

BIOLOGICAL RHYTHMS BULLETIN

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The Bee's Knees?

Herewith a tale for the telling; sit back and try to relax;
It needs to be told 'fore the story grows cold; a saga of knees and of tracts.
To hear it you may not believe it. But all that you read here is true.
Whataya think? That I'd fill up this space with some nonsense pulled out of the blue?

It starts with a paper in *Science*. A paper concerning the Clock.
Some said, "What huge implications!" Others said, "Oh, what a crock!"
The finding described how in people, rhythms were reset at will.
Not by exertion, nor temperature changes, and certainly not by a pill.

The same thing had been done in sparrows, and lizards and fish and in frogs.
But never before had the feat been accomplished in hamsters or cats or in dogs.
I'm talking of phototransduction; of light to the brain, if you please.
Not by the usual route through the eyes, but instead by a route through the knees.

How could this possibly happen?! How does it work? Please explain!
"You need a tract!" the anatomists said. "Why, without one it's simply insane!"
"We have a tract from the eyes. It terminates right at the Clock."
"Go find a pathway! We need to transect it, to see if the signal we block!"

The chorus it grew ever louder. Doubters emerged from the cracks.
Ocular Chauvinists gathered. They mounted their dogma attacks.
"You don't suppress melatonin!" The Chauvinists sang and they danced.
"Everyone knows that without melatonin the Clock's not delayed or advanced!"

"Wait just a second" I ventured. "What about this that I've read?"
Year's '68, it's the Menaker paper. I'll quote so you won't be misled.
In sparrows (and later with lizards); to rhyme this I must paraphrase:
"Pinealectomy doesn't affect extraocular setting of phase."

"Yeah, but our nocturnal hamsters, they don't respond to the light."
"It's something about your conditions; something just isn't quite right."
"No, we can't say what the 'something' might be; really, we haven't a clue."
"It's just that our Ocular Dogma precludes one from taking an alternate view."

How can you argue against 'em? Animal models don't lie.
Anatomists are the last word, after all. Ridiculous even to try.
Then again, maybe I'm wrong. Maybe I gave up too fast.
Let's have a look at the history of things; see what went on in the past.

Late in the 70's Dogma maintained, "Light Isn't Crucial to Humans."
Oops! Wait a minute! Lewy just showed all you need is a couple more lumens!
Now early 80's, it's rods and it's cones - Receptors of Choice, am I right?
Oops! Wait a minute! Foster's got mice who can shift in the absence of sight!

Now it's the 90's, *Drosophila* noses and wings and the legs and the like.
Who woulda thought it? There're clocks everywhere! Assuming that Kay's lab is right.
And what about cryptochrome A? A blue light receptor? Who knew?
It's found in the eyes of both mouse and of man; it's found in the skin of 'em too.

Then there's the findings of photobiology; not Clocks but other close kin --
Before we had milk for our vitamin D, we phototransduced through the skin.
And what about jaundice? It's treated with light; light to the skin, not the eye.
Why is circadian phototransduction so hard for some people to buy?

Well, maybe Dogma is way over-rated. Maybe there's room for the new.
In some cases nocturnal rodents behave in a way that a human can't do.
Valium rev's 'em and resets their clocks. But humans, it leads 'em to rest.
A nocturnal rodent may be a good model, but maybe it isn't the best.

Maybe we're wrong and there really is "something" peculiar about our design.
Maybe we missed a particular point; or failed to see some sort of sign.
But maybe we're more like the birds or the bees when it comes to the matter of docks.
Maybe there's more than just meets the eye; maybe it's still a black box.

Ocular Chauvinists, lend me your ears! Eyes are important, I know!
That doesn't mean that there's only one way to the brain for the light there to go
Doubt if you must; criticize as you should. Only one thing that I ask.
Get all the facts straight, and read what's been written, before you set forth on
your task.

Epilogue

Mid '99, a report issues forth, concerning some rats with no eyes.
Genetically mutant, and blind as a bat, their clocks are of regular size.
Turns out that these rats can be synchronized to cycles of dark and of light.
Look out you authors! Get ready to rumble! You guys are in for a fight!

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TOP 20 LIST

(collected and submitted by Eric Mintz, Georgia State University)

The following list indicates the top 20 individuals who have published the most articles containing the key word "suprachiasmatic," as determined using the National Library of Medicine PubMed database. The search results were imported into Reference Manager 8.5 and the author list was determined in that program. **Disclaimer:** This was done just for fun; there is no implied association between quality and quantity of research.

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In the Vanguard of SCN Clock Genes: Photo-inducible c-fos Was Discovered Ten Years Ago

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It now appears clear that transcriptional control is an important part of the intracellular machinery of the circadian clock (for review, see Dunlap, 1999). In mammals, this control was originally suggested by the discovery that environmental light can stimulate the expression of transcriptional regulatory proteins in the rodent suprachiasmatic nucleus (SCN). Proteins that control transcription bind to specific cis-acting regulatory DNA sequences (promoters and enhancers) usually located on non-transcribed segments of genes. The sequence-specific binding of these proteins to the DNA regulatory elements affects the initiation of transcription by RNA polymerase II, resulting in the altered transcription of targeted genes.

In the SCN, the prototypical photoinducible protein of this class is c-Fos, product of the *c-fos* proto-oncogene. Ten years have now passed since five laboratories first independently reported that light can physiologically regulate *c-fos* gene expression in the SCN of rats and hamsters (Rea, 1989; Aronin et al., 1990; Earnest et al., 1990; Kornhauser et al., 1990; Rusak et al., 1990). Since then, the gene has been studied in mice, mole rats, ground squirrels, chipmunks, diurnal rodents *Arvicampis niloticus* and *Octodon degus*, sheep, mink, baboons, quail, starlings, and chicks. The purpose of this little essay is to celebrate the past decade of *c-fos* research, both by recounting our knowledge and by highlighting our ignorance. We apologize in advance that space limitations prohibit us from including all the work and from citing all the workers, but we invite letters, telephone calls,



telefaxes, e-mail messages, or even personal visits from anyone desiring a more complete or specific set of references.

Here's What We Know

The principal features of SCN *c-fos* gene expression are by now familiar (for reviews, see Hastings et al., 1995; Schwartz et al., 1995; Kornhauser et al., 1996). The levels of *c-fos* mRNA and immunoreactive c-Fos protein in the retino-recipient subdivision of the SCN are dramatically elevated (from essentially undetectable levels) after either (i) lights-on at dawn during a light-dark cycle, or (ii) a light pulse administered during the subjective night in constant darkness (but not during most of the subjective day). This response is both rapid and transient with peak expression of mRNA and immunoreactive protein at about 30 min and 1-2 hr after light onset, respectively, with each disappearing about 2 hr and 6 hr after light onset, respectively. Light pulses as short as 5 min are effective. Stimulation with saturating light pulses indicates an upper limit to the number of photoinducible c-Fos cells at about 20% of the total SCN

cell population, at least in the mouse (Castel et al., 1997). The *in situ* hybridization and immunohistochemical data have been interpreted to suggest that photic stimulation increases actual *c-fos* gene transcription (rather than mRNA stability), although this has not been rigorously demonstrated (e.g., by nuclear run-on assays).

