department of Neurobiology & Physiology, Northwestern University, Evanston, IL 60208*

We have recently identified a novel mutation called *Clock* which lengthens the circadian period of locomotor activity rhythm in mice. While wild-type mice have free-running periods of ~23.6 hours, animals heterozygous for the mutation have free-running periods of ~24.7 hours, and homozygotes free-run at about 27-28 hours upon initial transfer to constant darkness but subsequently lose circadian rhythmicity when maintained in constant darkness. In this study we analyzed how the circadian period was modified by genetic background. While *Clock*/+ heterozygotes of the C57BL/6J inbred strain express circadian periods of 24.6 ± 0.37 hours (mean ± S.D.), *Clock*/+ heterozygous mice which are (BALB/cJ X C57BL/6J)F1([BC)F1] progeny tend to have shorter periods (24.1 ± 0.31 hours). This finding suggests that the BALB/cJ strain carries alleles that act in a dominant fashion to modify the effects of *Clock* upon circadian period. In addition *Clock*/+ animals from a [(BC)F1 X B6]N2 backcross and a (BC)F2 intercross showed increased variance of circadian period (24.3 ± 0.61, 24.1 ± 0.60 respectively) suggesting the segregation of modifier genes in these hybrid crosses. To test whether these putative modifier loci could be mapped genetically, we have analyzed 46 (BC)F2 *Clock*/+ mice, using Quantitative Trait Loci (QTL) analysis. Here we report the genetic mapping by linkage to simple sequence length polymorphisms (SSLPs) of loci that strongly modify the circadian period of locomotor rhythm in (BC)F2 *Clock*/+ animals. Regions that affect circadian period map to Chromosomes 3 (LOD score=3.4) and 4 (LOD score=4.0) and can account for about 30% and 34% of the genetic variation, respectively. These results indicate the QTL analysis may be useful approach to find genes that have small effects on circadian rhythms.

Supported by an NSF Center for Biological Timing grant.

5.10

GENETIC LINKAGE ANALYSIS AND PHYSICAL MAPPING OF THE MOUSE GENE, *CLOCK*.
David P. King*, Martha H. Vitaterna*, Anne-Marie Chang*, William F. Dove*†, Lawrence H. Pinto*, Fred W. Turek, and Joseph S. Takahashi*, NSF Center for Biological Timing, Department of Neurobiology and Physiology, Northwestern University, Evanston, IL 60208 and †McArdle Laboratory for Cancer Research and Laboratory of Genetics, University of Wisconson, Madison, WI 53706

We have recently reported the isolation of a novel mutation in the mouse which has several effects on the circadian rhythm of locomotor activity (Vitaterna *et al.*, *Science* **264**, 719, 1994). This semidominant mutation identifies a single, autosomal locus, designated *Clock*, which we initially mapped to the midportion of chromosome 5. As part of a continuing effort to clone the *Clock* gene by position, we have generated a high-resolution genetic map of the *Clock* region, and have used these data to begin construction of a physical map. Three interstrain crosses in which *Clock* segregates were used to map the mutation. The *Clock* mutation is carried on the C57BL/6J (B6) background. Both BALB/cJ (C) and C3H/HeJ (C3H) were used as counterstrains. DNA was obtained from >700 [(C X B6)F1 X B6]N_2 backcross mice, 127 (C X B6)F_2 mice, and >200 [(C3H X B6)F1 X B6]N_2 backcross mice. Simple sequence length polymorphisms (SSLPs) (Dietrich *et al.*, *Genetics* **131**, 423, 1992) and restriction fragment length polymorphisms were used as DNA markers. We have localized *Clock* to a region 1-2 centimorgans (cM) distal to the *c-kit* locus on chromosome 5. Using the large number of genetic markers (10) tightly linked (<1 cM) to *Clock*, we have begun to construct a physical map of the *Clock* region. To do so, a mouse yeast artificial chromosome (YAC) library was screened by PCR (Kusumi *et al.*, *Mamm. Genome* **4**, 391, 1993), using SSLPs as sequence tagged sites (STS). Additional STS were derived from the sequence of genes in the *c-kit* region. We have now isolated 24 YAC clones surrounding the *Clock* locus, and have constructed a preliminary physical map of the region. Supported by grants from the NSF Center for Biological Timing and the MacArthur Foundation to J.S.T., F.W.T., and L.H.P. and the NIH to W.F.D.

5.11

CIRCADIAN ORCHESTRATION OF GENE EXPRESSION IN CYANOBACTERIA.
Y. Liu*, T. Kondo§, N. F. Tsinoremas¶, S. Golden¶, N. V. Lebedeva¶, M. Ishiura§, and C. H. Johnson*. *Dept. of Biology, Vanderbilt Univ., Nashville, TN 37235 USA; §National Institute for Basic Biology, Okazaki, 444, Japan; ¶Dept. of Biology, Texas A&M Univ., College Station, TX 77843 USA

We wanted to identify genes which are controlled by the circadian clock in the prokaryotic cyanobacterium, *Synechococcus* , using luciferase as a reporter of gene expression. Bacterial luciferase genes (*luxAB*) were randomly inserted into the genome by conjugation with *E. coli* . 30,000 of the resulting transconjugants were then screened for luminescence using a cooled-CCD camera system. We recovered about 800 clones whose luminescence was bright enough to be easily monitored. Unexpectedly, the luminescence expression patterns of almost all of these 800 colonies clearly manifested circadian rhythmicity. These rhythms exhibited a range of waveforms and amplitudes and they also showed a variety of phase relationships. These results indicate that there is an extensive temporal programming of gene expression in this organism.

To study how the circadian clock controls the gene expression, one of the transformants whose luminescence rhythm showed a reversed phase relationship from that of our original reporter strain was further analyzed. The gene was cloned, sequenced and identified as the *purF* gene. This gene encodes the enzyme catalyzing the initial step of purine nucleotide biosynthesis, amidophosphoribosyltransferase. Northern analysis confirmed the reversed gene expression pattern of *purF* mRNA abundance. The promoter for the *purF* gene has been identified.

5.12

THE *PARAMECIUM* CIRCADIAN CLOCK: THE RESTING MEMBRANE POTENTIAL MIGHT BE DRIVEN BY INTRACELLULAR PACEMAKER.
K. Hasegawa[1] M. Shimamoto[1], K. Matsumoto[2], Y. Nakaoka[3] and Y. Tsukahara[4], [1]Sch. Med., Kitasato Univ., [2]Found. Promot. Tech. Oriental Med., [3]Faculty Engineer. Sci., Osaka Univ., [4]Photodynamic Res. Center, IPCR (Riken).

Intracellular cAMP concentration in *P. multimicronucleatum* is highest around midday (and subjective midday) when the species swim fastest with the most straight fashion and the resting membrane potential is more negative, and the concentration is the lowest at midnight (and subjective midnight) when the species swim slowest with the most frequent turns. cGMP exhibits a similar fluctuation to cAMP, with the maximum and minimum appearing a few hours earlier than those of cAMP. The ratio of cAMP/cGMP also fluctuates in a circadian manner, with the maximum appearing around midday and the minimum around midnight, implicating cAMP and cGMP in the circadian regulation of the resting membrane potential. Extracellularly added tetraethylammonium (TEA^+, 4mM) inhibits circadian changes in intracellular concentration of cAMP. However, even 32mM of extracellular TEA^+ does not inhibit the circadian motility rhythm. The addition of $CdCl_2$ (0.1mM) alone does not inhibit the circadian motility rhythm but it does if added together with TEA^+(4mM). This suggests that inhibition of both of K^+-channels and Ca^{2+}-channels is require to inhibit the circadian motility rhythm. From these observations, we conclude that the circadian change in the resting membrane potential may be attributed to circadian activity change in adenylate cyclase and guanylate cyclase, both of which are driven by a pacemaker.

5.13

CIRCADIAN REGULATION OF THE CELL DIVISION CYCLE IN EUGLENA GRACILIS: ROLE OF REVERSIBLE TYROSINE PHOSPHORYLATION IN THE TIMING OF MITOTIC KINASE ACTIVITY. Leland N. Edmunds, Jr.* and Gangaram Mohabir.* SUNY, Stony Brook, N.Y. 11794

We have demonstrated that the achlorophyllous ZC mutant of Euglena gracilis exhibits a circadian rhythm of mitosis. To determine how the circadian clock couples to the cell-division-cycle (CDC), we have monitored cell-cycle oscillations in the levels (western blotting) and activity (histone H1 kinase assay) of the subunits (cyclin B and cdc2 homologs) of M-phase promoting factor (MPF) across the CDC and circadian cycle of both rhythmically dividing (τ = 26 h) and stationary cultures freerunning in DD. The level of cdc2 was invariant in both dividing and nondividing cells (albeit with 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 B levels fluctuated with a peak just preceeding the onset of MPF activity but became elevated and invariant as soon as stationary phase was reached. We are determining the nature of the changes in cdc2 mobility by pretreatment of p13-agarose affinity-purified extracts with potato acid phosphatase, followed by sequential blotting with anti-phosphotyrosine and anti-PSTAIR; our results indicate that they reflect reversible tyrosine phosphorylation. If this finding is confirmed we will have identified a circadian checkpoint in the CDC and a significant role for reversible protein phosphorylation, not only in circadian timekeeping, but also in signal transduction between clocks. Supported by NSF grant DCB-9105752.

5.14

RHYTHMIC SYNTHESIS OF PCP IN *GONYAULAX*. Q.Hien Le, Paul Markovic, Raphael Jovine* and D. Morse University of Montreal, Montreal, Canada H1X 2B2 and *University of California, Santa Barbara, CA 93106

A 32 kDa peridinin-chlorophyll a-binding protein (PCP) is one of the most abundant proteins in *Gonyaulax*, as visualized by Coomassie blue staining of protein gels. PCP is a major component in the light harvesting reactions of photosynthesis, and was thus considered to be a likely candidate for mediating clock control over the photosynthesis rhythm. Western blot analyses with an antibody directed against PCP show that there are at least six 32 kDa and one 19 kDa PCP isoforms in *Gonyaulax*. Levels of these different isoforms do not appear to change over the period of one circadian day, however. In spite of the constant protein levels, we observe that the synthesis rate of the major PCP isoform changes at least ten-fold over a 24 hour period in either LL or LD. The synthesis rates are high between CT 1 and CT 11 suggesting that protein is being synthesized in time for the daily photosynthesis. We suggest that this new synthesis does not contribute sufficiently to the levels of protein already present and so measurable changes in the amounts of protein are not observed. The synthesis rate changes, as is the case for other clock regulated proteins in this organism, appear to be controlled at a translational level, as shown by data from both in vitro translation of extracted mRNA and Northern blot analyses.

5.15

RHYTHMIC SYNTHESIS OF RUBISCO IN *GONYAULAX*. Patrick Salois, Paul Markovic, J.W. Hastings* and D. Morse University of Montreal, Montreal, Canada H1X 2B2 and * Harvard Biological Laboratories, Cambridge, MA 02138

Ribulose-1,5-bisphosphate carboxylase/oxygenase (rubisco), which catalyses the first step in carbon fixation, is normally formed from multimers of large (55 kDa) and small (14 kDa) subunits. The abundant 55 kDa protein in *Gonyaulax* seen by Coomassie blue staining of two dimensional protein gels was thus thought to represent the large rubisco subunit. Microsequence analysis of the protein extracted from the gel confirmed the identity of the 55 kDa protein as rubisco, but as a rare form II rubisco which requires only large subunits for activity. The synthesis rate of the rubisco was found to be highest during the period between CT 18 and CT 10 in LD or LL with an amplitude change of 50 to 100 fold. The regulation of protein synthesis, as judged by in vitro translation of exztracted mRNA or by Northern blot analyses using a rubisco clone isolated from a *Gonyaulax* cDNA library as a probe, appeared to occur at a translational level. It seemed possible that rubisco might mediate clock control over the photosynthetic carbon fixation rhythm rhythm, so the amount of protein at different CTs was estimated by Coomassie blue staining of two D gels and by Western blot analyses with an antibody directed against Rhodospirillum rubisco. There was no measurable difference in the amount of rubisco over the period of one circadian day, which may be due to the low amplitude of the photosynthesis rhythm.

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 in the unicellular dinoflagellate, *Gonyaulax polyedra*, is regulated by the circadian biological clock. All three components, luciferase (the enzyme), luciferin (the substrate) and a luciferin binding protein (LBP) which are involved in the bioluminescence process undergo changes in their amounts and/or activities corresponding to the circadian cycle.

In order 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 *lcf* 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 *lcf* coding region. One such repeat unit is sufficient for luciferase activity. The mRNA levels of *lcf* at different circadian times were also examined by Northern analysis and found to be constant throughout the circadian cycle, indicating that translational control is involved in the circadian regulation, as with LBP.

5.17

SYNTHESIS OF *GONYAULAX POLYEDRA* GLYCERALDEHYDE 3-PHOSPHATE DEHYDROGENASE IS UNDER CIRCADIAN CONTROL. Thomas F. Fagan*, James C. Comolli*, David M. 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 biological processes that are under circadian regulation, including bioluminescence, cell motility, photosynthesis, and cell division. The bioluminescence rhythm, which is best understood, has been shown to correlate with the daily synthesis and destruction of two proteins involved in the reaction, the luciferase and the luciferin binding protein (LBP). The biochemical bases for the other rhythms are less well understood. However, *in vivo* pulse labelling has previously shown that the synthesis of many *Gonyaulax* proteins is under circadian regulation. Peptide sequencing has revealed that one such protein, the synthesis of which peaks during late subjective night / early subjective day, is homologous to glyceraldehyde 3-phosphate dehydrogenase (GAPDH), an enzyme that plays key roles in both glycolysis and the Calvin cycle. We have confirmed the identity of this protein by cloning and sequencing *Gonyaulax* GAPDH. Western analysis and enzyme activity measurements indicate that the total protein levels are almost constant throughout the day / night cycle, suggesting that while the synthesis of GAPDH occurs at a specific time of day it may represent only a small fraction of the total enzyme pool. Northern analysis indicates that the messenger RNA levels for GAPDH are also constant, indicating that control of translation rather than transcription is the underlying mechanism for circadian regulation of GAPDH synthesis.