There are strong correlations between the photic induction of *c-fos* and phase shifts of overt rhythmicity. The illumination threshold for gene expression matches the threshold for behavioral phase shifts (Kornhauser et al., 1990), even when the threshold is altered by age or prior history (Zhang et al., 1996; Shimomura et al., 1998). This feature is not shared by all SCN transcription factors; the induction of *nmr77* and *zif268* displays a photic sensitivity measurably greater than the sensitivity for phase-shifting (Lin et al., 1997). The degree of *c-fos* activation correlates with the magnitude of photic phase shifts (Trávníčková et al., 1996; Beaulé and Amir, 1999), although this relationship breaks down at high light intensities, under a long (LD 16:8) photoperiod, or with type 0 resetting (Trávníčková et al., 1996; Shimomura et al., 1998). The phase dependence of *c-fos* stimulation is similar to that already well-described for light-induced phase shifts of locomotor rhythmicity, and some of the pharmacological agents that block these behavioral phase shifts also block the photic stimulation of c-Fos protein in specific regions of the SCN, at least in the hamster (Abe and Rusak, 1994).

The fact that photoinduction is phase-dependent means that the *c-fos* gene is a target of the circadian pacemaker (i.e., it is clock-controlled); the mechanism that gates its activation to the time of day must itself be one of the pacemaker's output pathways. This mechanism is uncertain but appears to be specific to the SCN. In the intergeniculate leaflet, photic stimulation of *c-fos* mRNA and immunoreactive c-Fos protein levels does not appear to depend on the circadian phase of stimulation (Peters et al., 1996). Moreover, circadian control of c-Fos protein expression persists in the SCN maintained as a tissue slice *in vitro* (Bennett et al., 1996). Thus, neither the retina nor the rest of the brain are required constituents of the gating mechanism (although they might contribute to it).

From a practical point of view, these properties of *c-fos* have made it a unique and valuable intracellular marker of the effects of light on SCN function. Mapping with *c-fos* has helped to demonstrate the neurochemical heterogeneity of visual afferents and the neuropharmacology of behavioral phase shifts. Its expression has served as a sensitive measure of the duration of the photosensitive subjective night and has revealed circadian "sight" in otherwise blind animals.

But Here's Where Things Begin To Get Complicated

There is a family of *c-fos*-like genes that are structurally related to *c-fos*, including *fra-1*, *fra-2*, and *fosB*. The proteins encoded by these genes (c-Fos, Fra-1, Fra-2, FosB, and FosB) all share the same structural motif ("bZIP"), consisting of a leucine repeat domain (for dimerization with other, non-Fos, nuclear proteins) and a domain of highly basic amino acids lying N-terminal to the leucine repeat (for binding to DNA). Fos proteins preferentially bind to a specific DNA regulatory element (the Activator Protein-1 [AP-1] binding site), but only when they are complexed as heterodimers, especially to members of the *jun* gene family of bZIP proteins (c-Jun, JunB, and JunD).

Studies using *in situ* hybridization, immunohistochemistry, and gel mobility shift assays have shown that *fos* and *jun* gene expression is differentially regulated within individual SCN cells, resulting in the generation of AP-1 binding complexes with constant, as well as variable, protein components (Takeuchi et al., 1993). Our own data in rats suggest that AP-1 binding sites on ventrolateral SCN target genes are constitutively occupied by FosB / JunD complexes and that c-Fos, Fra-2, FosB, and JunB are induced and compete for binding

after photic stimulation (Schwartz et al., 1998). Another group (François-Bellan et al., 1999) failed to detect FosB and concluded that AP-1 binding complexes in darkness represent heterodimers of Fra-2 and JunD. While this discrepancy will need to be resolved, the main point is that light acts to change the protein composition of AP-1 binding complexes in the SCN. Compositional changes can alter the stability and binding affinity of the complexes, and along with changes in total binding activity (Kornhauser et al., 1992; François-Bellan et al., 1999), might ultimately influence the transcriptional regulation of SCN genes with AP-1 sites on their promoters.

This kind of complexity confounds investigations seeking a causal link between light-induced *c-fos* gene expression in the SCN and phase shifts of overt rhythmicity. Honrado et al. (1996) reported that mice homozygous for a *c-fos* null mutation nevertheless entrained to a light-dark cycle and generated phase-dependent phase shifts of their wheel-running rhythms to light pulses. Honrado et al. speculated that other proteins might compensate for the loss of c-Fos in these animals; indeed, Fra-2 and/or FosB would appear to be plausible candidates for such a role. It is important to remember that Fos proteins alone cannot form effective DNA-binding complexes; thus, whether or not the proteins are *necessary* for photic phase shifts, they are surely not *sufficient*. Predictably then, there are now several pharmacological treatments or physiological perturbations that can elevate c-Fos levels in the SCN without causing concomitant phase shifts (for discussion and references, see Schwartz et al., 1996).

A final complication is the existence of an endogenous circadian rhythm (at least for *c-fos* and *junB* gene expression in rats and hamsters) in the dorsal SCN, in a distribution distinct from the region in the ventral SCN that responds to photic stimulation (Sumová et al., 1998; Guido et al., 1999). This differential photic and circadian regulation in separate cell populations is consonant with independent evidence for the functional compartmentalization of the SCN into two subdivisions. Recently, a novel demonstration of rhythmic *c-fos* transcription *in vitro* was accomplished by monitoring bioluminescence from SCN slice cultures derived from a transgenic *fos/luc* mouse line expressing the human *c-fos* promoter linked to a firefly luciferase reporter (Geusz et al., 1997).

And Here's What We Really Don't Know

A role for *c-fos* in the circadian timekeeping mechanism remains undefined. Perhaps the strongest evidence that it is somehow involved in photically-induced phase shifts comes from Wollnik et al. (1995), who reported that the intracerebroventricular injection of antisense oligodeoxynucleotides to both

c-fos and *junB* prevented light-induced phase delays of the rat locomotor rhythm. The problem with this approach, however, as Wollnik et al. discuss in their paper, is the non-sequence-specific binding of such oligos to other cellular mRNAs, as well as their non-specific interactions with various proteins, membranes, and signalling pathways. Although Wollnik et al. used nonsense control oligos to *c-fos* and *junB*, multiple control oligos are required to test for the sequence specificity of antisense effects, viz., a sense control (to maintain the structure but not the nucleotide composition of the oligo), scrambled control (to maintain composition but not structure), and mismatched control (to demonstrate hybridization selectivity) (Stein and Krieg, 1994). In their paper, Wollnik et al. argued for the sequence specificity of their effects by showing that immunoreactive FosB and c-Jun protein levels in the SCN were unaffected by the injections; however, these two immunoreactivities are among those in the rat SCN that show a constitutive expression (Peters et al., 1994; Ebling et al., 1996). Raising further concerns about specificity is the authors' observation that photic phase shifts were completely inhibited by a 50% reduction in SCN c-Fos levels, a

result not easily reconciled with previous data (Kornhauser et al., 1990). Interestingly, in studies of dividing Swiss 3T3 fibroblasts, intracellular microinjection of various antibodies against Fos family proteins suggests that the activity of multiple proteins must be inhibited in order to effectively block DNA synthesis (Kovary and Bravo, 1991). In contrast, similar experiments using antibodies against Jun family proteins indicate that the activity of each single Jun protein is essential for cell cycle progression. Thus, it may be that the inhibition of *junB* is the indispensable part of Wollnik et al.'s experimental design.

It is well documented that c-Fos is not activated in the SCN when non-photic stimuli are used to generate behavioral phase shifts, at least in the hamster (Mikkelsen et al., 1998). In combination with the results using photic stimuli, this finding supports the idea that c-Fos functions as part of the circadian pacemaker's photic input pathway (assuming that photic and non-photic stimuli utilize the same pacemaker outputs). A critical challenge to this hypothesis would be the discovery of photically-induced phase shifts without SCN c-Fos expression. There are recent hints that this might be the case when light pulses are administered at critical phases to *tau* mutant hamsters (Grosse et al., 1995) or to rats previously entrained to a long (LD 16:8) photoperiod (Trávníčková et al., 1996). More work is needed to evaluate these data; perhaps such circumstances have altered the sensitivity of the phaseshifting mechanism to the level of c-Fos protein.

The topographical differences in *c-fos* gene regulation between different regions of the SCN -- photic induction in the ventral part and circadian expression in the dorsal part -- imply that the function of c-Fos in circadian timekeeping is likely to be cell-specific. Such specificity is reflected in the identities of the cells that express the protein. At least in the rat SCN, some of the dorsomedial cells responsible for the circadian expression of c-Fos also synthesize vasopressin (Schwartz et al., 1998). In contrast, the identity of the ventrolateral cells responsible for the photic expression of c-Fos include some that contain gastrin releasing peptide and/or vasoactive intestinal polypeptide (Romijn et al., 1996). However, in the mouse SCN, photoinducible c-Fos is present in vasopressin cells (among others) (Castel et al., 1997); unlike the rat, retinal input to the mouse SCN is not limited to the ventrolateral subdivision but is more homogeneously distributed throughout the entire dorsoventral extent of the nucleus.

Much attention has been focused on *c-fos* induction in the SCN by the Ca^{2+} or cyclic AMP-dependent phosphorylation of members of the CREB family of DNA-binding proteins (Ginty et al., 1993; Obrietan et al., 1998). However, transcriptional regulation of *c-fos* is not governed by any single response element on the *c-fos* promoter; it relies on the cooperative, interdependent activity of multiple control elements (Robertson et al., 1995). In addition to the Ca^{2+} /cyclic AMP response element (CaCRE) located at -60 in the 5' untranslated region of the *c-fos* gene, these elements include the serum response element (SRE, at -310), to which a separate set of proteins is bound (SRF, Elk-1, SAP-1), and the c-Sis-inducible element (SIE, at -345), with which the Stat protein family interacts. The signal transduction mechanisms that regulate activity at the CaCRE, SRE, and SIE are not independent linear pathways but elaborate, cell-specific networks; they include complex kinase cascades (PKA, CaMK, MAP kinases ERK and JNK, and JAK) that exhibit "cross-talk" through complicated interactions between multiple intermediates. While Neil and I have memorized all the abbreviations, we know little else about their function in the SCN.

A final (but crucial) point bears emphasis. Although data from *in situ* hybridization, immunohistochemistry, and gel mobility shifts can identify which *fos* and *jun* gene products constitute AP-1 binding complexes, as well as their affinity for DNA, these methods do not

reveal whether the complexes alter transcription once they are bound to the promoters of target genes. Binding activity is not equivalent to transcriptional activity, as the *trans*-activating (or -repressing) potential of the complexes can be regulated by the phosphorylation of component proteins at specific sites, the participation of "adaptor" proteins, or the influence of the nuclear matrix. In the SCN, nothing is known regarding the transcription (or the identities) of AP-1 dependent genes.

Ten years ago, when *c-fos* became the first photoinducible SCN gene, we were smitten by the vision that it might be a key to unlocking the molecular basis for circadian entrainment. Today we remain enamored, but we are also humbled by the complexity of the signalling mechanisms that must be unraveled. In any case, we wish hearty congratulations to *c-fos* on your double-digit birthday, and many happy returns (of productive research). And to the new clock genes: enjoy all of our unbridled adoration now, while you're still the new kids on the block.

Acknowledgments

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CAN YOU TOP THIS?*

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Limerick of the Month

A Swiss banker's wife from Gstaad
had been suffering badly from SADD.
So they turned up the flux
to 10,000 lux
to stop her from going quite mad.

Dick Joyce
ROYAL COLL SURGEONS IRELAND
DUBLIN 2, IRELAND.
charles.joyce@pupk.unibe.ch

Editor's note: The following lyrics were written by Jonathan Wisor, Stanford University, who sang them with guitar accompaniment at an SRBR meeting reception in approximately 1996.

"C-Jun"

(to the tune of "Hey Jude" by the Beatles)

C-jun
it is a gene
that's expressed when neurons are active
The minute that calcium rushes in
then it begins... c-jun expression.

C-jun
what does it do?
It is a transcription factor
The minute it dimerizes with fos
then it can cause... further gene expression

So anytime it is expressed
You can expect
To see a cascade of gene induction
And when it binds to DNA
It's fair to say
there is an increase in gene transcription
junana junana (note: that's Japanese for 17, the avian sarcoma virus strain from which c-jun was cloned)

C-jun
knockout mice
They could be quite a model system
But don't try it 'cause someone already tried
C-jun knockouts died... during gestation

In the SCN we know
the data show
C-jun is induced by light exposure
And in the hippocampus too
It is induced
by metrazole which causes seizures
junana junana

repeat first verse and then the finale:
Na na na nana na na nana na na c-jun

Photobiology for the Chronobiologist:

Part I - Units of Light Measurement

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1. Introduction

In this paper (and in Part II to appear in a subsequent *Bulletin*), we will attempt to review the use of light stimuli in circadian rhythm experiments. Until recently, most circadian rhythm experiments have used light as a gross stimulus to elicit a response from the clock. In such experiments organisms are usually exposed to "bright" artificial light controlled by a simple timer which regulates exposure by turning lights either on or off. These conditions bear little resemblance to the natural photoperiod, and may actually confuse our understanding of circadian rhythm mechanisms. This approach is analogous to using a hammer to drive in a screw, an action that is quick and easy but ultimately doomed. The recent general interest in the action of light on the circadian rhythm system makes it all the more important for the circadian rhythm field to adopt the standardized approaches developed by photobiologists. This is necessary if we are to meaningfully: (1) compare experimental results from different laboratories; (2) compare different experimental models; (3) elucidate the cellular and molecular mechanisms of photoentrainment in any organism. Below we consider two photobiological topics that relate to circadian rhythm research: The measurement of light and the use of different light stimuli in circadian rhythm experiments. In the next edition of this supplement we will consider how to characterize photopigments using action spectrum techniques (Photobiology for the Chronobiologist: Part II - Action Spectra).