5.18

Analysis of Clock-Controlled Genes in *Neurospora*. **Norman Garceau*, Deborah Bell-Pedersen,* Kristin M. Lindgren*, Mari Shinohara*, Hyeseon Cho*, Jay C. Dunlap and 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 crassa*, including the timing of the initiation of conidiogenesis. This property has allowed genetic and molecular techniques to be used to examine the pathways whereby clocks control cellular metabolism and behavior. Initial efforts targeted the isolation of genes whose transcript levels are controlled by the clock. One of the genes, designated *clock controlled gene-1* (*ccg*-1), identified by a subtractive hybridization procedure (Loros et al, Science **243**, 385-388, 1989) was shown by nuclear run-on analysis (Loros and Dunlap, Mol. Cell. Biol. **11**, 558-563, 1991) to have clock regulated transcription as the primary point of regulation. *ccg-1* is a highly abundant gene of unknown function whose transcript may comprise several % of the cell mRNA. We have found *ccg-1* gene expression to be blue-light photo-inducible (Arpaia et al, MGG, in press). Although disruption of the *ccg-1* locus produces no detectable phenotype including no effect on the clock, we find, by both transcript and protein analysis, that *ccg-1* is turned on early in development and is regulated by the *aconidial-2* (*acon-2*) locus which, when mutated, blocks conidiation completely. Western analysis of the protein indicates CCG-1 to be present in undifferentiated hyphae in a time-of-day specific manner. Immunocytochemical localization shows extensive, non-nuclear staining of CCG-1 in the cytoplasm of aerial hyphae and conidiospores. Additionally, we have found ccg-1 to be heat-shock inducible. Deletion analysis of upstream *ccg-1* sequences suggests that the clock regulatory elements lie near the start site of transcription and are distinct and separate from sequences conferring glucose and developmental regulation.

Additionally, eight new clock-controlled genes have recently been isolated by differential screens between a set of time-specific cDNA libraries. Sequence analysis shows *ccg-7* to be the *Neurospora* glycolytic enzyme, glyceraldehyde-3-phosphate dehydrogenase and *ccg-12* to encode the *Neurospora* copper metallothionien gene. 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.

Supported by NIH grants MH 44651 and GM34985, AFOSR grant F49620-94-1-0260, and NSF MCB-9307299

5.19

PROMOTER ANALYSIS OF THE *NEUROSPORA CRASSA* CIRCADIAN CLOCK-CONTROLLED *CCG-2 (EAS)* GENE.

Deborah Bell-Pedersen*, Jay C. Dunlap, and Jennifer J. Loros. Department of Biochemistry, Dartmouth Medical School Hanover, New Hampshire 03755

The *N. crassa ccg-2* gene, encoding a fungal hydrophobin, is transcriptionally regulated by the circadian clock. In addition, *ccg-2* is positively regulated by light, and transcripts accumulate during asexual development. To sort out the basis of this complex regulation, deletion analysis of the *ccg-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 *ccg-2* gene. We are currently using this element as a probe in gel-mobility shift assays to identify *trans*-acting clock factors.

5.20

IDENTIFICATION OF RM1, A CLOCK CONTROLLED mRNA FROM *XENOPUS* RETINA. Carla B. Green* and Joseph C. Besharse*, University of Kansas Medical Center, Kansas City, KS 66160.

Many aspects of retinal physiology are regulated by a retinal circadian clock in *Xenopus laevis*, including gene transcription. In order to elucidate molecular mechanisms of this clock control, we have established a screen to identify retinal mRNAs that are expressed in a rhythmic, clock regulated manner. *Xenopus* eyecups were cultured in constant darkness and retinas were removed at 6 hour intervals for 2 days. Retinal RNA isolated from each sample was used in mRNA differential display analysis (Liang and Pardee, 1992, *Science* 257:967-971). This technique allows direct comparison of sub-populations of messages between the different time points. These results show that the expression of the majority of mRNAs in the retina (>99%) does not change throughout the day. However, a small number of bands exhibited a rhythmic pattern of expression that showed a consistent temporal pattern throughout both days. One of these bands was reamplified and used as a probe in northern blot analysis, resulting in detection of two distinct bands (~2 Kb and 3.9 Kb). These bands (named RM1) both show the same high amplitude rhythm (~8-fold) and temporal pattern of expression, with low levels throughout the day, and a peak in early night. Therefore, RM1 expression is under the control of a retinal circadian clock. The differential display product was used to screen a *Xenopus* retinal cDNA library and 4 cDNA clones were isolated. Preliminary characterization of these RM1 clones indicates that both messages are represented and may be products of differential splicing. Identification of RM1 and other rhythmic messages should provide insight into molecular mechanisms of clock control.

5.21

A LIGHT PULSE IN THE EARLY EVENING ABOLISHES PINEAL c-*fos* EXPRESSION IN THE DJUNGARIAN HAMSTER. Wendy Haggren*, Tsehay Mekonnen*, Tamako Ishida-Jones*, and W. Ross Adey. Pettis V.A. Medical Center, Loma Linda, CA 92357.

The timing of changes in the expression of the transcriptional regulator c-*fos* in response to light at night is similar 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 3.25 hr after lights off. One cohort of animals was returned to darkness after 20 min. Pineal glands and retinas were taken from hamsters killed in darkness at 3.5 hr after lights off (D) and from light pulse hamsters killed at 3.75 hr after lights off (LP). At 5.5 hr 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* gene probe, which had been generated from RNA extracted from light-pulse treated retina, was transcribed to produce a ^{32}P-labeled RNA for Northern analyses. At 3.5 hr 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 hr after lights off, hamsters that have remained in darkness continued to show detectable c-*fos* expression in the pineal, while the c-*fos* signal was once again detectable in light-pulsed animals returned to darkness, indicating that transcription of the c-*fos* had resumed. We show a similar pattern of light-regulated c-*fos* expression in the pineal glands of rats. These data suggest that rapid changes in the circadian production of pineal melatonin are regulated at the level of gene transcription.

5.22

IN VITRO STUDIES OF SIGNAL TRANSDUCTION IN PRIMARY CULTURES OF THE SUPRACHIASMATIC NUCLEI. Michael Hastings*, Irina Schurova*, Philip Sloper* Eric Bittman*+ and Shaun McNulty*. Dept. Anatomy, University of Cambridge, CB2 3DY, U.K., + Dept. Biology, UMASS, Amherst, MA 01003.

The suprachiasmatic nuclei (SCN) are the principal circadian oscillator in mammals. They can be entrained by light/dark cycles, probably via glutamatergic retinal input, and by the pineal hormone melatonin. Our aim was to establish primary SCN cultures *in vitro* in order to study in individual cells signal transduction events regulated by glutamate (glu) and/ or melatonin. Primary cultures of SCN were prepared from newborn Syrian hamsters. Single- and dual-immunocytochemistry were used to examine the phosphorylation of the transcription factor CREB (P-CREB-ir) and the induction of immediate-early genes (Fos-ir, Egr-ir) following 7- 10 days in culture. The intensity of nuclear immunoreactivity was assessed by densitometry. In both mixed cultures (ca. 50% neuronal) and pure astrocyte cultures, forskolin induced nuclear P-CREB-ir in all cells. Melatonin had no effect on the induced P-CREB-ir in pure astrocyte cultures, but reversed this response in approx. 50% of cells in mixed cultures. In mixed cultures, glutamate caused a dose- and time-dependent induction of P-CREB-ir in approx. 50% of cells. Glutamate also induced the expression of Fos-ir and Egr-1-ir in these cultures. The effects of glu were blocked by MK801, indicating the involvement of NMDA-type glutamate receptors. These studies demonstrate that glu and mel have opposing actions on P-CREB-ir in the SCN, consistent with the view that in the neonatal rodent both forms of stimulus may contribute to circadian entrainment.

5.23

INPUT PATHWAY TO CIRCADIAN CLOCK CONTROLLING SPERM RELEASE IN INSECT MAY INVOLVE OPSIN. Jadwiga M Giebultowicz* and Russel G. Foster*. Oregon State University, Corvallis Or. 97331 and University of Virginia, Charlottesville, Va. 22903

The reproductive system of lepidopteran males contains a circadian clock which controls rhythmic sperm release from the testis into the vas deferens. In our effort to define components of this circadian system we explored the input pathway to the pacemaker. A photoreceptive component of the pacemaking mechanism is located within the reproductive system itself, since this system responds with a changed timing of sperm release when exposed to light signal *in vitro*. Determination of spectral sensitivity was based on the fact that a 4h pulse of white light, given 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 4 h pulse of monochromatic light, between 420 and 600 nm, and the timing of sperm release was determined on the following day. The maximum delay of sperm release in intact males was obtained at a 500 nm wavelength. Isolated reproductive systems tested at this wavelength *in vitro* required 10 times less light than intact animals to produce a 5 h phase delay. Since the shape of the action spectrum indicated that opsin may be involved in mediating light response, we attempted to immudodetect opsin in the reproductive system of the gypsy moth males. Preliminary results indicate that the cone opsin-like antigen is present in several epithelial cells of vas deferens, in the region adjacent to seminal vesicles. Opsin immunoreactivity is restricted to the outermost portion of this columnar epithelium.

Supported partially by NSF grant No. MCB9407943 to JMG

5.24

CLONING OF A MOUSE MELATONIN RECEPTOR. Alfred L. Roca and Steven M. Reppert. Laboratory of Developmental Chronobiology, Mass. General Hospital; and Program in Neuroscience, Harvard Medical School, Boston MA 02114

Recently, high-affinity melatonin receptors have been cloned in Xenopus (Ebisawa et al. 1994; PNAS 91:6133) and mammals (Reppert et al. 1994; Neuron 13:1177). Cloning of a murine melatonin receptor (MR) cDNA and its gene is a necessary step for generating transgenic animals. Degenerate primers were designed using regions conserved among other mammalian MR cDNAs. PCR of mouse genomic DNA yielded a 466 bp fragment, coding from the presumed third to seventh transmembrane domains, with 86% amino acid identity to the sheep and human high-affinity melatonin receptors. In situ hybridization of adult C57BL/6J mouse brain using the PCR-generated fragment produced a hybridization pattern consistent with that expected for a high affinity melatonin receptor; hybridization signal was most intense in the hypophyseal pars tuberalis. To delineate gene structure, a murine cell line (RT2-2) was used which expresses the high affinity receptor. Northern analysis of poly(A)+ RNA indicated a primary transcript length of ca. 2 kb. Southern blot analysis of genomic DNA indicated a single-copy gene. Using the PCR-generated fragment as a probe, phage containing genomic sequences were isolated by screening a BALB/c mouse EMBL3 SP6/17 library at high stringency. In addition, low stringency screening with an upstream sheep MR cDNA fragment isolated a non-overlapping set of genomic clones. This suggests that the coding region is comprised of 2 exons divided by a large (>8 kb) intron. RNase protection assays suggest that a major transcription start site is located ca. 180 bp upstream of the start codon.

5.25

DOPAMINE ACTS AS AN EFFECTOR OF THE CIRCADIAN CLOCK IN GOLDFISH RETINA. Stuart C. Mangel* 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 3-48 hrs. Surgery was performed under dim red or infrared light. Retinas 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 eliminated rod input to the cells, a state usually observed during the subjective day. In contrast, application of spiperone (1-10 μM), a D2-like receptor antagonist, clozapine (1-10 μM), a preferential D4 antagonist, or forskolin (10 μM), an activator of adenylate cyclase, during the subjective day reduced cone input and increased rod input. Eticlopride (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.

5.26

TEMPERATURE MODULATES PACEMAKER AMPLITUDE IN CHICK PINEAL CELLS. R. Keith 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 circadian 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; Zatz et al., 1994; Barrett and Takahashi, in press). In addition to these effects, temperature modulates the amplitude of the pacemaker. Three sets of data support the conclusion that the chick pineal pacemaker has a higher amplitude oscillation at 40°C than at 37°C. (1) The phase response curve to subsaturating intensity (0.15 $\mu W/cm^2$) light pulses of 6-hour duration has a higher amplitude at 37°C than at 40°C. This is consistent with a limit-cycle model in which a stimulus (e.g. light of specified intensity and duration) causes a perturbation of similar magnitude and direction at each temperature but the relative effect of the stimulus is decreased as pacemaker amplitude is increased (e.g. 40°C). (2) The melatonin rhythm persists longer and with a higher amplitude in constant conditions at 40°C than at 37°C; the rhythm begins damping in less than 10 cycles at 37°C but is still robust after 10 cycles at 40°C. (3) The light intensity necessary for a critical pulse that causes arrhythmicity is lower at 37°C than at 40°C. A pulse with an intensity of 0.15 $\mu W/cm^2$ at CT16 is required to disrupt the rhythm at 37°C but an intensity of 100 $\mu W/cm^2$ at CT18 is necessary to disrupt the rhythm at 40°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 40°C than at 37°C.

Supported by NIH 1 F32 MH 10369 to R.K.B. and by NIH R37 MH 39592 to J.S.T.

5.27

EVIDENCE FOR A CIRCADIAN RHYTHM IN CARTILAGE. Debora L. Nickla* & Josh Wallman*. Biology Department, City College of CUNY, 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 perifusion system. A defined medium (N2) containing labeled sodium sulfate was continuously replenished via a multi-channel pump at 2ml/hr and samples of the "conditioned medium" collected for biochemical analysis at 2 hr intervals for 48-72 hrs. Cultures were started at 3 times of day.

We find that the uptake of sulfate into PGs shows a rhythm of approximately 24 hours, which persists for at least 2 cycles *in vitro*. Removing the tissue from the eye and placing it in culture appears to strongly phase-shift 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-neural origin.

CIRCADIAN SYSTEMS—NEURAL SUBSTRATES AND FUNCTIONAL ORGANIZATION

6.1

INJECTIONS OF S-ANTIGEN ANTIBODY INTO THE BRAIN OF THE BLOW FLY (Calliphora vicina) INTERFERE WITH ENTRAINMENT AND THE EFFECTS OF CONSTANT LIGHT. D.S. Saunders*, B. Cymborowski, H.G. McWatters and Hong Seau Feng, Institute of Cell, Animal and Population Biology, University of Edinburgh, Edinburgh EH9 3JT, Scotland, U.K.

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 the flies so treated, the locomotor activity rhythm failed to entrain to a daily light-dark cycle (LD 12:12). In constant 'bright' light (LL > 0.04 Wm^{-2}) which normally caused arrhythmic locomotor behaviour, injection of antibody produced a rhythmic activity record in which the free-running period (τ) was lengthened, reminiscent of behaviour in 'dim' LL (< 0.04 Wm^{-2}). It is concluded that S-antigen antibody interferes with circadian entrainment or the production of LL-induced arrhythmicity by lowering the apparent sensitivity of the photoreceptor(s) involved. Since the compound eyes, ocelli and optic lobes may be surgically removed without affecting photic entrainment in C.vicina, these results focus attention on four groups of S-antigen positive neurons in the mid-brain as likely candidates for the extra-optic ("deep brain") photoreceptors in this insect.