2. The Measurement of "Light" or Radiant Energy

Technically, light and radiant energy are not the same. Radiant energy is considered to be all electromagnetic energy, whilst light refers to that part of the electromagnetic spectrum visible to the human eye (between the wavelengths of 380 - 750 nm). Therefore, the strict definition of light is based upon human sensitivity to radiant energy. However, the term "light" is generally used to define radiant energy detected by physiological systems in humans and non-humans, and the term "light" will be used interchangeably with the term "radiant

energy" in this paper. An understanding of light (radiant energy if you prefer!) as a stimulus is essential if biological responses to light are to be understood.

The measurement of light, can be undertaken using a variety of techniques and expressed in several different units. This diversity caused considerable confusion about what different light units represent, and which units are most appropriate for a given experimental situation. We can start by dividing light measures into two broad categories called: Radiometric or Photometric measures. These terms can be further subdivided and are associated with specific units. The terms and units discussed below are summarized in Table 1.

2.1 Radiometry: Radiometry is the measurement of electromagnetic energy within the optical spectrum, which includes ultraviolet radiation, visible light, and infrared radiation. An ideal radiometric detector has a flat spectral response (Figure 1). A range of radiometric quantities and units are used, the two most commonly used in biology are irradiance and radiance.

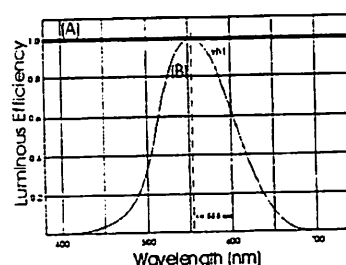


Figure 1: The "ideal" spectral responses of (A) a radiometric and (B) a photo-metric detector (see Table 1). The sensitivity range of a radiometric detector extends beyond the range of visible wavelengths indicated on this figure and into the ultraviolet and infrared parts of the electromagnetic spectrum.

2.1a Irradiance: Irradiance is a radiometric measure of the amount of light falling upon a known surface area. The international unit of measure of irradiance is watts/m². However, most detectors are calibrated in watts/cm². Biologists use this measure to quantify the incident light coming from all directions over a 180° field of view (Figure 2). Note that many irradiance detector heads do not provide a complete 180° field of view. Instead of using a cosine diffuser (which integrates light from a 180° field of view), detector heads are constructed so that light from a broad field of view passes through an aperture and falls on the detector surface of 1 cm². The most commonly used irradiance units found on radiometers are: $\mu\text{watt}/\text{cm}^2$ and photons/cm²/s/nm.

Table 1. Units of Radiometry and Photometry

Radiometric measures: measurement of electromagnetic energy within the optical spectrum, which includes ultraviolet radiation, visible light, and infrared radiation. An ideal radiometric detector has a "flat" spectral response.

Irradiance: measure of radiant energy from all directions over a 180° field of view.	Radiance: measure of radiant energy viewed from a specific direction or region in space.
Common Units:	Common Units:
$\text{erg}/\text{s}/\text{cm}^2$	$\text{erg}/\text{s}/\text{cm}^2/\text{sr}$
$\mu\text{W}/\text{cm}^2$	$\mu\text{W}/\text{cm}^2/\text{sr}$
photons/cm ² /s	photons/cm ² /s/sr

Photometric measures: measuring human visual responses to radiant energy. A measurement of visible light that falls between the wavelengths of 380 - 750 nm. The spectral response of a photometric detector is not flat but attempts to reproduce that of the average human eye. Two average human eye responses are used. A **photopic response** (maximum sensitivity at a wavelength of 555 nm), and a **scotopic response** (maximum sensitivity at a wavelength of 507 nm). By convention, photometric measurements are considered photopic unless otherwise stated.

Illuminance (illumination): measure of light from all directions over a 180° field of view.	Luminance: measure of light viewed from a specific direction or region in space.
Common Units:	Common Units:
lux (lx)	candela (cd)
$\text{lumen (lm)}/\text{m}^2$	$\text{lumen (lm)}/\text{sr}$
$\text{phot (ph)} = \text{lm}/\text{cm}^2$	$\text{cd}/\text{m}^2 = \text{lm}/\text{m}^2/\text{sr}$
$\text{foot-candles (fcd)} = \text{lm}/\text{ft}^2$	$\text{lm}/\text{m}^2/\text{sr}^1 = \text{lx}/\text{sr}$
	footlamberts (FL) = fcd/sr

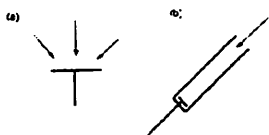


Figure 2: Representation of the arrangement of the photocell used for the detection of (A) irradiance/illuminance and (B) radiance/luminance. Irradiance/illuminance measures collect radiant energy from all directions over a 180° field of view, whilst radiance/luminance measures collect radiant energy viewed from a specific direction or region in space (see Table 1). In most cases a lens is used to determine the collection angle for a radiance detector, however a narrow aperture tube can be substituted (as shown in B).

2.1b Radiance: Radiance is a measure of the amount of light in a specific region of space (Figure 2). The key to understanding radiance is that it refers to the amount of light as viewed from a specific direction. Radiance detectors are constructed by using an aperture and a positive lens in front of the detector which allows light from a defined region of space (unit of solid angle or steradian/sr) to fall onto the detector surface. Thus, radiance is expressed in terms of energy per unit area, per unit time, per steradian. The most commonly used units are $\mu\text{Watt}/\text{cm}^2/\text{sr}$ and $\text{photons}/\text{cm}^2/\text{s}/\text{sr}$. **2.1c Irradiance or Radiance?** Even with the definitions above, the appropriate application of irradiance or radiance measures is not always clear. The rule is that if the direction or position of the light source is important then radiance measures are used. If the position of the light source is not important, then irradiance measures should be collected. A few examples below may help clarify this issue further.

A radiance measure would be appropriate to determine the amount of light passing through a hole in the forest canopy and falling onto a basking lizard. However, having said that radiance is good for measuring the amount of light in a particular direction of space, this measure is not an appropriate unit for characterizing point sources of light (e.g. light emitted from a laser) because the field of collection of the detector usually extends outside the area of the point source. For this reason it is better to use an irradiance detector and direct the point source 90° to the collection surface.

A common problem in circadian rhythm research is the characterization of a phase-shifting light stimulus. For example, when a light-pulse is delivered to a rodent in circadian rhythm experiments, it is often placed into a chamber designed to provide uniform lighting (such a device is shown in Foster, Provencio et al. 1991). While light usually originates above the animal, it is scattered using a frosted glass screen (an "opal" screen). As a result, the light falling on the animal is uniform and extended. In this example the light should be characterized using a measure of irradiance. A final example would be the measurements of sunlight. Sunlight within the environment varies in its direction, spectral quality and flux. For example, if a measure of all the available light arriving on the ground is required, then irradiance measures are appropriate. By contrast, if measures of sunlight at the horizon are required (the viewing direction is clearly important) then radiance measures are appropriate. In this case the radiance measure would ignore much of the scattered light above the horizon.