6.2

THE OCCURRENCE OF CLEAR RHYTHMIC LOCOMOTOR ACTIVITY IN INDIVIDUAL *DISCONNECTED* MUTANTS OF *DROSOPHILA MELANOGASTER* IS ACCOMPANIED BY THE PRESENCE OF LATERAL NEURONS IN THESE FLIES. Charlotte Helfrich-Förster* and Wolfgang Engelmann*. Botanisches Institut, 72076 Tübingen, Germany

The lateral neurons (LNs) are good candidates for being circadian pacemaker neurons of Drosophila melanogaster (Frisch et al. 1994, Neuron 12: 555-570). The LNs contain the period-protein (PER), which is essential for rhythmic behavior (Hall and Rosbash 1993, Proc. Natl. Acad. Sci. USA 90: 5382-5383). Furthermore, one half of the LNs - the ventral LNs - contain a neuropeptide, the crustacean pigment-dispersing hormone (PDH) (Helfrich-Förster 1995, Proc. Natl. Acad. Sci. USA 92: 612-616). These PER-PDH-containing cells are not found in the majority of disconnected mutants (Helfrich-Förster und Homberg 1993, J. Comp. Neurol. 377: 177-190). Most disconnected flies are behaviorally arrhythmic (Dushay et al. 1989, J. Biol. Rhythms 4: 1-27). In about 2 % of disconnected brains single PER-PDH cells are immunostained with an antiserum against PDH. About the same percentage of flies show some circadian locomotor rhythmicity. The aim of the present study was to determine whether the occurence of rhythmicity and the presence of LNs is correlated in individual flies. After locomotor activity rhythm was monitored in individual flies, their brains were immunostained with an antiserum against PDH. Out of 70 recorded flies, 1 fly showed a clear circadian rhythmicity, 4 showed temporarily some rhythmic components and the remaining 65 were arrhythmic. A single PDH-immunoreactive neuron which belonged to the ventral LNs was found in the right brain half of the fly with clear circadian rhythmicity. All other flies had no remnants of the ventral LNs. The results further underline the role of the ventral LNs as circadian pacemaker neurons. On the other hand, they show that the ventral LNs are not the only cells that are involved in the generation of rhythmicity.

6.3

A NON-NEURAL ENDOCRINE PACEMAKER IN INSECT DEVELOPMENT.

Xanthe Vafopoulou and Colin G.H. Steel.
Department of Biology, York University, North York, Ontario M3J 1P3

The cellular responses comprising insect development are elicited by steroid moulting 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, the 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. Arrythmic PGs (from animals reared in LL) were maintained in vitro for 42h. 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 form nervous tissue.

6.4

THE ACCESSORY MEDULLA - THE CIRCADIAN PACEMAKING CENTER IN THE BRAIN OF OTHOPTEROID INSECTS? Uwe Homberg', Monika Stengl', Bernhard Petri', Stefan Würden', Thomas Reischig', Rudolf Loesel', and Franz Lippert'. Institut für Zoologie, Universität Regensburg, 93040 Regensburg, Germany

Lesion experiments have shown that the circadian pacemaking center in cockroaches and crickets resides in the optic lobe, near the proximal ventral edge of the medulla (Chiba and Tomioka 1987, Zool Sci 4:945-954). This area is occupied by a small distinct neuropil, the accessory medulla (aMe). We have investigated the organization and function of the aMe in locusts, crickets and cockroaches. The aMe is not retinotopically organized. Immunocytochemistry suggests that serotonin and a variety of partially colocalized neuropeptides including allatotropin, allatostatin, and FMRFamide-related peptides serve neuroactive roles in the aMe. A prominent group of aMe neurons is immunoreactive for ß-pigment-dispersing hormone (PDH). These neurons send fibers from the aMe to the lamina, the midbrain, and, in cockroaches and locusts, to the contralateral optic lobe. Differences in the anatomy of the PDH neurons are suited to explain differences in the coupling strength of the bilaterally paired pacemakers in crickets and cockroaches. Reappearance of circadian wheel-running activity after bilateral optic-stalk severance in the cockroach *Leucophaea maderae* correlates with regeneration of PDH-immunostained fibers from the aMe to their original targets in the midbrain. The intensity of PDH-immunostaining in the aMe shows a daily rhythm, and injections of PDH into the aMe cause phase shifts in circadian motor activity. Neurons of the aMe of *L. maderae* and the locust *Schistocerca gregaria* respond to photic stimuli. In the locust, neurons with sidebranches in the aMe are sensitive to polarized light. Inputs to these neurons in the aMe might account for time compensation in polarized-skylight orientation. Taken together, the data support a role of the aMe as circadian pacemaking center in orthopteroid insects. Supported by DFG grants Ho 950 and Ste 531.

6.5

CIRCADIAN PHASE DIFFERENCES IN A RETINAL PACEMAKER NEURON DELAYED RECTIFIER K CURRENT AND INCREASED CURRENT INDUCED BY A PHASE-SHIFTING NEUROTRANSMITTER, SEROTONIN. Jon W. Jacklet* and Steven Barnes. Dept. Biology, SUNYA, Albany, 12222 and Dept. Medical Physiol., Univ. Calgary, Alberta, Canada, T2N 4N1.

Pacemaker neurons of the Aplysia eye express a robust circadian rhythm of neuronal impulse activity. We dissociated the retina into primary culture and made whole cell patch recording from a subset of basal retinal pacemaker neurons. These neurons had resting potentials near -40 mV and, if neurites had grown out, produced spontaneous action potentials, >60 mV amplitude. Under voltage clamp 5 ionic currents were characterized, including a fast Na current, a Ca current, a delayed rectifier K current, an A current and a hyperpolarizing activated Cl current. Serotonin, which phase shifts the circadian rhythm of the intact eye, enhanced the delayed rectifier K current by 30%. 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 mechanisms of circadian clock.

6.6

GANGLION CELLS OF THE RETINOHYPOTHALAMIC TRACT IN MAMMALS. H.M. Cooper, J. Negroni and A. Attar. Cerveau et Vision, I.N.S.E.R.M. U-371, 69675, FRANCE

We have used retrograde tracers and viral tract tracing methods to examine the morphology and distribution of retinal ganglion cells (RGC's) 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 gamma class of ganglion cell. The cell soma is small to medium sized (9-15 µm 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 RGC's 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. RGC's are distributed over the entire surface of the retina in nocturnal species, but in the dorso-nasal region in diurnal species. In all animals RGC's 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 $P75^{NGFR}$ RECEPTOR MUTATION. Diego A. Golombek, Mark W. Hurd, Kuo-Fen Lee* and Martin R. Ralph. Department of Psychology, University of Toronto, Toronto, Ontario, M5S 1A1, CANADA. *Whitehead Institute for Biomedical Research, Massachusetts Institute of Technology, Cambridge, Massachusetts 02142.

A role for nerve growth factor (NGF) in the mammalian circadian system was assessed in hamsters using pharmacological methods and in mice using a line of gene targeted animals carrying a null mutation at the low affinity p75 receptor locus. In hamsters housed in constant dark, icv administration of NGF at various times in their circadian cycle produced phase shifts of locomotor rhythms that were similar in direction and circadian timing to those produced by brief pulses of light (delays at circadian time 13.5, and advances at CT 18). NGF administration at CT 18 also induced the expression of c-fos in the hypothalamic suprachiasmatic nucleus. Behavioral rhythms of gene-targeted mice were phase advanced while entrained to 24 hour light-dark cycles. In constant dark, unperturbed rhythms appeared normal but phase shift responses to brief pulses of light were significantly decreased. Together, these results support the hypothesis that NGF, through an action mediated at least in part, by $p75^{NGFR}$ receptors in the SCN, is involved in the regulation of the sensitivity of the system to light.

6.8

CALCINEURIN MODULATES CIRCADIAN RHYTHMS AND CIRCADIAN RESPONSES TO LIGHT. Martin R. Ralph and Diego A. Golombek. 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 underlying entrainment require the activation of biochemical pathways that result in the induction of immediate-early genes, particularly AP-1 components (Fos-Jun) in the SCN. Some of these pathways include calmodulin-dependent processes since inhibitors of calmodulin or calcium/calmodulin-dependent protein kinases can produce circadian phase shifts and also attenuate circadian responses to light. Calcineurin (phosphatase 2B) is a calmodulin-dependent protein that influences the function of AP-1 proteins in activated T-cells by dephosphorylating the 120kD NF-AT transcription co-factor and potentiating its translocation to the nucleus. It is also the common effector mechanism for immunosuppressants, cyclosporin A (CsA) and FK-506, which inhibit the phosphatase via cyclophilin and FKBP12 proteins, respectively. We report here that, in hamsters, both CsA (i.p.) and FK-506 (i.c.v.) significantly reduce circadian responses to light and 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 of the putative targets for calcineurin activity, NF-AT was present in a group of cells within and close to the SCN. Calcineurin activity therefore, appears to be required for the normal progression of the circadian cycle and for circadian responses to light. The specific phosphatase activity may be a link through which the immune system could influence rhythm generation and entrainment in mammals.

6.9

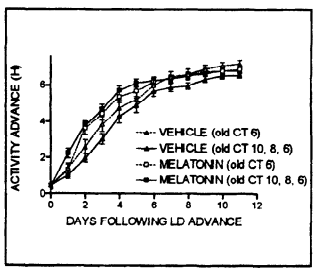
cGMP-DEPENDENT PROTEIN KINASE INHIBITORS BLOCK LIGHT-INDUCED PHASE ADVANCES OF CIRCADIAN RHYTHMS IN VIVO. Anuradha Mathur, Diego A. Golombek and Martin R. Ralph. Department of Psychology, University of Toronto, Toronto, Ontario M5S 1A1, CANADA.

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 kinases was examined in vivo using inhibitors of cAMP-dependent kinase (PKA) and cGMP-dependent kinase (PKG). In a constant dark, aperiodic 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 late 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. The 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.

6.10

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

Administration of melatonin to C3H/HeN 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 24 - 2 . In a reentrainment model, administration of melatonin for 3 days at the new dark onset facilitates the rate of reentrainment following an advance of the LD cycle (Soc. Neurosci. Abstr., 20:1438). This study examined whether melatonin administration 2 h before activity onset (CT 10, 8, 6) would further enhance the facilitation of reentrainment by melatonin. C3H/HeN mice were entrained to a 12:12 LD cycle, which was then advanced by 6 hours. Mice were treated with either melatonin (90 µg s.c.) or vehicle (1% ethanol/saline) for 3 days at either the new dark onset (CT 6) or at CT 10 on day 0, CT 8 on day 1, and CT 6 on day 2. As found previously, melatonin administered for 3 days at CT 6 facilitated the rate of reentrainment ($p < 0.05$), measured by daily advances in running wheel activity from pre-shift baseline. When administered according to the advancing time schedule, melatonin facilitated the rate of reentrainment compared to mice treated with melatonin at CT 6 on all 3 days ($p < 0.05$) and mice administered vehicle at CT 10, 8, and 6 ($p < 0.0001$). Thus, administration of melatonin at a shifting CT 10 improves the efficacy of treatment. Supported by a Glaxo grant (MLD) and NIH F32-AG05608 (SB).



6.11

DOPAMINERGIC ACTIVATION IS A TRANSIENT MECHANISM FOR ENTRAINMENT IN SYRIAN HAMSTERS. Pamela Snodgrass* and Fred C. 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 may be the activation of dopamine receptors within the fetal suprachiasmatic nucleus (SCN). In rats and hamsters, prenatal ip injections of the D1-dopamine receptor agonist SKF 38393 to the mother induces *c-fos* expression in the fetal SCN (Weaver et al., 1993; Viswanathan et al., 1994). Furthermore, daily injections of SKF 38393 to SCN-lesioned mothers on days 11 through 15 of gestation sets the phases of the offsprings' circadian rhythms as measured at weaning (Viswanathan et al., 1994). To determine whether D1 agonists also affect the circadian rhythms of adult hamsters, groups of male adult hamsters in constant dim light (< 1 lux) were given single injections of SKF 38393 (1-phenyl-2,3,4,5-tetrahydro-(1H)-3-benzazepine-7,8-diol hydrochloride, 8 mg/kg) or of vehicle at four circadian times (2, 8, 14, 20). The wheel running behavior of the hamsters was recorded for 7 days before the injections and for 20-30 days after. In addition, other groups of hamsters were given 5 daily injections of SKF 38393 or vehicle at circadian times 6 or 18. Although in neither experiment was there a phase-shifting effect of the drug, there was also no detectible induction of Fos immunoreactivity in the caudoputamen (CP). Therefore the first experiment was repeated with a different D1 agonist, SKF 81297 (6-chloro-7,8-dihyroxy-1-phenyl-2,3,4,5-tetrahydro-1H-3-benzazepine hydrobromide, 5mg/kg), which did induce Fos immunoreactivity in the CP. Like SKF 38393, SKF 81297 did not cause phase shifts at any of the four circadian times examined. Therefore, entrainment by dopaminergic activation observed during development appears to be transient. Despite the failure of D1 agonists to cause phase shifts in adults, SCN cells may still be affected by dopaminergic activation; preliminary results indicate that Fos immunoreactivity is induced in the adult SCN by SKF 81297. Supported by NIH Grant HD18686 to F.C.D.

6.12

SEROTONERGIC STIMULATION AND NON PHOTIC PHASE SHIFTING IN HAMSTERS. Katarzyna Bobrzynska, Matthew Godfrey and Nicholas Mrosovsky. Departments of Zoology and Physiology. University of Toronto, Toronto, Ontario M5S 1A1, Canada.

Various stimuli that make hamsters active also phase shift their circadian clocks. Activity or its correlate appears to be important in producing the shifts because preventing locomotion blocks the phase shifts otherwise found after dark pulses or triazolam administration. The physiological basis of clock resetting after induced activity remains unknown. The serotonergic (5-HT) projection from the raphe to the suprachiasmatic nucleus (SCN) is a possible route by which non photic information could reach the pacemaker. Administration of 8-hydroxy-2(-di-n-propylamino)tetralin hydrobromide (8-OH-DPAT), a 5-HT1A and 5-HT7 receptor agonist) at circadian time (CT) 8 produces phase advances in the circadian rhythms of hamsters. For 5-HT to be a candidate for mediating the effect of activity on the pacemaker, it must be shown that 5-HT agonists do not produce shifts simply because they make animals more active. Therefore, we investigated the contribution of activity to the 8-OH-DPAT produced shifts. Phase-shifting effects of this drug are not mediated by increased activity levels, because preventing hamsters from moving after receiving 8-OH-DPAT did not abolish phase shifts. Moreover, higher doses of 8-OH-DPAT, which diminished activity on the day of injection did not affect the amplitude of phase shifts. Surprisingly quipazine (a non specific 5-HT agonist), when injected in the middle of subjective day did not phase shift the activity rhythm of hamsters, as it has been reported to in rats.

6.13

ACTIONS OF NON-PHOTIC STIMULI ON THE ERG CIRCADIAN RHYTHM OF CRAYFISH. Beatriz Fuentes-Pardo*, Miguel Angel Verde* and Araceli de la O-Martínez.* Depto. Fisiología. Fac. Medicina, UNAM. Apdo. Postal 70-250. México 04510 México.

Adult male crayfish (Procambarus clarkii) were kept in constant darkness and temperature (16°C). For ten days, every three minutes they received a 15 µs, 200 lux light flash, to elicit the photoreceptors' electrical response to light (electroretinogram, ERG) which was recorded and storaged for ulterior analysis. At different circadian time of the fifth day of recording, an adult female crayfish was introduced into the recording chamber during 4 hours. The relative position between both specimens did impossible that one could see the other one. The amplitude of the ERG showed periodic variations following a clear circadian pattern. After the presence of the female (but not of a male in control experiments) the ERG circadian rhythm showed a transient phase and changed in both phase and period which were circadian-time dependent. These observations show that non-photic events are able to produce major reorganization of the ERG circadian rhythm presumably associated with chemical (hormonal) stimulation.
Supported by IN-213394 DGAPA grant.

6.14

CONSTANT LIGHT HOUSING INDUCES FOS PROTEIN IN RAT INTERGENICULATE LEAFLET. Kim Edelstein* and Shimon Amir.* Center for Studies in Behavioral Neurobiology, Department of Psychology, Concordia University, Montreal, Canada H3G 1M8.

Exposure to prolonged constant light (LL) disrupts circadian rhythms in nocturnal rodents. This disruption may be manifested as a loss of circadian rhythmicity, desynchrony of individual rhythms, or splitting of circadian activity rhythms into two or more components. The neural mechanisms underlying these effects are not well defined, although previous research has demonstrated that ablation of the intergeniculate leaflet (IGL) prevents splitting of circadian activity rhythms in the hamster. We examined the effects of LL on the circadian system of Wistar rats previously housed under a normal light-dark cycle, using a telemetry system to measure temperature and activity rhythms, and the immunocytochemical expression of Fos protein as a marker of light-induced activation of cells in the IGL. Results indicate that LL induces Fos expression in the IGL independent of circadian time. Furthermore, we found a correlation between the disruption of circadian temperature and activity rhythms observed in rats housed in LL for 30 days, and attenuation of Fos immunoreactivity in the IGL of those animals. Whereas Fos expression was observed in the IGL of rats sacrificed during the LL period prior to the loss of circadian rhythmicity, rats sacrificed after this disruption occurred exhibited few Fos immunoreactive cells in this region. Results support the idea that the IGL mediates the disruption of circadian rhythms during exposure to prolonged LL. These data also suggest that Fos expression in the IGL may be a marker of the integrity of the circadian system in rats housed under prolonged LL.

6.15

EFFECT OF NMDA RECEPTOR BLOCKADE ON THE PINEAL GLAND RESPONSE TO A LIGHT PULSE. Shawn A. Rowe, Sally A. Ferguson and David J. Kennaway.
Circadian Physiology Group, Department of Obstetrics and Gynaecology,
University of Adelaide Medical School, Adelaide, South Australia, 5005.

The suprachiasmatic nucleus (SCN) has a key role in the generation of endogenous biological rhythms and their entrainment to the external environment. Light is the predominant external regulator of SCN function, with excitatory amino acids (EAA) believed to be the transmitters mediating the effects of light in the retino-hypothalamic tract. In the current study we investigated the effects of a non-competitive antagonist of N-Methyl-D-Aspartate receptors (Dizocilpine maleate; (+)-MK-801) on the light induced entrainment of the melatonin rhythm. Albino-Wistar male rats (80g) were acclimatised to a 12L:12D photoperiod (lights off 1800h). Rats were injected with saline or MK-801 15 minutes before a light pulse (1 min / 2 lux) at 2200h. Hourly urine samples were collected on the nights before, during and after treatment and assayed for the melatonin metabolite, 6-sulphatoxy-melatonin (aMT.6S). Rats injected with saline had an acute transient decrease in urinary aMT.6S excretion rate after the light pulse and a delay in the onset of excretion on the night following treatment (2.0 ± 0.4h delay compared to the first night). In the absence of a light pulse the onset on the third night remained unchanged (0.3 ± 0.2h delay). MK-801 (3 mg/kg) injected prior to the light pulse failed to block both the acute decrease and delayed onset of the aMT.6S excretion the following night (the onset of aMT.6S excretion was delayed by 1.5 ± 0.1h compared to the first night). In the absence of a light pulse, neither MK-801 injection (3 mg/kg) nor NMDA administration (30 mg/kg) at 2200h had an acute effect on aMT.6S excretion and the onset of aMT.6S excretion was unchanged following these treatments. These results suggest that EAA neurotransmission is not essential for photic entrainment of the melatonin rhythm in rats.

6.16

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 and Gynaecology,
University of Adelaide Medical School, Adelaide, South 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 retino genuiculate pathway. The serotonergic projection from the raphe nucleus to the SCN has also been shown to influence circadian rhythms but its role is less clear. This study examined the role of serotonin in the transfer of light information to the SCN/pineal gland axis, using the serotonin agonist, Quipazine. Albino-Wistar rats (80 g) were acclimatised to a 12L:12D photoperiod (lights off 1800; CT12) and placed in constant darkness from CT12 on night 1 until the end of the experiment. Saline or quipazine (1, 3 or 10 mg/kg; sc) were injected at CT18, urine collected in hourly fractions and assayed for the melatonin metabolite, 6-sulphatoxy-melatonin (aMT6.S). Quipazine administration caused a rapid, dose dependent suppression of the excretion of aMT.6S, as well as a significant phase delay in aMT.6S onset on the 2 nights following administration compared to night 1 (night 3;1.0 ± 0.3, night 4; 1.8 ± 0.6 h). Saline injection at CT18 did not alter the aMT.6S excretion rate (night 3; 0.4 ± 0.2h, night 4 : 0.6 ± 0.2h). The $5HT_{1a}$ receptor agonist, 8-OH-DPAT (2-Dipropylamino-8-hydroxy-1,2,3,4-tetrahydronaphthalene-hydrobromide; 5 mg/kg; ip) also had significant acute and phase delaying effects on aMT.6S excretion (night 3; 0.8 ± 0.2 h, night 4; 1.0 ± 0.2h). The acute decrease in aMT.6S excretion and subsequent delay in the onset of excretion on the following nights by serotonin agonists suggests an action at the level of the SCN, possibly via the newly described $5HT_7$ receptor. We conclude that serotonergic pathways have an important role as mediators of light on the SCN/pineal gland axis of rats.

6.17

PARTIAL ANTAGONISM OF THE EFFECTS OF MELATONIN AGONISTS ON HAMSTER SUPRACHIASMATIC (SCN) NEURONS. S.W. Ying*[1], B. Guardiola-Lemaître*[2], P. Delagrange*[2] and B. Rusak*[1]. 1) Dalhousie Univ., Halifax, Nova Scotia, Canada B3H4J1; 2) IRIS, 92415 Courbevoie, France.

S-20928 (N-[2-(1-naphtyl) ethyl] cyclobutyl carboxamide) has been shown previously to antagonize melatonin (Mel) inhibition of forskolin-stimulated cAMP production stimulated by forskolin in ovine pars tuberalis cell cultures, to reverse the pigment aggregation produced by Mel in Xenopus melanophores, and to prevent the body weight gain induced by decreasing photoperiod in the garden dormouse. We investigated whether S-20928 can also antagonize the neurophysiological effects of Mel and a Mel agonist (S-20098 : (N-[2-(7-methoxy-1-naphtyl) ethyl] acetamide)) on neurons in the hamster SCN, intergeniculate leaflet (IGL), hippocampus and dorsal lateral geniculate nucleus (dLGN). When S-20928 (0.5-10.0 mg/kg BW in 20 % DMSO vehicle) was injected intraperitoneally alone, it caused dose-dependent suppression (10-50 %) of firing rates of SCN and IGL cells for 5-30 min. Iontophoresis of Mel or S-20098 onto photically responsive SCN and IGL cells reduced both spontaneous firing rates and photic activations. To test its antagonist properties, S-20928 was injected 1 min before iontophoretic application of Mel or S-20098 to a target neuron at doses that alone caused less than 20 % decreases in firing rates (1.0-2.0 mg/kg BW). S-20098 reversed agonist effects by 30-65 % for 25/87 SCN and IGL cells (28.7 %), and by 20-30 % for 40/87 cells (46 %); for 22 cells (25.3 %), there was no antagonism (<20 % change) or even potentiation of Mel effects. S-20928 was somewhat less effective for non-visual SCN cells, hippocampal and dLGN cells. The serotonin antagonist metergoline failed to reverse Mel or S-20098 effects. These results indicate that S-20928 may have mixed agonist/antagonist actions, but at low doses, it partially antagonized neurophysiological effects that are presumably mediated by activation of Mel receptors.

6.18

NEURAL CONNECTIONS BETWEEN ANTERIOR HYPOTHALAMIC GRAFTS AND THE HOST BRAIN: DOES OUTGROWTH ARISE FROM THE DONOR SCN, AND IS THERE TARGETED INGROWTH? Michael N. Lehman[1], Joseph LeSauter[2], Charles Kim[1], Wendy Strother[1], and Rae Silver[2]. [1]Dept. Cell Biol., Neurobiol. & Anat., Univ. Cincinnati. Coll. Med., Cincinnati, OH 45267 and [2]Dept. Psychol., Barnard Coll., New York, NY 10032.

Recent studies reveal that fetal grafts of the anterior hypothalamus (AH) containing the SCN possess extensive efferents (Sollars & Pickard, Brain Res., 614:212; Lehman et al., Cell Transplant., 4:75). However, it is not known whether this outgrowth arises from the donor SCN or from extra-SCN tissue, nor whether there is targeted ingrowth from the host brain. To address the former, we implanted fetal (E19) rat SCN micropunch grafts that contain minimal extra-SCN tissue into the third ventricle of immunosuppressed, SCN-intact hamsters (n=6). Additional hamsters received either fetal rat AH grafts (n=4), cortical (CTX) micropunch grafts, or larger CTX grafts (n=6). To determine whether grafts receive targeted ingrowth, we implanted fetal (E15) hamster AH tissue into the third ventricle of immunosuppressed rats (n=7). After 2 wks, animals were perfused and brain sections immunostained using a neurofilament (NF) antibody (RMO 108, gift of Dr. V. Lee) that recognizes rat but not hamster NF. 50% of fetal rat SCN micropunch grafts, 75% of AH grafts, 75% of CTX micropunch grafts, and 100% of CTX grafts were viable; 70% of fetal hamster AH grafts survived. Fetal rat AH grafts consistently innervated regions that are normal targets for SCN and AH efferents. Both types of CTX grafts heavily innervated the preoptic area and hypothalamus as well as more distant sites such as the lateral hypothalamus and amygdala, often via white matter tracts. In contrast, outgrowth from SCN micropunch grafts was variable: one graft possessed no outgrowth, another had only a few fibers into the host AH, and the third innervated some of the areas contacted by AH grafts albeit with fewer fibers. Thus, SCN micropunch grafts vary in the extent of their outgrowth, suggesting that much of the outgrowth from AH grafts arises from extra-SCN tissue. Fetal hamster AH grafts implanted into rat hosts contained areas of heavy fiber ingrowth. In each graft, we also found large (20-30 µm diam.) host neurons within innervated areas, suggesting that they migrated into the graft along with ingrowing fibers. It remains to be determined whether these cells/afferents, or the efferents described above, are important for functional recovery in lesioned recipients. Supported by NS28175 (MNL) & NS 24292 (RS).

6.19

DISTRIBUTION OF NADPH-DIAPHORASE ACTIVITY AND LIGHT-STIMULATED FOS IMMUNOREACTIVITY IN THE RAT SUPRACHIASMATIC NUCLEUS. Shimon Amir*, Barry Robinson* and Kim Edelstein*. Center for Studies in Behavioral Neurobiology, Dept. of Psychology, Concordia University, Montreal, Canada H3G 1M8.

Nitric oxide (NO) serves as a messenger molecule in some neuronal systems that use glutamate as a transmitter and glutamate mediates the transmission of photic signals by retinal ganglion cell axons terminating in the suprachiasmatic nucleus (SCN). Pharmacological treatments which block NO synthesis by NO synthase (NOS) prevent glutamate-induced phase shifts of the cell firing rhythm in SCN slice preparation in vitro; similar treatments inhibit light transmission to the SCN as well as light-induced phase shifts in activity rhythms in vivo, implicating NO in circadian light signaling in vivo. There is limited information, however, about the presence and function of NOS-containing neurons within retinorecipient regions of the rodent SCN. We used NADPH-diaphorase histochemistry and immunostaining for Fos protein to assess the co-distribution of NOS-containing neurons and light-responsive cells in the rat SCN region. Convergence between NADPH-diaphorase stained fibers and Fos-immunoreactive cells was noted inside the SCN, but the number of NADPH-diaphorase stained elements found in the SCN was substantially low compared with that found in retinorecipient regions bordering the nucleus. Co-localization of Fos immunoreactivity and NADPH-diaphorase histochemical activity within individual cells was not detected. The results rule out the possibility that photically-activated cells in the SCN synthesize and release NO. Instead, photic stimulation may trigger NO synthesis in NOS-containing neurons located near the photically-activated cells.

6.20

EFFERENTS OF THE SUPRACHIASMATIC NUCLEUS FORM CLOSE APPOSITIONS WITH BOTH ESTROGEN RECEPTOR AND GnRH NEURONS IN HAMSTERS. Horacio O. de la Iglesia and Eric L. Bittman. Neuroscience & Behavior Program, Univ. of Massachusetts, Amherst 01003.