2.2 The Photon. It is critical to remember that photopigments absorb energy as photons. Photopigments act as photon "counters". Thus, all measures of light used in biological experiments should ideally be expressed as a photon flux. The energy of a photon is proportional to the reciprocal of its wavelength ($1/\lambda$). This means that high frequency short wavelength light (blue light) has photons of higher energy than red light. Nonetheless, since photopigments act as photon 'counters' the biological effectiveness of light is unrelated to these differences in energy. Note, therefore that if the biological effectiveness of 450 nm light (blue light) and 620 nm light (red light) are to be compared the stimuli must contain the same number of photons (photon flux). Since the standard radiance/irradiance measures are assessments of energy, if the stimuli are calibrated to contain an equal

radiometric irradiance (same number of $\mu\text{Watt}/\text{cm}^2$) then the blue light stimulus would contain fewer photons than the red light stimulus. In these experiments, the apparent sensitivity of the photopigment will be erroneously shifted to longer wavelengths. When comparing the effect of different wavelengths of light it is necessary to convert measures of radiant energy into photons. An example of such a calculation is shown below:

To convert energy flux (e.g. W/cm^2) to photon flux you first need to know the amount of energy contained in a photon: $E = h\nu$

Where:

E is the energy in a photon

h is Planck's constant ($6.626 \times 10^{-34} \text{ W} \cdot \text{s}^2$)

n is the frequency of the wave. Remember that: $n = c/\lambda$

Where:

c equals the speed of light in a vacuum ($3.00 \times 10^{17} \text{ nm/s}$)

λ is wavelength in nanometers (nm).

Therefore for 1 photon of 500 nm light:

$$E = (6.626 \times 10^{-34} \text{ W} \cdot \text{s}^2) \times (3.00 \times 10^{17} \text{ nm/s}) / 500 \text{ nm} =$$

$$3.976 \times 10^{-19} \text{ W} \cdot \text{s} / \text{photon or}$$

$$3.976 \times 10^{-13} \mu\text{W} \cdot \text{s} / \text{photon}$$

The conversion of a light value from watts into photons proceeds as follows. If, for example, the irradiance of the stimulus was $3 \times 10^{-2} \mu\text{W}/\text{cm}^2$ at a λ of 500 nm light then one would need to divide the measured value in $\mu\text{W}/\text{cm}^2$ by the energy of a 500 nm photon in $\mu\text{W} \cdot \text{s}^2$. Thus $= 3 \times 10^{-2} \mu\text{W} \cdot \text{cm}^2 / 3.975 \times 10^{-13} \mu\text{W} \cdot \text{s}^2/\text{photon} = 7.5 \times 10^{10} \text{ photons}/\text{cm}^2/\text{s}$. You may want to repeat this calculation using the same irradiance ($3 \times 10^{-2} \mu\text{W}/\text{cm}^2$), but substitute different λ values. By doing this you will appreciate the relationship between watts and photons, and that an equal number of watts does not contain the same number of photons.

An alternative unit of photon flux is the Einstein ($\text{E}/\text{m}^2/\text{s}$) or microEinstein ($\mu\text{E}/\text{m}^2/\text{s}$), and is the measure of light most often used by plant physiologists. 1 Einstein = 1 mole of photons at a specific wavelength (6.022×10^{23} photons); 1 microEinstein = 1 micromole of photons at a specific wavelength (6.022×10^{17} photons).

2.3 Photometry. In contrast to radiometry, photometry is concerned with measuring human visual responses to radiant energy and deals only with the measurement of visible light that falls between the wavelengths of 380 - 750 nm. In addition, the spectral response of a photometric detector is not flat (like a radiometer) but attempts to reproduce that of the average human eye (Figure 1).

A complication in photometry is the use of two average human-eye responses. During the hours of daylight, the cones of the retina provide a photopic response, with a maximum sensitivity at a wavelength of 555 nm (Figure 1). Under low levels of light the rods of the retina provide the scotopic response, with a maximum sensitivity at a wavelength of 507 nm. By convention photometric measurements are considered photopic unless otherwise stated (as shown in Figure 1). For a more detailed discussion of photometric units, see below.

The basic energy unit for the photometric unit is the lumen (lm) which is defined with reference to a truly monochromatic (single wavelength) light source in the following way: For scotopic measurements 1 watt = 1700 scotopic lm at 507 nm. For photopic measurements 1 watt = 683 photopic lm at 555 nm. However, note that because photometric measures do not have a flat spectral response, and because the emission spectrum for most light sources is varied (see below) converting photometric units into radiometric result from changes in the light spectrum rather than changes in the photon flux. By contrast, if good quality interference filters are being used to generate monochromatic light, changes in the spectral composition of the light source will not present a problem. In any case, the preferred way of changing the flux of a stimulus is through use of neutral density filters, which (in theory) reduce transmission at all wavelengths equally. A great range of neutral density filters exist varying both in quality and price, ranging from black plastic trash bags to glass neutral density filters carefully calibrated to reduce radiant flux by specific log unit amounts.

units is difficult. Indeed, this is only possible if the precise emission spectrum of the light source is known. If both photometric and radiometric units need to be measured or reported it is much simpler (and probably more accurate) to use dedicated detectors.

A critical point to stress is that photometric units provides a measure of visual brightness as it would appear to a human observer. Of course, the spectral responses of other organisms differ from that of the human eye. Therefore it is inappropriate to use photometric measurements when studying non-human photoreceptor systems. Moreover, since we do not know which ocular photoreceptors mediate human or mammalian circadian rhythm responses to light (Foster 1998), there is no justification for the use of photometric measures in human circadian rhythm studies either. The only reason for reporting photometric light measures in circadian rhythm papers is that historically many studies have used these units, and thus these measures can provide a point of comparison with earlier studies. Finally, a critical point to remember is that because photometric measures do not have a flat spectral response (Figure 1) they should never be used to calibrate colored (monochromatic) light sources. For example, equal photometric measures "red" and "green" light will have a very different photon content, and would not be comparable light stimuli.

Having rejected photometric measures as unsuitable for circadian rhythm studies, let us now, for completeness, consider these measures in some detail! A range of photometric units are used, which in the first instance may be separated into those associated with illuminance or luminance (Table 1).

2.3a Illuminance (illumination). Illuminance is a measure of the amount of light falling upon a surface of defined area. It is analogous to the radiometric measure of irradiance except that detectors do not have a flat spectral response and depend upon the subjective human sensitivity to light. One lumen (lm) of light falling on one square foot is termed a footcandle (fcd). The metric equivalent - one lumen per square meter, is called a lux (1 lux (lx) = 10.76 fcd) (Table 1). Traditionally the lux has been the most commonly used measure of light in circadian rhythm experiments. To a large extent, this is the result of the low cost and availability of instruments used to measure illuminance compared with other detectors.

2.3b Luminance. Luminance is also known as photometric brightness. Luminance is analogous to the radiometric term radiance except for its dependence upon the subjective human sensitivity to light. Like measures of radiance, luminance detectors have a defined collection angle for light expressed in steradians (sr). In most cases a lens is used to determine the collection angle. A great variety of different units are used to express luminance (Table 1), the most common being lx/sr.

Note that the term "intensity" is often used as a general term to mean an amount of light. Technically, however it has a very specific meaning. Intensity is a measure of the radiant or luminous energy from a given direction in space. Units for intensity are energy (luminous or radiant) per steradian per unit time. To avoid confusion, the term intensity should not be used as a general term for a quantity of light.

3. Light stimuli

Deciding which light source is appropriate for any circadian rhythm experiment will depend upon a large number of factors, including compatibility with previous experiments, cost, availability, and the nature of the experimental question. In general light stimuli can be divided into white- or monochromatic - light sources.

3.1 "white light". A very large number of white, or more accurately broad spectrum, light sources have been used in circadian rhythm experiments. These include: tungsten - halogen incandescent lights which contain relatively low levels of short-wavelength/blue light, are rich in long wavelength and infra-red light, and whose spectral emission

is profoundly affected by voltage; a huge variety of fluorescent lights, most of which contain strong emission peaks or lines in the blue/UV portion of the spectrum; high-pressure xenon arc lamps which provide the most uniform broad-spectrum light; and sunlight, the spectral composition of which varies both with the time of day and other features of the environment. For a detailed description of the emission spectrum of these and other broad spectrum light sources see (Wyszecki and Stiles 1982). The point to emphasize about these various "white" light sources is that although superficially comparable, in fact they vary greatly in their emission spectra.

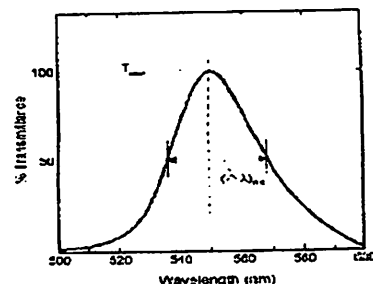
3.2 "monochromatic light". There are a large number of ways to produce narrow spectrum or monochromatic light. (A) Absorption filters made of glass, gelatin or liquids in which colored agents are dissolved or suspended. Gelatin filters are perhaps the most versatile type of filter and are made by mixing organic dyes in gelatin. They have the advantage of being relatively cheap and can be made very large, but are not stable over time (they can change their transmission spectrum as they age) and tend to have fairly broad transmission spectra. Also be aware that short wavelength (blue) filters often have a second long-wavelength (red) transmission that is frequently not reported by the manufacturer; (B) Cut-off filters essentially transmit or block light above or below defined cut-off wavelengths. These can be particularly useful in the generation of pure UV light sources when combined with fluorescent lights that have strong emission peaks in the UV (e.g. (Provencio and Foster 1995)); (C) Interference filters produce narrow band monochromatic light using an arrangement of highly reflective surfaces which ensure that only a narrow band of wavelengths are transmitted. These filters are made by successively evaporating dielectric and silvered films on glass. Interference filters are defined on the basis of their wavelength of maximum transmission (λ_{max}) and on the basis of their half-maximal bandwidth $(\Delta\lambda)_{0.5}$. (Figure 3). An important point to note is that the angle of incidence of the light falling on these filters greatly modifies their transmission spectra and it is critical that the incident light strike the filter at 90° to the surface. Also note that the mirrored surface should face the light source. Although able to produce higher quality monochromatic light than gelatin filters, interference filters are expensive, and because of their small size, cannot be used to bathe large areas in monochromatic light. Interference filters, coupled with powerful tungsten - halogen (or similar) bulbs and high quality optic fibers for light delivery, have formed the basis of most monochromatic light sources for circadian rhythm experiments; (D) Monochromators or diffraction gratings are designed to disperse incident light into its spectral components - from which any desired narrow band of wavelengths can be isolated. Although very versatile they are expensive, produce relatively little monochromatic light and, like interference filters, cannot be used to irradiate large areas; (E) Light-Emitting Diodes (LED's) emit monochromatic light of high purity. The early LEDs had a relatively low irradiance confined to a fairly narrow portion of the spectrum - usually in the yellow, red or infrared. However, the latest generation of LEDs can produce large amounts of monochromatic light at wavelengths which span the whole spectral range. It seems likely that LEDs will replace interference filters as the primary means of producing monochromatic light for circadian rhythm experiments.

3.3 Changing the emission level (photon flux) of a light source. There are two general methods for controlling the irradiance of a light source. The voltage applied to the light source can be varied and/or the light source can be screened using neutral density filters. In almost all cases, changing the voltage applied to the light source will change its color temperature, and hence the spectral emission of the bulb. In this case, an associated change in spectral composition may invalidate comparisons at different irradiances. Any experimental effects might

3.4 White light or monochromatic light as a stimulus? It is difficult to make generalizations, but ideally a "standard" monochromatic light stimulus, selected to coincide with the maximum (or near maximum) spectral response of the photosystem under examination, should be used for entrainment experiments. Monochromatic light has the advantage that it can be precisely defined and reproduced. Monochromatic light also avoids the complication of photoreversal, the biological phenomenon in which light at one wavelength drives a response in one direction, whilst light at another wavelength drives it in the reverse direction. Photoreversal has been reported in a number of photoreceptor systems and may even be important in photoentrainment (Solessio and Engbretson 1993). Despite the advantages of monochromatic light, most researchers use artificial broad spectrum light as a general entraining stimulus. This is largely because white light is cheap to produce and can be used to irradiate large areas. It is also true that we do not know which, or how many photopigments, mediate photoentrainment in most organisms. Without this knowledge it is impossible to select a "standard" monochromatic light stimulus. The cost and efficiency advantages of white light will undoubtedly ensure its continued use as a stimulus in circadian rhythm experiments. However, it is important to be aware of the problems that are inherent in using white light. For example, the varied types of fluorescent and incandescent light sources have widely different emission spectra. As a result, comparison of the effects of white light treatments are only valid when exactly the same light source has been used (remember that the emission spectrum from different types of fluorescent bulb can be very different and that the emission spectrum of a bulb will change with age). In addition, radiometric light detectors are sensitive to radiant energy in both the visual spectrum and infra-red parts of the spectrum. If a light source is rich in infra-red light (e.g., any incandescent lamp such as a tungsten-halogen bulb) then a significant portion of the flux recorded will be derived from energy that is not typically perceived as light. This would greatly complicate comparisons between results using different white-light sources, particularly when comparing incandescent and fluorescent light sources, because fluorescent lights produce only low levels of infrared. It is more difficult to change the levels of radiant energy produced by a white light source uniformly across the spectrum. Note that neutral density filters are often only "neutral" across certain regions of the spectrum. This problem is often observed when attempting to produce very low irradiances of light using several layers of neutral density filter.

There comes a point when adding additional layers of filter has little effect in lowering the amount of "light" detected by the radiometer. This occurs because some plastic neutral density filters are not particularly effective at blocking infra-red light, and radiometers are sensitive in the infrared part of the spectrum. Finally, white light sources, and particularly incandescent sources, provide an additional problem for the circadian rhythm researcher in that they generate heat that might act as a zeitgeber.

Figure 3: The transmission spectrum (transmittance - T) of a typical interference filter. Such filters are classified upon the basis of their wavelength of maximum transmission (λ_{max}) and on the basis of their half-maximal bandwidth $(\Delta\lambda)_{0.5}$.



The transmission spectrum of the interference filter shown in this diagram has a λ_{max} or T_{max} at 550nm, and the $(\Delta\lambda)_{0.5}$ is 20 nm. Note that the transmittance spectrum of interference filters tend to be symmetrical, whilst the transmittance of most gelatin filters are not symmetrical.