Ovulation depends on a surge of pituitary luteinizing hormone (LH), which in turn results from the secretion of gonadotropin releasing hormone (GnRH). This peptide is synthesized in the preoptic area (POA) and anterior hypothalamus and released from the median eminence. GnRH release is under control of ovarian estradiol and the circadian system. Lesions of the suprachiasmatic nucleus (SCN) abolish LH surges. The POA and anterior hypothalamus contain both estrogen-receptor (ER) and GnRH immunoreactive neurons but ER and GnRH are not generally colocalized in individual cells. Both the POA and anterior hypothalamus receive SCN projections. We asked whether the SCN may regulate the LH surge through input to GnRH and/or ER immunoreactive neurons. We used *Phaseolus vulgaris* leuco-agglutinin (PHA-L) as an anterograde tracer of SCN efferents and performed double label immunocytochemistry for PHA-L and ER or GnRH. SCN projections make close appositions with both estrogen receptor and GnRH neurons. Appositions on ER cells are present in the medial preoptic area (MPA), septohypothalamic nucleus (SHy), strial part of the preoptic area and bed nucleus of the stria terminalis. 5 to 15 % of ER cells in these areas are contacted by SCN efferents. Appositions on GnRH cells are present in the MPA, anterior medial preoptic nucleus, periventricular hypothalamic nucleus, lateral septum, SHy, and diagonal band. 11 % of the GnRH cells are contacted by SCN efferents. Projections of neurons of the subparaventricular hypothalamic nucleus and the retrochiasmatic area, both proposed relay stations of the circadian system, also make appositions with these two cell types. These results suggest two pathways by which the circadian system may regulate the timing of ovulation. Other methods will be required to determine whether these appositions represent synaptic contacts. Supported by NIMH RO1-44132.

6.21

EFFECT OF PHOTOPERIOD REVERSAL ON EX VIVO SUPRACHIASMATIC NUCLEUS ELECTRICAL ACTIVITY RHYTHMS. R.L. Pieschl*, F.D. Yocca* and V.K. Gribkoff*. CNS Biology, Bristol-Myers Squibb Pharmaceutical Research Institute, 5 Research Parkway, Wallingford, CT 06492.

When rats maintained on a 12 hour light/dark cycle are exposed to a photoperiod reversal (12 hour delay in the onset of darkness), wheel-running activity rhythms become phase delayed, with the entrainment to the new photoperiod occurring after about 1 week (Quay, Physiol. Behav. 5:1281-1290, 1970). It is not clear whether this latency to entrain the activity rhythm represents the latency required to entrain the underlying circadian pacemaker (the suprachiasmatic nucleus, SCN), or whether the behavior has been affected by alterations in the pacemaker output. To approach this question we recorded the circadian rhythm of the multiple-unit electrical activity in vitro from SCN neurons in hypothalamic brain slices taken from rats housed in a reversed photoperiod for 1 to 14 days. Different groups of rats were used at each time point, and electrical activity was recorded for 2-3 days. These rhythms were compared to control values recorded from slices taken from rats housed in a normal 12 hour light/dark cycle. We found that the rhythms recorded in vitro initially were not entrained to the reversed photoperiod, but they gradually shifted and became entrained after 7 days. This was similar to the entrainment of activity rhythms reported by others. These results suggest that the SCN electrical activity recorded in vitro and behavior rhythms are temporally coupled.

6.22

VASOPRESSIN DEFICIENCY AND CIRCADIAN RHYTHMS WITH NOCTURNAL FOOD-RESTRICTED FEEDING. Cyrilla H. Wideman*, George R. Nadzam*, and Helen M. Murphy*. Departments of Biology and Psychology, John Carroll University, Cleveland, OH 44118

Male, vasopressin-containing, Long-Evans (LE) and vasopressin-deficient, Brattleboro (DI) rats were maintained in individual cages while telemetered body temperature (BT), heart rate (HR), and activity level (AC) data were collected. The rats were exposed to a 12/12 light/dark cycle (photic zeitgeber). Two separated 1h feeding periods (nonphotic zeitgeber) were also used. While general light/dark patterns of circadian rhythms of BT, HR, and AC were similar in DI and LE rats, there were significant differences in BT and HR with DI rats exhibiting lower values in both of these parameters. In addition, the timing of nocturnal peaks of BT in DI rats followed, rather than anticipated, the ingestion of food. Results demonstrate that both photic and nonphotic zeitgebers act in synchrony for BT, HR, and AC for LE rats. In DI rats, these two zeitgebers are acting in synchrony for HR and AC; however BT is more dependent upon the physiological consequences of the lack of vasopressin than upon a nonphotic zeitgeber. (Research supported by a Focused Giving Grant from Johnson & Johnson.)

6.23

VASOPRESSIN DEFICIENCY AND CIRCADIAN RHYTHMS WITH AD-LIBITUM AND DIURNAL FOOD-RESTRICTED FEEDING. Helen M. Murphy*, George R. Nadzam*, and Cyrilla H. Wideman*. Departments of Psychology and Biology, John Carroll University, Cleveland, OH 44118

Male, vasopressin-containing, Long-Evans (LE) and vasopressin-deficient, Brattleboro (DI) rats were maintained in individual cages while telemetered body temperature (BT), heart rate (HR), and activity level (AC) data were collected. The rats were exposed to a 12/12 light-dark cycle. Two conditions were utilized: 1) ad-lib feeding during the 24h cycle and 2) two separated 1h feeding periods in the light cycle. With ad-lib feeding, nocturnal cycles of BT, HR, and AC were maintained in DI and LE rats. There were significant differences in BT and HR with DI rats exhibiting lower values in both of these parameters. With separated feeding periods during the light cycle, BT, HR, and AC parameters shifted from nocturnal to diurnal patterns and the photic zeitgeber was lost in DI rats. On the other hand, LE rats lacked a well-defined circadian rhythmicity and never lost the photic zeitgeber. Results show that vasopressin plays a modulatory role in endogenously determined, but environmentally synchronized, rhythms. (Research supported by a Focused Giving Grant from Johnson & Johnson.)

6.24

ROLE OF MELATONIN IN THE CIRCADIAN SYSTEM OF JAPANESE QUAIL. Herbert Underwood. North Carolina State Univ., Raleigh, N.C. 27695

The rhythm of blood-borne melatonin in quail is due to melatonin secretion by both the pineal and eyes: The pineal contributes two-thirds of the blood melatonin and the eyes the remaining one-third. An intraocular clock drives the ocular melatonin rhythm. The eyes play a major role in the circadian system of Japanese quail because bilateral eye removal causes the body temperature and activity rhythms of female quail to damp into arrhythmicity in DD. However, the majority of birds subjected to optic nerve section remain rhythmic in DD, although rhythmicity is less robust, and 25% are rendered arrhythmic. Optic nerve section does not affect the eyes' ability to synthesize or secrete melatonin. The results suggest that a pacemaker in the eye acts to maintain rhythmicity of central oscillators (in SCN?) 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 and sham-pinealectomized birds in DD and melatonin entrained normal birds when administered daily. The results support the hypothesis that melatonin is importantly involved in linking pacemakers in the eyes to the rest of the circadian system.

6.25

MELATONIN RELEASE BY CULTURED PARIETAL EYE, PINEAL AND RETINA IN *IGUANA IGUANA*. Gianluca Tosini*, and Michael Menaker, Department of Biology, University of Virginia, Charlottesville, VA 22903.

Many investigations have indicated that the pineal complex (parietal eye and pineal gland) and the lateral eyes are components of the circadian system of nonmammalian vertebrates. The existence of circadian oscillators within the pineal is supported by the demonstration that isolated pineals of some birds, lizards and fishes, when cultured *in vitro*, can maintain circadian rhythms of melatonin synthesis for some days in DD. Also the lateral eyes of the frog *Xenopus* contain self-sustained circadian oscillators. Although there is some evidence that the parietal eye of some lizards might be involved in the circadian organization and may produce melatonin, this structure has never been studied in *in vitro* culture to test for the presence of circadian oscillators. The aim of this study was to investigate the presence of circadian oscillators in the pineal, parietal eye and retina of the lizard *Iguana iguana*. In order to determine whether these structures contain circadian oscillators, we measured melatonin release from separately cultured parietal eyes, pineals and retinas. The methods we used were similar to those used previously to measure melatonin rhythms from cultured pineals. Parietal eyes, pineals and retinas were removed from the lizards in light, cultured individually in a flow-through culture apparatus, while being superfused at a rate of 1ml/hr with GIBCO 199 culture medium at constant temperature of 27 °C. Melatonin was measured by RIA. Melatonin release from cultured parietal eyes, pineals and retinas was entrained in L:D cycles with melatonin being high during the dark phase of the cycle and low during the light phase. When they were release in constant darkness the pineals showed a clear circadian melatonin rhythm which persisted for at least 5 days, indicating that a self-sustained oscillator within the pineal regulates melatonin synthesis. Although we have not yet observed persistent rhythms in cultured parietal eyes or retinas, we have not yet optimized the culture conditions for these structures. Our results demonstrate that in *I. iguana* all organized photoreceptive structures are capable of rhythmically producing melatonin in synchrony with a L:D cycle. Whether the parietal eye and retina like the pineal, contain circadian oscillators remains to be seen.

6.26

INTERACTIVE EFFECTS OF LIGHT INTENSITY AND CLONIDINE TREATMENT ON FREE-RUNNING CIRCADIAN RHYTHMS IN RATS. Alan M. Rosenwasser, Suzanne Dwyer, and Ashley Transki. University of Maine, Orono, ME. 04469.

Previous research from this laboratory has shown that chronic administration of the alpha-2 adrenergic agonist, clonidine, shortens the 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, suggesting that clonidine treatment may interact with ambient lighting conditions to influence free-running period. In the present study, we tested this hypothesis by examining the effects of chronic clonidine administration (3.0 ug/ml via the drinking water) on circadian activity rhythms in rats successively maintained under a series of different light intensities, including constant darkness. Compared to control animals, clonidine administration shortened free-running period only under moderate to high light intensities. Indeed, clonidine treatment shortened 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. Instead, when taken together with our previous studies, these results indicate that an alpha-adrenergic mechanism interacts with light intensity in a complex manner to influence free-running circadian period.

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 homeotherms 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, the activation of thermoregulatory responses should defend the circadian oscillation in body temperature. However, the present studies in golden hamsters show that the modulation of the thermoregulatory 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.

6.28

SUPRACHIASMATIC NUCLEI (SCN) LESIONS DO NOT AFFECT HYPERTHERMIA AFTER OPEN FIELD STRESS IN RATS. Maciej Wachulec*, Hua Li*, Hideto Tanaka*, Elizabeth Peloso* and Evelyn Satinoff. Psychology Department, University of Delaware, Newark, DE 19716.

Rats placed in an open field become hyperthermic, more so in the light (L) than in the dark (D). We examined whether SCN lesions that reduce or abolish the circadian temperature rhythm also abolish the circadian pattern of open field stress response. 7 mo old male rats were kept individually at 23±1°C in LD 12:12 (lights-on at 07:00). Body temperature (Tb) was recorded telemetrically. At least 3 wk after electrolytic SCN lesions, open field stress was induced by placing the rats individually in a clear open container 25x50x30 cm for 30 min. Experiments were performed between 10:00-14:00 and 22:00-02:00. In control rats Tb rise depended on time of day; stress induced a significant increase in Tb only in L, when Tb was at its trough. In rats with SCN lesions, Tb was flatter: higher in L and lower in D, and the stress-induced Tb rise was significant during both L and D. We conclude that SCN lesions, which eliminate circadian Tb rhythms, do not eliminate the homeostatic rise in Tb due to open field stress.

Supported by NIMH Grant #7R01MH41138-08 and Alzheimer's Association Grant #11RG-93-036 to ES.

6.29

THE EFFECTS OF EXERCISES AT THE DIFFERENT TIME OF A DAY UPON CIRCADIAN RHYTHMS OF BLOOD GLUCOSE AND MALIC DEHYDROGENASE IN RATS.

LI Liang

Hainan Teacher College, Haikou city, Hainan, P.R. of China

64 Wistar rats in 2 groups, Comparson(CG) and Experiment(EG), were employed andat the different 4 time-fixed points of a day, 8 rats in EG, after taking the treadmill-run(3km/h-5%)for 20 minutes, were put into water(25-28℃)for swimming until they exhaused and sank to the bottom of the pool, and then, being picked out and cut heads for sampling blood immidiately. 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 Cosinor The results showed that the chronoexercise made amplitude of the malic dehydrogenase decrease ($P<0.05$), while that of the blood glucose was increase($P<0.05$). But, the acrophases for both indexes advanced. The finding indicated that the high-intensity chronoexercise could infuence the enzyme activity related to aerobic oxidation and the levels of blood glucose and make the acrophase advance. The patterns of these kind of the physiological osillations may be related to the patterns of the exercises at the different time.

TUESDAY/WEDNESDAY BEHAVIOR AND ECOLOGICAL RELEVANCE OF CIRCADIAN RHYTHMICITY

13.1

CIRCADIAN RHYTHMICITY FOR LATENCY TO MATING IN DROSOPHILA. Bernard Possidente*, Helmut V.B. Hirsch*[a] and Debra R. Possidente*. Skidmore College, Biology Dept., Saratoga Springs, NY 12866.

Virgin flies were tested for copulation latency hourly in 12:12LD at four days post-eclosion. Mean latency was shortest at about six hours after lights-on, and longest in the dark. The frequency of latencies in the top quartile ("fast flies") peaked at about the same time. Mated pairs 180^{o} out of phase followed the female's rhythm. Wild-type and per^{o} mutants, mated 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 hour six of the subjective day. There was no fast-fly peak for per^{o} in LD or DD. None of these four groups showed a peak for mean latency when all flies were included. Per^{o} had a longer mean latency than wild-type. Latency was longer in DD than LD for wild-type but not per^{o}. 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. [a]Biology Dept., SUNY Albany, Albany, NY 12222. Supported by a Whitehall Foundation grant to H.V.B.H.

13.2

POSSIBLE CIRCADIAN PERIOD MUTATION IN THE ALBINO DEERMOUSE, *P. maniculatus*. Patricia J. DeCoursey* 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 actographs 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.

13.3

Selective breeding for nocturnality in Arvicanthus niloticus, L., Smale, and C. Katona, Department of Psychology, Michigan State University, East Lansing, MI, 48824

Arvicanthus niloticus is a murid rodent with daily rhythms in body temperature and mating behavior that are typical of those seen in diurnal species (McElhinny, unpublished data). Wheel running rhythms were diurnal in 24 of 25 individuals housed in a 12:12 LD cycle. One female, however, exhibited a nocturnal pattern of wheel running, with activity that regularly continued for 7 hours after the lights went out, and was relatively infrequent during the day. This nocturnal female was paired with a male and produced 14 offspring in 4 litters. Of these animals, 47% 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.

13.4

SOCIAL CUES ENHANCE REENTRAINMENT RATES OF TEMPERATURE AND ACTIVITY RHYTHMS ONLY IN FEMALE OCTODON DEGUS. Namni Goel* and Theresa M. Lee, Department of Psychology, Neuroscience Lab Bldg, 1103 E. Huron St., University of Michigan, Ann Arbor, MI 48104-1687.

Octodon degus is a diurnal, highly social rodent from South America. Previous experiments with degus showed that female phase-shifters housed with entrained (donor) female partners (FF) resynchronized their activity and temperature rhythms 30-40% faster than female phase-shifters housed alone following six-hour phase advances of the light-dark cycle. The FF group also resynchronized their rhythms faster than female phase-shifters housed with entrained males. Social cues, however, had no effect on male phase-shifter resynchronization (Goel and Lee, in press). The goals of the two experiments reported here were: 1.) to determine if the enhancement of resynchronization rates was a result of social cues directly affecting the circadian system or the result of masking by female donors on female phase-shifters and 2.) to investigate whether the sex differences on effects of social cues occurred in conditions other than a six-hour advance (e.g., a six-hour delay). In the first experiment, female phase-shifters housed with female donors or females housed alone underwent a six-hour phase advance, and on the day following reentrainment, phase-shifters were transferred to constant conditions (DD). The temperature and activity rhythms of female phase-shifters free-ran from the point at which reentrainment occurred whether females were housed alone or with a female donor during the shift. Thus, social cues speed up reentrainment in female degus and are a viable nonphotic zeitgeber capable of enhancing the photic effects on the circadian system in this species. In the second experiment, female phase-shifters reentrained the temperature and activity rhythms 20-35% faster when housed with either entrained females or males compared to females housed alone following six-hour phase delays. No significant differences in reentrainment rate for phase-shifting males existed between test conditions. This experiment extends the previous finding that females, but not males, can respond to donor cues to increase reentrainment rates. Donor cues from females enhance reentrainment after advances and delays, but the effect of male donor cues is dependent on the direction of the phase shift.

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-26823 and an NSF Graduate Research Fellowship

13.6

MODIFICATIONS OF ULTRADIAN AND CIRCADIAN CO2 OSCILLATIONS BY SEVERAL ENVIRONMENTAL CHANGES. M. Stupfel, A. Perramon and V. Gourlet. INSERM, Le Vésinet, 78110, France.

Carbon dioxide emission ($\dot{V}CO2$) taken as an index of metabolic exchanges shows oscillations which have been analysed for ultradian (UR) and circadian (CR) rhythms by several statistical procedures. Sampling $\dot{V}CO2$ every 20 min shows interspecies statistical ($P<0.001$) differences for UR ($\tau \leq 40$ min) periods and amplitudes. In all cases UR persist, even in the absence of CR. Periods, amplitudes and phases were compared in LD12:12 in diverse environmental conditions. Starvation in rats and quail progressively decreases the CR amplitudes more than those of UR and provokes CR phase advance in rats. Mobility restraint by suspension of quail decreases their L→D and D→L circadian responses but has respectively only slight and no effect on UR periods and amplitudes. Ageing influence was measured in 34, from 44 till 965 day old, rats. Tremendous changes in circadian L→D responses and circadian differences between L and D $\dot{V}CO2$ levels were found, while no differences were observed in UR. Grouping of mice and of quail influences CR much more than UR. This data corroborates previous ones which showed that in several endotherms submitted to DL12:12, LL or DD, UR remain stable, which is not the case for CR. These results support the hypothesis that UR would be an endogenous thermodynamic chronobiological entity distinct from CR.

CLOCKS, PHOTOPERIODISM, AND CIRCANNUAL RHYTHMS

14.1

ANNUAL FAT CYCLE OF THE VIRGINIA OPOSSUM, Didelphis virginiana: A CIRCANNUAL RHYTHM SYNCHRONIZED BY PREVAILING PHOTOPERIOD? John N. Mugaas. 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, the daily light:dark cycle matched that 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 ± 0.76 kg, at a rate of 0.025 ± 0.006 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 ± 0.0001 kg/day) was comparable to that measured for males, but the second one had a reduced magnitude and rate of gain (0.88 ± 0.32 kg, at a rate of 0.009 ± 0.004 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

CIRCANNUAL RHYTHMS OF PINEAL MELATONIN PRODUCTION IN FEMALE RATS ARE ABOLISHED BY GROWTH OF MALIGNANT TUMORS. H Bartsch[1]*, C Bartsch[1]*, F Deerberg[2]*, D Mecke[3]*, TH Lippert[1]*. [1]Section of Clinical Pharmacology, University Women's Hospital, and [3]Institute of Physiological Chemistry, D-72076 Tübingen, Germany; [2]Central Institute for Laboratory Animal Breeding, D-30455 Hannover, Germany

The pineal hormone melatonin shows a tumor-size dependent decline in patients with primary breast and prostate cancer prior to operation (1). In order to analyze pineal activity over the complete course of tumor development and growth, urinary 6-sulphatoxymelatonin (aMT6s) representing pineal melatonin production was determined over the period of one year in two strains of female rats: 1.) Fischer rats with chemically induced mammary carcinomas and 2.) BDII/Han rats with spontaneous endometrial carcinomas. Only tumor-free animals (i.e. untreated controls in case of Fischer rats; BDII/Han rats with tumor growth suppressed by treatment with melengestrol acetate) had significant circannual aMT6s rhythms with peaks in May and July respectively. The absence of circannual rhythms in animals bearing mammary carcinomas was due to an elevated excretion of aMT6s at the time when tumors became palpable. Animals with endometrial carcinomas showed different phases of decreased and increased urinary aMT6s. These results show that the growth of malignant tumors is associated not only with disturbances of the circadian rhythms of various hormones including melatonin (1) but also with disruption of circannual rhythmicity of the pineal hormone. (1) Bartsch C et al. Ann NY Acad Sci 1994; 719:502-525.

14.3

MELATONIN AFFECTS THE CIRCADIAN CLOCK OF THE DJUNGARIAN HAMSTER, *PHODOPUS SUNGORUS*. G. Robert Lynch. Department of Environmental, Population, and Organismic Biology, University of Colorado, Boulder, CO 80309-0334.

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 day (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 α) induces decompression 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 ejection about 3 hrs prior to projected lights off. 4) Daily MEL injections at the new lights off accelerate reentrainment 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 MH52546)

14.4

A ROLE FOR THE SCN IN THE INTERPRETATION OF MELATONIN SIGNALS DELIVERED ON NON-CIRCADIAN SCHEDULES.? Julia Grosse* and Michael Hastings, Dept. Anatomy, University of Cambridge, CB2 3DY, UK.

Seasonal reproductive changes are regulated by photoperiodic cues which are translated into an endocrine signal by the nocturnal secretion of melatonin from the pineal gland. The role of the circadian pacemaker, the suprachiasmatic nuclei (SCN), in the interpretation of this signal may differ between species: there is evidence that the SCN is required for the short-day response in Siberian hamsters. In contrast, it has been shown that in the Syrian hamster the SCN is not required for the interpretation of daily exogenous melatonin injections or infusions. This study investigated the possibility of a role for the SCN in the interpretation of melatonin infusions delivered on a non-circadian schedule. Pinealectomised Syrian hamsters bearing bilateral lesions of the SCN, or sham lesions, received 42 8h infusions of melatonin (50ng/h) or saline in a random pattern, or at the same time daily, over six weeks. After six weeks, control, saline-infused animals in both SCNX and sham groups had large testes. However, sham animals receiving random melatonin infusions had regressed testes, as did the SCNX animals receiving melatonin at the same phase every day. There was no significant difference in paired testes weight (PTW) between these groups (mean PTW±SEM=0.82±0.16g vs. 0.57±0.2g). In contrast, SCNX animals which received random infusions did not show gonadal regression (mean PTW±SEM=2.43±0.57g), with PTW differing significantly both from SCNX animals which received melatonin at the same time daily and sham animals which received random infusions ($p>0.01$). These results suggest that the SCN may play a role in the interpretation of a series of melatonin signals, though not the duration of individual signals.

This work was funded by the Wellcome Trust, Project Grant 037667/Z/93

14.5

ACTIVITY AND BODY TEMPERATURE RHYTHMS IN THE GOLDEN SPINY MOUSE: RESPONSE TO PHOTOPERIOD UNDER THE INFLUENCE OF SOCIAL CUES. Abrahm Haim and Nava Zisaple Biology Dept., University of Haifa at Oranim P.O. Kiryat Tivon 36006, Israel.

The golden spiny mouse Acomys russatus exists in hot and arid environments. It displays nocturnal 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: a) 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 T_b were monitored using a Mini-Mitter Data Quest system In some cases body temperature was measured by using a thermocouple. The mice were kept under a long (16L:8D) and short (8L:16D) photoperiods in the absence and presence of A. cahirinus at a constant ambient temperature. Activity and T_b rhythms were also studied in sham operated and pinealectomized mice.

The results indicated that the daily rhythms of T_b 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 addition, these reponses were attenuated 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.

14.6

SEASONAL VARIATIONS OF PHASE AND AMPLITUDE OF BODY TEMPERATURE CIRCADIAN RHYTHM. Marc Hébert*, Marie Dumont*, Julie Carrier*, Chantal Lafrance*, Josée Guillemette*. Hôpital Sacré-Coeur & Université de Montréal, Montréal, Canada H4J 1C5.

The aim of this study is to evaluate the effect of seasonal variations in natural light exposure on retinal sensitivity and circadian phase and amplitude. Subjects (18-35 years old) wear a photosensor (Actillume) for 6 days and undergo a circadian evaluation with a 40-h constant routine. Retinal sensitivity is measured with an electroretinogram (ERG). These measures are repeated twice, with a 6-month interval, once in winter (December-March) and once in summer (June-September). They are conducted in Montreal (45°31'N). We report here the preliminary results of circadian evaluations of rectal body temperature rhythm for the first 9 subjects (20-26 y old; 5M, 4F). On average, the phase of temperature minimum was earlier in summer compared to winter (0553 h vs 0626 h) and the amplitude of the rhythm was larger in the summer (0.24°C vs 0.22°C). Advances of phase position in summer compared to winter were observed in 6 subjects and ranged from 33 min to 120 min (mean: 74 min). For the other three subjects, the phase of the temperature minimum happened later during the summer (3 min, 28 min, and 140 min). Increases in amplitude during summer compared to winter were observed in 5 subjects (mean: 0.04°C, range: 0.01°C to 0.09°C), but the amplitude decreased in summer for 4 subjects (mean: 0.04°C, range: 0.01°C to 0.06°C). There was no relationship between seasonal changes in amplitude and seasonal changes in phase position.

Research supported by FRSQ (Québec) and CRSNG (Canada).

14.7

SEASONAL BREEDING, MELATONIN RHYTHMS, AND THE TINY PINEAL GLAND OF A TROPICAL BAT, ANOURA GEOFFROYI. Paul D. Heideman[1], K. P. Bhatnagar[2], F. K. Hilton[2], and F. H. Bronson[3]. [1] Dept. of Biol., Coll. of William & Mary, Williamsburg, VA 23187 & [2] Dept. of Anat. Sci. and Neurobiol., Health Sci. Center, Univ. of Louisville, Louisville, KY 40292 & [3] Inst. of Reprod. Biol., Univ. of Texas at Austin, TX 78712.

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 melatonin. A population of the tropical bat Anoura geoffroyi 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 geoffroyi have a minute, thin, and rod-like pineal gland (type ABY). However, despite having a very small pineal gland, this species produced a fairly typical mammalian serum 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.

14.8

ACUTE EFFECTS OF MELATONIN INFUSIONS IN SIBERIAN HAMSTERS: ENHANCED RESPONSIVENESS SHORTLY AFTER WEANING. Brian Prendergast*, Michael R. Gorman* and Irving Zucker*. Dept. Psychology, U.California, Berkeley, CA 94720.

Reproductive development in Siberian hamsters is under photoperiodic control. Day length (DL) information is transduced by the production of pineal melatonin (MEL), which varies inversely with duration of DL. Adult male hamsters treated with long duration (>8h/day) infusions of MEL undergo gonadal regression, while those treated with short duration infusions (<6h/day) manifest gonadal development. The effects of MEL infusions appear dependent on hamsters' photoperiodic history and age: reproductive development in juvenile hamsters is modulated both by gestational photoperiod and by DLs prevailing beginning at 15 days of age, when a pup's endogenous rhythm of pineal MEL secretion is evident. Pups are largely unresponsive to photoperiod prior to day 15, but may be extremely sensitive to DL cues at weaning. This experiment addresses whether: 1) there is a critical period of heightened responsiveness to photoperiod and MEL during early development, and 2) the critical period varies as a function of the hamster's photoperiodic history. Male hamsters gestated in long DLs (16h light/day=16L) and short DLs (8L) were maintained in constant light (LL) beginning at 14 days of age. Groups were infused for 3 successive days with either long-duration MEL (12h/day), short-duration MEL (6h/day) or saline vehicle (SAL), beginning at 18, 25, or 32 days of age. Twelve days after the last infusion, paired testis and body weights were determined. 16L hamsters were maximally sensitive to MEL infusions beginning at day 18; long duration infusions prevented any gonadal growth; this effect was also present in infusion groups started at day 25, but not at day 32. Preliminary data indicate that 8L hamsters exhibited sensitivity to long-duration MEL infusions until day 32. We conclude that enhanced responsiveness to MEL exists shortly after weaning, such responsiveness is influenced by the hamster's photoperiodic history, and may permit a comparison of gestational day length with day length several weeks later. These results suggest a mechanism which would allow juveniles to initiate seasonally-appropriate developmental trajectories.

14.9

CIRCADIAN RHYTHMS OF INDOLE METABOLISM IN *GONYAULAX POLYEDRA*. Rüdiger Hardeland*, Birgit Fuhrberg*, Gudrun Behrmann*, Susanne Burkhardt*, Burkhard Pöggeler* and Ivonne Balzer*. I Zool. Inst., Univ. Göttingen, D-37073 Göttingen, Germany.

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 indoleamine, which continues to oscillate at an elevated level. This finding may be relevant for the understanding of seasonality, since the lower temperature facilitates the short-day response of asexual encystment, which can also be mimicked by melatonin. The first enzyme of indoleamine biosynthesis, tryptophan hydroxylase, shows a pronounced circadian rhythm, which is almost antiphasic to that of melatonin. Aromatic amino acid decarboxylase is not rate-limiting for indoleamine formation. The rhythm of melatonin is not explained by the pattern of hydroxyindole O-methyltransferase activity. This enzyme may contribute to the nocturnal maximum of 5-methoxytryptamine, another cyst-inducing indoleamine, which is, however, predominantly formed via deacetylation of melatonin by aryl acylamidase 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-acetyltransferase, an enzyme which is unstable in preparations from *Gonyaulax* 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 acylamidase, presumably in connection with a decline of N-acetyltransferase.