4. Summary

The problem of providing an appropriate light stimulus for circadian rhythm experiments is greatly exaggerated because the photopigments which mediate photoentrainment have not been defined in most organisms. This problem is compounded by our poor understanding of the features of the light environment that provide the entrainment signal. In the absence of this information circadian rhythm researchers should attempt to use light stimuli that can be accurately reproduced. For the reasons outlined above, light stimuli should be monochromatic and defined in radiometric units. This will enable different research groups to compare and collate their data, and will simplify the interpretation of results.

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- Recommended commercial light sources for circadian rhythm experiments:**
Dr. Neil Goldman, Enlightened Technologies Associates, Corporate Headquarters, 10370-A Democracy Lane, Fairfax, VA 22030-2522, USA (703) 359-4447; (703) 359-6808 fax; e-mail: info@etai.com; www.etai.com

*Editor's note: With this issue, we introduce a more or less regular feature in which investigators describe their species or preparation of choice for the study of biological rhythms. *Arvicanthus niloticus* is relatively new on the scene and its name begins with "A." The next contribution is expected to be on the "Z"ebrafish.*

Arvicanthus niloticus: a diurnal "lab rat"?

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The world of circadian rhythm research has been lacking a diurnal

version of a laboratory rat for some time: a rodent that breeds well in the lab and exhibits easily measurable and precise circadian rhythms, but is more active during the day than at night. That was why *A. niloticus* caught my attention in the fall of 1988, when I had just arrived in Masai Mara National Reserve, Kenya, for what turned out to be a four year stay. These little murid rodents seemed to be out and about all day long. They came sometimes to eat the crumbs beneath our table at breakfast and lunch when it was always light out, but never at dinner, when it was always dark. It was difficult to rule out the possibility that they might also be active at night, when it is more difficult to see small dark rodents. Therefore, my colleague, Kay

Holekamp and I bought a squeaky running wheel and an aquarium at a pet store in Nairobi one day. Back in camp we caught a young *Arvicanthis*, named her Rita Mae, and placed her with the squeaky wheel on my desk in our tent. Rita Mae ran like crazy during the day, as we expected, and we didn't hear her running at night. She could have been running while we slept, but even during Kay's frequent bouts of insomnia, Rita Mae's wheel was silent. This led us to wonder if *A. niloticus* might be just the animal I had been looking for.

However, I got distracted by other things and more or less forgot about the *Arvicanthis* until the summer of 1993, when Kay Holekamp trapped 29 animals, packed them up in wooden crates, and brought them to Michigan State University. This was not a simple process, as it involved dealing with a complex maze of bureaucracies in the US and in Kenya. When I met her at the Chicago airport we spent six hours going from one office to another, having stacks of forms signed and stamped. Surprisingly, no one there ever actually looked at the animals. We could have been sneaking little red Martians into the country, and no one would have noticed!

A safety official did catch up, however, one year after the *Arvicanthis* had arrived at MSU. At this point, the official suddenly became concerned that the *Arvicanthis* might have brought the Hanta virus with them. The message came down that we were all to suit up in full body protective gear, ventilators, and safety goggles each time we entered a room with *Arvicanthis*. As far as I can tell there still is no recorded case of the virus anywhere on the African continent. Ebola perhaps, but not Hanta. In any case, our *Arvicanthis* quickly passed the test, and everyone relaxed. I was somewhat humbled two years later, when Abel Bult was doing surgery on an *Arvicanthis* and two little red fleas jumped out at him. The fleas turned out to be a species indigenous to Africa, and not found in the Americas! Two months of anti-flea treatment followed, and the foreign fleas are gone, we think.

A. niloticus is a member of the subfamily *murinae*, as are mice (*Mus musculus*) and rats (*Rattus norvegicus*). They are brown, have a long rat-like tail, and big ears. Adult males and females weigh 100 gm, and 80 gm, respectively. The gestation period is 25 days and they mate on a postpartum estrus, so once they get started a pair can produce a litter, typically 5-6 pups, every 25 days. But sometimes they can take a while to get started, and only 50-75% of mating pairs that we establish actually produce young. *A. niloticus* do not exhibit nice predictable estrous cycles that can be easily monitored. Although they are clearly capable of coming into estrus and ovulating spontaneously, there is no clear external indicator of these events, and vaginal smears are completely uninformative. The only way that we have been able to predict estrous is to create mating couples, let them have babies, and wait 25 days for the post-partum estrus. This rather protracted exercise has enabled us to establish that the ovulatory surge in luteinizing hormone (LH) and the rise in Fos expression within neurons containing gonadotropin hormone releasing hormone (GnRH) occur virtually 12 hours out of phase in *Arvicanthis* and *Rattus*.

In Masai Mara, *A. niloticus* live in groups that may contain a breeding pair and multiple generations of their offspring, raising the possibility that these animals are monogamous. Casual observations in the lab suggest that males participate in parental duties such as attending the nest and retrieving pups. In the field, nests are located underground at the base of bushes from which runways radiate in several directions. Sometimes these runways connect nests associated with several bushes, and at high population densities it becomes unclear where one colony begins and another ends. In Masai Mara, approximately 1 degree South of the equator, the evening temperatures typically drop to around 50°C, and the midday temperatures typically rise to 86°C, pretty much all year round. The major seasonal changes in the *Arvicanthis* environment involve the truly extraordinary transitions between dry seasons and wet seasons.

When a diurnal species evolves from a nocturnal ancestor a coordinated suite of rhythms in a variety of behavioral and

physiological parameters typically change their patterns of coupling to the light-dark cycle. Our major goal is to elucidate the changes in underlying neural mechanisms responsible for these transitions. As a first step in our efforts to address this issue we have characterized nine different rhythms in our laboratory colony of *Arvicanthis*, and found that six of these are what one would expect from a typical diurnal species. These are rhythms in drinking, general activity, mating behavior, sleep/rest (as deduced from video recordings), body temperature (Tb), and the ovulatory surge in LH. The temporal organization of three variables has yielded unexpected findings. First, parturition, which in other species typically occurs when animals are least active, occurs in an "anti-crepuscular" pattern in *Arvicanthis*. That is, they birth at all times of day and night except the periods around lights-on and lights-off. Second, the daily rhythm in serum corticosterone shows a sharp drop just prior to lights-on in male *Arvicanthis*, and is not detectable in females. The daily drop in corticosterone just prior to the most active period of the day is the opposite of what is observed in other nocturnal and diurnal species, in which glucocorticoids rise just prior to the active period. The third rhythm that has yielded unexpected patterns involves the first variable that we measured: wheel running. Almost all of our original animals were either diurnal or crepuscular in a running wheel, but one was distinctly different and relatively nocturnal. She exhibited a very brief morning bout of running and then stopped for the day. At night, when the lights went out, she started again, and kept on going for 5-8 hours, while all of the other animals stopped within one or two hours of lights-out. Half of the offspring of our "nocturnal" female were also nocturnal and half were diurnal. These patterns did not reflect various shades of nocturnality that merged in a continuous manner with the diurnal patterns. They were distinctly different, and there were no intermediate patterns. In a larger breeding study we found that females were more likely to be nocturnal wheel-runners than males, and that the trait did indeed run in families. However, when we implanted animals with transmitters and monitored Tb and general activity, we were taken aback to find that our nocturnal individuals switched to showing a perfectly diurnal pattern as soon as the wheels were removed. In other words, access to the wheel induced our "nocturnal" animals to become nocturnal. This led us to wonder how many animals would be nocturnal in the field, where wheels were virtually nonexistent.