14.10

EFFECT OF A SHORT PHOTOPERIOD ON ESTROGEN RECEPTOR IMMUNOREACTIVITY 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 lordosis. This study examined photoperiodic influences on lordosis and estrogen receptor immunoreactivity (ERIR). Ovariectomized Syrian hamsters were housed in either a long photoperiod (LP; 16L:8D) or SP (8L:16D) for 10 weeks. 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 ventrolateral 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. John T. Burns,* Tracy L. Kuzio,* and John N. Mugaas.# Department of Biology, Bethany College, Bethany, WV 26032 and #Department of Physiology, West Virginia School of Osteopathic Medicine, 400 North Lee Street, Lewisburg, WV 24901.

Temporal synergisms of neurotransmitter precursors, 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, 4-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 relation (5-HTP was followed by L-DOPA 0, 8, or 16 hrs. later). Some groups received only 5-HTP, L-DOPA, or saline. There were also several non-injected controls. Injections were given at 0600, 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 relation group had depressed ($p < .025$) testis weights (1.46 ± .17 g) as compared to the 8-hr group (2.00 ± .18 g) or the 16-hr group (2.00 ± .17 g) and slightly depressed epididymal fat pad weights. Effects of temporal relations suggested by Albert H. Meier and colleagues are thus supported. Funded by the Gans Research Fund and WVSOM.

14.12

PHOTOPERIODIC REGULATION OF LH, FSH, GH, α-MSH AND β-ENDORPHIN, BUT NOT PROLACTIN, REQUIRES AN INTACT HYPOTHALAMO-PITUITARY SYSTEM IN RAMS.

Gerald A. Lincoln and Iain J. Clarke*

MRC Reproductive Biology Unit, Edinburgh, EH3 9EW, UK, and * Prince Henry's Medical Research Institute, Clayton, Victoria, Australia.

We have previously shown that the photoperiodic regulation of the secretion of prolactin persists in hypothalamo-pituitary disconnected (HPD) Soay rams providing evidence that melatonin acts, at least in part, in the pituitary gland to mediate effects of photoperiod (J. Neuroendocrinol. 6: 251-260, 1994). To establish whether this pituitary effect is restricted to the control of lactotrophs 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 72 weeks. In the control rams there was a significant ($p<0.01$) effect of photoperiod on the plasma concentrations of LH (2.39 ± 0.33 vs 0.80 ± 0.09 ng NIH-S23/ml, mean ± SEM long vs short days respectively, FSH (61.52 ± 5.33 vs 10.64 ± 1.62 ng NIH-FSH-S14/ml), GH (2.18 ± 0.27 vs 2.66 ± 0.35 ng NIH-oGH-I-4/ml), α-MSH (147.4 ± 20.9 vs 42.5 ± 4.3 pg/ml) and β-END (436.9 ± 66.7 vs 159.1± 29.1 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 (3.21 ± 0.33 vs 3.38 ± 0.33 ng/ml), GH 1.13 ± 0.13 vs 1.34 ± 0.15 ng/ml), α-MSH (170.6 ± 41.9 vs 144.6 ± 36.3 pg/ml) and β-END (490.7 ± 28.8 vs 498.7 ± 26.6 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 positive/negative regulation by the hypothalamus. The disruption of the photoperiodic responses in the HPD animals indicates that an intact hypothalamo-pituitary system is required for the photoperiod/melatonin relay to affect the secretory activity of gonadotrophs (LH and FSH) and melanotrophs (α-MSH and β-END).

14.13

EVIDENCE FOR SEPARATE SITES OF CONTROL OF THE PHOTOPERIOD AND DAILY RHYTHMS IN FETAL PROLACTIN IN THE SHEEP

I.C. McMillen, I.R. Young and D.C. Houghton

Departments 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 fetal prolactin concentrations and on the daily rhythm in fetal melatonin (MT) and prolactin (PRL). Fetal HPD or a sham operation was carried out at around 110d gestation. Ewes carrying either HPD fetal sheep (n=10) or intact fetal sheep (n=12) were then exposed to long (LL;16h light:8h dark) or short light (SL;8h light:16h dark) and fetal and maternal blood samples collected throughout late gestation. All ewes were subjected to one 24h sampling period between 0900h and 0900h at between 135 and 140d. Mean fetal PRL concentrations were significantly higher in LL (HPD; 37.3±11.3 ng/ml : Intact; 71.0±16.2 ng/ml) than in SL (HPD; 9.0±4.8 ng/ml : Intact; 34.2±16.0 ng/ml) in all ewes. There was an increase in maternal MT during the dark in LL and SL in ewes carrying HPD or intact fetal sheep. There was an increase in fetal MT during the dark in the HPD and Intact groups during SL. Under LL, however, fetal MT were only increased during the dark phase in the Intact and not the HPD group. In the Intact group, fetal PRL was significantly higher ($p<0.05$) at 1300h and at 1700h than between 0300h and 0700h in LL and in SL. In contrast there was no significant effect of time of day on fetal PRL in either LL or SL after fetal HPD. We have therefore demonstrated that the photoperiod induced changes in fetal PRL are maintained after surgical isolation of the fetal pituitary. Our results provide direct evidence however for a fetal hypothalamic role in the generation of the daily rhythms in fetal MT and PRL.

15.1

DAILY VARIATIONS OF ADENOSINE METABOLISM IN HUMAN BLOOD.

*Victoria Chagoya de Sánchez, *Rolando Hernández-Muñoz, *Jorge Suárez, *Susana Vidrio, *Lucía Yáñez, *Mauricio Díaz-Muñoz, *Raúl Aguilar-Roblero, *Arturo Vega, *Luis Villalobos, *León Rosenthal, *Federico Fernández-Cancino and *René Drücker-Colín. Department of Bioenergetics and Neuroscience, Instituto de Fisiología Celular, UNAM, Mexico 04510, D.F.

Daily variations of adenosine and its metabolism 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 rhythmicity in humans investigating if adenosine and its metabolism, including the activity of its metabolizing enzymes in the blood presented daily variations. Young healthy males were adapted for two days to the room conditions where the experiment was performed. Awaking period was from 06:00 to 23:00 h, and the blood sampling was done every hour from an heparinized catheter placed in the antecubital vein.The results showed that adenosine and its catabolites (inosine, hypoxanthine and uric acid) adenosine synthesizing (S-adenosylhomocystein hydrolase and 5'nucleotidase), degrading enzyme (adenosine deaminase) and nucleotide forming (adenosine kinase) enzymes, as well as adenine nucleotides (ATP.ADP and AMP) presented statistically significant fluctuations analyzed by ANOVA. While the energy charge and the phosphate levels did 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 ultradian fluctuations. The acrophase of the parameter that presented rhythmic pattern were observed either during the dark or light period. The results suggest that adenosine and its metabolism also might play an important role in the rhythmic pattern of the humans.

15.2

STUDY ON CIRCADIAN RHYTHMS OF TESTOSTERONE IN ATHLETES

Jian Kunlin Chengdu Institute of Physical Education, Chengdu 610041 P.R.China

10 elite athletes(male, age 18-21 yrs) in Chengdu Education Institute envolved researching project as the volunteers in the exercise chronobiology laboratory (LD 15:9, temperature: 28±3℃, humidity: 50%).The samplings for venous blood were taken every 4 hour in a day and radioimmoassay was employed to assay the amounts of the testosterone, the date were analyzed by means of Minnesota Cosinor.The results showed that testosterone display a significant circadian rhythm ($P<0.05$) and the acrophases was 08:00 a.m.The findings of the study indicate that we should pay more attention to this kind of rhythmical characteristics when we employ the index forselecting thepromising athletes and carrying out chronotraining.

15.3

IDENTIFICATION OF PHOTIC RESPONSIVENESS IN THE SCN of NEWBORN PRIMATES. S.A. Rivkees 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 to the SCN of humans. The baboon SCN also expressed |^{125}I|-melatonin and |^{125}I|SCH-23982 binding indicating the presence of melatonin and D1 dopamine receptors. To test for photic responsiveness, the 2-deoxy|^{14}C|glucose (DG) method was used to study animals at the end of gestation. After their natural birth, animals were injected with DG at either mid-day, mid-night, or after 5000 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-responsiveness. These data provide the first direct evidence that the primate SCN are responsive to light at birth.

15.4

THE SHAPE OF THE CIRCADIAN RHYTHM OF RECTAL TEMPERATURE IN HUMANS.

J Waterhouse[1], D Minors[1], S Folkard[2]
[1]School of Biological Sciences, University of Manchester,UK
[2]Department of Psychology, University of Swansea, UK

Rectal temperature and wrist movement have been measured in a group of 14 subjects who lived on a 30-h "day" (10h sleep, 20h wake) for 14 (solar) days. Throughout their waking span, subjects were almost always sedentary. During this time all phase relationships existed between the sleep/activity cycle and circadian temperature rhythm, and so it was possible to investigate the extent to which the size of the masking effect upon rectal temperature depended upon time elapsed since last sleep, the phase of the temperature rhythm, and the amount of wrist movement.

The masking effect of sitting was lowest in the first two hours after waking, but thereafter showed no systematic changes. When the masking effect of sitting was related to the phase of the temperature rhythm, values were significantly lower from 4h before to 2h after the acrophase, and significantly higher from 4h to 8h after the acrophase. Wrist movement followed a different pattern and showed a significant cosine curve fit that was in phase with the temperature rhythm.

The effect of the phase-dependent variation in masking caused by sitting is to produce a temperature rhythm that does not rise and fall during the activity span like a cosine curve but rather stays closer to a plateau value throughout much of the time awake.

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, School of Biological Sciences, University of Surrey, Guildford, Surrey, GU2 5XH, UK.

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 2400h on days (D)1-4 subjects remained in dim artificial lighting (<50 lux) and darkness (<1 lux) from 2400-0800h. At 1700h on D3 either melatonin (0.05mg, 0.5mg or 5mg) 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, offset and better sleep quality were associated with increasing doses of melatonin. The following day, 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 rhythm to phase-advance. A significant dose-response relationship existed between the dose of oral melatonin, the magnitude of temperature suppression and the degree of advance phase shift in the endogenous melatonin and temperature rhythms, suggesting that acute changes in body temperature by melatonin may be a primary event in phase-shifting mechanisms.

15.6

EFFECT OF EXOGENOUS MELATONIN ON HUMAN ACTIVITY-REST AND RECTAL TEMPERATURE RHYTHMS IN CONSTANT DIM LIGHT.

Benita Middleton, Josephine Arendt, Barbara Stone*. University of Surrey, Guildford, UK and *Defence Research Agency, Farnborough, Hants, UK

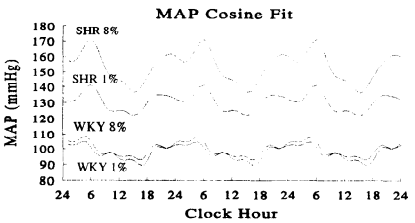
Daily administration of melatonin (MT) with a 24 h periodicity entrains free-running activity rest cycles in rats. Comparable experiments in humans have used free running blind subjects with sometimes inconsistent results. This study investigated entrainment by MT in sighted volunteers. 6 Healthy males aged 22.7+- 1.6 years were maintained together for two sessions of 32 days in constant dim light (5.7 +- 6 lux). Throughout they took (if awake) 3 capsules per day at 1200, 2000 and 0400h. In a randomised cross-over design the 2000h capsule contained MT for the first 16 days (MT 1st) and placebo (P) for the second 16 days (P 2nd) or vice-versa (P 1st, MT 2nd). Activity-rest (A) and core body temperature (T) were monitored every 20 sec and 6 min respectively throughout. Sleep onset time (SOT) and duration were derived by Action 3 software, T (masked) was analysed by 3-day moving cosinor fit. Sleep onset time (SOT; for all sleeps > 6h) and T acrophases were analysed by linear regression. Significant differences from 24h were within 95% confidence limits. Results: P 1st: 5/6 subjects free ran, tau (τ) range 24.2-24.4h (SOT), 24.25-24.43h (T). MT 2nd: 5/5 (one quit), (SOT), and 4/5 (T) subjects entrained to 24h. One subject delayed to entrain, others advanced. Direction of entrainment was related to time of MT administration relative to T minimum. MT 1st: 2/6 (SOT) showed highly irregular sleep, 4/6 entrained, 2/6 (T) free-ran, τ= 24.07, 24.14h, shorter than for P 1st. When very irregular sleep was noted, MT administration was close to T maximum. P 2nd: 2/6 (SOT) free ran, τ= 24.21, 24.25h, 4/6(T) free ran, τ range 24.13-24.19h. There were no differences in sleep duration. These results suggest that MT can reentrain sleep timing after a period of dim light. After-effects of MT treatment may modify SOT with P 2nd. Apparent entrainment of T may be due to masking.

15.7

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

DA Calhoun, S-T Zhu, JM 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 tranducers (DataSciences, Inc.) were implanted into 8- wk old, male SHR and WKY allowing for sampling of mean aortic pressure every 4 min. After 10 days of recovery, animals were fed basal (1%) or high (8%) NaCl diet for 2 weeks. A rhythm analysis was applied by means of the nonlinear least-squares fitting program PHARMFIT. Plots of best cosine fit MAP were:



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.]

15.8

A MATHEMATICAL MODEL FOR THE RHYTHMICITY OF ENDOCRINE SYSTEM. *BingzhengLiu*, Phys.Dept. Northeast Normal Univ. Changchun, China

It is well-known that besides circadian rhythmicity, most of endogenous hormones process pulsatile secretory character with period about 1-3 hour. We think this hour-rhythmicity has network or system character. for example, the pulsatile period of LH and testosterone of man and rat decrease markedly after castration. We propose here a mathematical model for the hypothalamo-pituitary-testis axis of adult man. The equations are:

$$\frac{dx_1}{dt} = \frac{0.03 + 0.37x_1 + 0.012x_1^2}{1 + 0.04x_1 + 0.008x_1^2 + 0.87x_3 + 0.73x_3^2} - 0.3x_1$$

$$\frac{dx_2}{dt} = \frac{0.0073 + 0.0027x_1 + 0.0087x_1^2}{1 + 0.8x_1 + 0.0096x_1^2 + 0.85x_3 + 0.16x_3^2} - 0.013x_2$$

$$\frac{dx_3}{dt} = \frac{0.024x_2 + 0.017x_2^2 + 0.046x_2^3}{1 + .083x_1 + .56x_1^2 + .5x_2 + .04x_2^2 + .006x_2^3 + .065x_1x_2 + .026x_1^2x_2 + .003x_1x_2^2} + 0.003 + 0.0023x_2 + 0.0097x_4 + 0.00074x_5 - 1.5x_3$$

$$\frac{dx_4}{dt} = 0.2x_3 - 0.0098x_4, \qquad \frac{dx_5}{dt} = 0.019x_3 - 0.00077x_5$$

where x_1, x_2, x_3, x_4 and x_5 denote respectively the plasma concentrations of LRH (GnRH), LH, free testosterone, TeBG-bound testosterone and albumin-bound testosterone, the unit of LH is iu/l, those of the remaining hormones are all $\mu g/l$, time unit is minute. Integrating these equations numerically by computer, we obtain periodic solution with period 150 min. which agrees well with experiments. Some other deductions (mean concentrations, production rates,MCR,etc.) from the above equations also agree with experiments.

15.9

EFFECTS OF HOSPITALIZATION AND SURGERY ON CIRCADIAN RHYTHMS IN PATIENTS BEFORE, DURING AND FOLLOWING NEUROSURGERY. Phillip E. Vinall*, Michael S. Kramer*, and Frederick A. Simeone*. Neuroscience Research Institute, Philadelphia, PA. 19107

Studies have examined circadian rhythm dysfunction following surgery but none have included pre-surgery data from the home environment. Circadian rhythms were measured in patients who underwent surgery involving the removal of tumors in the third ventricle (n=4), non-third ventricle brain tumors (n=5) and the spine (n=2).Changes in activity and external illumination were recorded using a wrist worn Actillume (Ambulatory Monitoring, Inc.). Data were collected 3-5 days in the home environment before the patient entered the hospital (A), while in the hospital including ICU stay (B), and 5 days after leaving the hospital (C). Stored data were downloaded to a PC and cosinor analyses were performed using Action 3.1 software (Ambulatory Monitoring, Inc.). The software also contained built-in algorithms for sleep/wake scoring based on activity. Mean acrophase and tau values were not significantly affected by surgery, while the sleep/wake ratio increased and remained elevated. Activity and light mesor and amplitude values were significantly affected by surgery and remained depressed after returning to the home environment. This study was performed with the approval of the Research Review Committee of Pennsylvania Hospital.

	A	B	C
Acrophase	15:17±1h20m	15:02±1h13m	15:23±1h12m
Tau	23:43±0.56m	23:07±1h32m	23:53±0.40m
Sleep/wake	0.24±.09	0.52±0.26*	0.36±0.2*
Mesor			
activity	17.6±5.2	7.87±3.4**	11.18±5.7*
light	0.86±0.5	0.76±0.7	0.55±0.4*
Amplitude			
activity	38.53±9.0	4.87±2.8**	7.89±4.3*
light	1.60±0.43	1.01±0.3**	1.35±0.4*

* sig.dif. (p<.05) from A; ** sig.dif. (p<.05) from A and C.

15.10

OPTIMAL CIRCADIAN TIMING OF 5-FLUOROURACIL (5-FU) IMPROVES ANTICANCER ACTIVITY AND NORMAL HOST TOXICITY AND HENCE THERAPEUTIC INDEX. PA Wood*, D Peace*, M Torosoff*, R Vyzula *and WJM Hrushesky*. Stratton VAMC, Albany, NY12208.

5-Fluorouracil (5-FU) is one of the most commonly used drugs for treating solid tumors. However, its use is limited by marrow and gut toxicity, and low response rates. The circadian time of administration is known to affect the clearance of fluoropyrimidines and their toxicity. We have asked whether the anti-tumor efficacy of 5-FU may also be circadian time dependent and how this compares with the circadian pattern of toxicity in the normal host. 120 CD2F1 female mice with a transplanted subcutaneous sarcoma were treated with a single intravenous injection of diluent (D) or 5-FU (150 mg/kg) 9 d after tumor inoculation administered at 1 of 6 times throughout a 24 h cycle. Daily tumor size was recorded. Across all circadian times, a significant inhibition of tumor size was caused by 5-FU (drug effect F=8.2, p=0.004). Inhibition of tumor growth was different depending on the circadian timing of 5-FU. The circadian dependence of 5-FU tumor growth inhibition was apparent when absolute tumor size (circadian effect F=5.3, p<0.001) or tumor size as % (F=6.2, p<0.001) or difference (F=3.1, p=0.009) from mean diluent tumors were compared. Inhibition of tumor growth was greatest when 5-FU was given late in the activity span and least when given in the mid to late sleep span. Toxicity studies in non-tumor bearing CD2F1 mice with 5-FU (200 mg/kg) show greatest lethality and marrow toxicity when 5-FU is given in mid sleep and minimal host toxicity when given in late activity. These results indicate that optimal circadian timing of 5-FU can be identified which can both decrease toxicity and enhance tumor control thereby potentially increasing the therapeutic index of this common anticancer drug. This time of day in mice is at the end of the activity span and during the first half of the sleep span.

15.11

"BONE CLOCK": MODELS TO AID IN DESCRIBING BONE RESHAPING WITH AGING. Fazle 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 first simple model assumes that, although outer radius (R) grows with age, inner radius (r) might change to keep area constant and $r = ([q.Age]^2 - c)^{\frac{1}{2}}$. A second model is based on the fact that if bone thickness remains constant, then R - r = K. 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, or B=M/A for a given length. By the first model, we have $B=M/\pi(R^2-r^2)$ and for the second: $B=M/\pi(2Rk-K^2)$. More realistic models note that actual bone thickness is the result of separate processes of growth (G) and resorption (Re). Hence, both G and Re can be separately related to age. The resultant equations reveal that, effectively, a peak bone mass occurs, and then a decline with age. There can be added to size equations a further term that will give mineral loss/area as an age related phenomenon. Although multiple factors may be involved, simplified expressions might be useful in comparing the sexes as well as different populations, and in evaluating the long term effects of therapeutic interventions.

15.12

EVIDENCE FOR A THALAMIC ROLE IN HUMAN CIRCADIAN CONTROL. F. Portaluppi, P. Cortelli*, M. Contin,* P. Avoni*, P. Maltoni,* A. Pavani, L. Vergnani, R. Manfredini, E. Lugaresi*. University of Ferrara, Ferrara, Italy I-44100 and *University of Bologna, Bologna, Italy I-40100

The thalamus acts as a functional relay between the limbic system, the frontal cortex and the hypothalamus; it plays a fundamental role in integrating motor and vegetative functions and regulating the sleep/wake cycle. Some alterations in human circadian control have been found in several states of sleep disturbance, suggesting a modulation by extrahypothalamic, central nervous system structures. Fatal familial insomnia (FFI) is a rare genetic disease characterized by selective thalamic degeneration that causes chronic sleep loss. Under standardized conditions and polysomnographic control, 3 patients and 6 healthy controls underwent repeated 24-hour study sessions covering the entire clinical course of FFI. Melatonin (MT), prolactin (PRL), somatotropin (GH), corticotropin (ACTH), cortisol (C), and catecholamines were assayed at 30-min intervals; blood pressure (BP) and heart rate (HR) were continuously monitored at the finger. A sleep/wake cycle was always absent in FFI. Circadian rhythmicity of GH disappeared in early stages, simultaneously with sleep loss. MT, PRL, ACTH, C, and catecholamines showed a progressive reduction in circadian amplitude leading to rhythm obliteration only months after total disappearance of a sleep/wake cycle. An early shift in phase of the BP rhythm was found with preserved nocturnal bradycardia. BP and HR rhythms disappeared only in preterminal stages. These data suggest a thalamic role in human circadian control. The progressive thalamic lesions documented in FFI patients may gradually deprive the circadian oscillators of what seems to be an essential cortical control.

15.13

THE ROLE OF MELATONIN IN MEDIATING THE CIRCADIAN RHYTHM OF SLEEP. Adam CL. Fletcher, Cameron J. van den Heuvel and Drew Dawson. Sleep & Circadian Rhythms Laboratory, The University of Adelaide, Dept. of Obstetrics & Gynaecology, The Queen Elizabeth Hospital, Woodville SA 5011, Australia.

Recent experiments indicate that the pineal hormone melatonin may mediate the circadian sleep and wakefulness rhythm via a hypothermic effect. If melatonin decreases core temperature and increases sleep quality, then we hypothesize that suppression of melatonin production should increase sleep disruption and core temperature. 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 all subjects completed an adaptation and a baseline recording night. The following two nights consisted of a melatonin suppression and a control condition, to which subjects were assigned randomly. Melatonin suppression was achieved by oral administration of 100 mg atenolol at 1900 h and subjects were given placebos at 2200, 0200 and 0400 h. On the control night, atenolol at 1900 h was followed by 2 mg of melatonin orally at 2200 and 0200 h and 1 mg at 0400 h. In this condition, melatonin was administered to control for effects of atenolol unrelated to its suppression of melatonin. Melatonin suppression by atenolol significantly increased sleep onset latency (SOL) at 2300 h from a baseline mean of 23.4 mins to 32.4 mins ($p<0.05$) . In addition, melatonin suppression significantly increased the amount of wakefulness across the night from 37.5 mins to 81.6 mins ($p<0.01$). Means for SOL and wakefulness in the control condition were not significantly different from baseline values. There were no significant changes in sleep architecture, as REM and non-REM sleep as proportions of the total sleep time were unchanged. In conclusion, melatonin suppression increases the time taken to fall asleep and the amount of time awake across the night. These results indicate that melatonin plays a role in regulating the circadian rhythm of the sleep/wake cycle.

15.14

ABNORMAL CIRCADIAN RHYTHM OF PLASMA MELATONIN IN PATIENTS WITH LIVER CIRRHOSIS - RELATION TO SLEEP ARCHITECTURE. P.E. Steindl*, B.Finn*, B.Bendok*, S.Rothke*, P.C. Zee, A.T.Blei*. Dept of Medicine and Neurology, Lakeside VAMC and Northwestern University, Chicago, Illinois 60611.

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 o melatonin and changes in the sleep architecture are observed in cirrhotic patients with subclinical hepatic encephalopathy (HE). We studied 24 hr plasma melatonin profiles and sleep recordings by polysomnography in seven non-alcoholic cirrhotics and carefully matched controls. Neuropsychological testing confirmed the presence of subclinical encephalopathy. Cirrhotic subjects showed markedly elevated melatonin levels during daytime (93.75±20.74*pg/ml vs 1.59±1.06 pg/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 constantly and significantly delayed (Onset at 9:30 PM ± 13 min vs 7:50 PM ± 26 min in controls, $p=0.013$; Peaktime at 5:36 AM ± 29min vs 12:36 AM ± 33 min, $p< 0.001$). Although polysomnographic readings 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 RHYTHMS OF TEMPERATURE & SLEEP. Cameron J. van den Heuvel and Drew Dawson. Sleep & Circadian Rhythms 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 hypothermic 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 non-consecutive nights, between 1900 h and 0700 h. One of three conditions was completed each night; baseline, nocturnal melatonin suppression (MS), or a control atenolol and melatonin condition (AM). Nocturnal melatonin was suppressed in the MS condition by oral administration of atenolol (100 mg) at 1900 h. In the AM condition, any effects of atenolol unrelated to melatonin suppression were controlled for by giving 100 mg 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 placebos 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 secretion. After atenolol administration, 6s-aMT 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 ($P<0.01$), mean nocturnal SOL to stage 1 ($P<0.01$) and mean nocturnal SOL to stage 2 sleep ($P<0.05$). 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 hypothermic 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.

15.16

TUMOR-STAGE DEPENDENT DEPRESSION OF THE CIRCADIAN AMPLITUDE OF MELATONIN IN PATIENTS WITH UNOPERATED PRIMARY BREAST AND PROSTATE CANCER IS CONNECTED WITH DISTURBANCES IN THE SECRETION OF PROLACTIN, TSH AND GROWTH HORMONE C Bartsch[1]*, H Bartsch[1]*, St-H Flüchter[2]*, TH Lippert[1]*. [1]Section of Clinical Pharmacology, University Women's Hospital, D-72076 Tübingen, Germany, [2]Department of Urology, Klinikum Winterberg, D-66119 Saarbrücken, 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 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 adenohypophyseal hormone secretion via central neuroendocrine mechanisms.

15.17

THE LAST PART OF A BIOLOGICAL CLOCK: EVIDENCE FOR COORDINATED INVOLUTION. Richard P. Spencer. Dept. Nuclear Medicine, Univ. Connecticut Health Center, Farmington, CT 06030.

Changes in tissue composition and loss of organ weight with aging, have been well recognized. What is required is a methodology to bring these phenomena under a general rubric, rather than viewing separate events. The allometric equation has been primarily utilized for following growth phenomena, such as organ changes during embryogenesis or postnatal life, organ/body weight in varied populations, and in following animals which have been genetically altered. This relation was also studied as a descriptor during 'downsizing' of aging. Instead of viewing organ weight (W) as a function of time (age), comparison was made with body weight (B), via the allometric equation. Thus: $\log W = \log k + p.\log B$. A review of literature values of organ/body weight of humans at ages 65 years and beyond, revealed the equation to be a good decription, with correlation coefficient of 0.7 to over 0.9. Loss of organ weight appears to be coordinated with loss of body weight. Demonstration of such a relationship suggests at least 2 possible causes. 1) A genetic basis, with a clock for coordination of involution. 2) Accumulated oxidative or other damage affecting control sites, so that all organs (to some degree) begin involution. Coefficients (k) and exponents (p) of the allometric equation were closely coupled between males and females. This may provide insights as to describing such phenomena as secular trend in body size during the century.

15.18

MODULATION OF HOST BREAST CANCER BALANCE BY THE FERTILITY CYCLE . WJM Hrushesky*, MT Torosoff*, H Gupta*, G Mann* and PA Wood*. Stratton VAMC. Albany, NY 12208.

The timing of breast cancer resection within the fertility cycle determines its curability (Lancet 1989;ii:949). Natural killer cell activity and splenocyte interleukin-2 production covary with this cycle of curability (JNCI 1988;80:1232) 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 C3HeB/FeJ female mice received syngeneic breast cancer cells subcutaneously during proestrus (P), estrus (E), metestrus (M), or diestrus (D). Time to tumor appearance, tumor size and fertility phase were measured every 12 h for 24 d. The fertility cycle affected the time to tumor take with proestrus phase, the time of highest immune cell activity and surgical cure, of tumor injection being associated with a longer delay in tumor appearance ($F=2.7, p=0.06$) and inhibited tumor growth ($F=8.8, p<0.01$). When average tumor size is inspected over linear 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 unmasked. 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 (Prin. Pract. Surgery 1836).