So, in the summer of 1998 my graduate student Julie Blanchong set out to characterize the daily rhythms of *Arvicanthis* at the field site in Kenya where the original MSU animals were trapped. Julie's mission was made more challenging by strict instructions not to walk around at night, amidst the lions, hyenas, elephants, buffalos, hippos, leopards...etc. Armed with timer traps that measured the interval between when the traps were set and when they were closed, Julie found a peak in *Arvicanthis* trappings in the middle of the hottest time of the day, and no evidence of nocturnal individuals. By contrast, all of the other rodents and the shrews that entered the traps did so exclusively at night. It remains possible that some *Arvicanthis* would exhibit the nocturnal pattern at a different time of year, or when the population was more or less dense, or perhaps if a diurnal predator came to forage in the neighborhood. However, for now, the field data suggest that *A. niloticus* is a robustly diurnal species.

A. niloticus thus continues to be the focus of our efforts to identify the differences within the nervous system responsible for differences between nocturnal and diurnal animals. My collaborators, students and I have explored the possibilities that some subpopulation of cells within the SCN, or some targets of SCN efferents might function differently in nocturnal and diurnal animals. We have found interesting differences between *Rattus* and *Arvicanthis* with respect to which SCN cell populations express Fos, and to patterns of 2-deoxyglucose uptake in the SCN. We have also found striking differences between these species with respect to patterns of Fos

expression in forebrain regions known to receive input from the SCN. Interestingly, nocturnal and diurnal *Arvicanthis* differ from each other with respect to patterns of Fos expression in neuropeptide-Y containing cells in the intergeniculate leaflet, at least when they have access to a running wheel. One job now is to determine the causes of these various differences. Another is to continue to evaluate possibilities as to where the fundamental differences lie, perhaps within the SCN, or in its projection sites. *A. niloticus* continues to represent an ideal model with which to address this issue.

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SLTBR ANNUAL MEETING REPORT

Review and Summary of the 11th Annual Meeting, May 16-18, 1999

The 11th meeting of the Society for Light Treatment and Biological Rhythms took place at The Holiday Select Inn, Old Town, Alexandria, Virginia. The scientific program was organized by Michael Terman.

The afternoon before the formal scientific program began, a Continuing Medical Education (CME) course was held. After a warm welcome by past-president Raymond W. Lam was given to the participants of the CME course, Anthony J. Lewitt delivered an overview on clinical practice guidelines for the treatment of seasonal affective disorder (SAD), followed by Alfred J. Lewy who presented melatonin treatment guidelines for winter depression and chronobiological disorders. Daniel F. Kripke then focussed a lively discussion on when to use light treatment for nonseasonal major depression. Finally Raymond W. Lam gave a most interesting talk on the use of light therapy for bulimia nervosa and premenstrual depression.

On the same evening, the welcome reception was followed by the poster session. Norman E. Rosenthal introduced the poster session. This has been one of the highlights of the SLTBR meetings throughout the years, not the least for reasons of Norm's constructive critical and cordial way of summarizing and commenting on the presented findings.

In trying to overview the poster presentations please accept my apology for not being able to review all the presentations in this forum.

Among clinical studies, M.C. Corral and colleagues reported two cases of successful light treatment in postpartum depression, indicating that light therapy may be a valuable option for depressive women who choose to breastfeed their infants and therefore refuse pharmacological treatment. J. Martin et al. support the importance of bright light exposure in the elderly, studying the potential relationship between illumination and mood disorders in nursing home patients. In a collaborative study performed at The Academic Hospital Gronigen (Y. Meesters and D.G.M. Beersma) and The University of Helsinki (T.T. Partonen), a dawn simulator was shown to be a useful tool for easy wake up in the general population. Focussing on new pharmacological approaches to SAD, N. Praschak-Rieder and coworkers reported the case of a young woman who fully remitted after having been treated with the novel selective noradrenaline reuptake inhibitor reboxetine 8mg/day. Prior to treatment with reboxetine this patient had not responded to SSRIs and light therapy. M.H. Teicher et al. presented an interim analysis of a double-blind, placebo-controlled study in which SAD patients were titrated to a maximum daily dose of 400mg Bupropion.

As current tools for the assessment of atypical symptoms have been shown to be inadequate for research purposes, J.B. Rifkin and coworkers created The Diagnostic Interview for Atypical Depression (DIAD) which detects

and scales the presence and severity of atypical symptoms with a high inter-rater reliability.

H.M. Calil (Sao Paulo, Brazil) and E.S.A.H. Gad from Egypt proved that seasonality is not a geographically limited dimension and therefore underscored the importance of chronobiological factors in the pathobiology of mood disorders. A number of studies investigated the role of psychological factors in the etiology, course and outcome of SAD. A ruminative response style to depressive symptoms might contribute to the occurrence of cognitive symptoms and the severity of already present vegetative symptoms (M.A. Young et al.). E.M. Tam and colleagues suggested that, with regard to several cluster B traits, SAD patients may have a personality profile that is intermediate in pathology between non-clinical subjects and patients with non-seasonal major depressive disorder. N. Goel et al. showed that the Tridimensional Personality Questionnaire was not found capable of predicting the response to light therapy.

A study conducted by R.T. Loving replicated findings of Neumeister et al. showing that partial sleep deprivation and bright light therapy positively augment antidepressant medication and assist with rapid response and recovery from depression.

Studying the biological basis of SAD using [123I]-β-CIT single photon emission tomography M. Willeit and colleagues from Vienna found evidence for a lower midbrain serotonin transporter availability in drug-naive depressed SAD patients compared to healthy controls. Altogether it was a great pleasure to see how many different issues were addressed in the poster session. This and the lively discussion made it one of the highlights of the meeting.

The first oral presentation provided new knowledge on the role of melatonin for chronobiological mechanisms. G.C. Brainard et al. focussed on research with monochromatic wavelengths in order to develop an action spectrum for melatonin regulation which might be useful in identifying the photoreceptor systems involved in light therapy. B.B. Byrnc and colleagues investigated the efficacy of two new light-emitting diode (LED) visors. Results from a study performed by R.J. Cole and colleagues suggest that light mask treatment through closed eyelids during sleep combined with behavioural treatment may be helpful in the treatment of Delayed Sleep Phase Syndrome. The effects of 6 consecutive dawn presentations in crossover with dim light was explored by K.V. Danilenko et al. A. Wirtz-Justice and coworkers chose a pharmacological approach to the question whether melatonin suppression is necessary for a phase shift to occur. By examining baseline endogenous melatonin profiles A.J. Lewy et al. recommended a low threshold dim light melatonin onset and the melatonin synthesis offset as reliable markers for the endogenous circadian pacemaker. The second session focussed on the therapeutic effects of light and included talks by C.I. Eastman ("Our SAD story has a happy ending")

and D.F.Kripke, who found low illumination to be associated with poor mood and sleep disturbances. D. A.Oren and colleagues presented very intriguing pilot data from a study on the effects of light therapy in the treatment of antepartum depression. The data certainly need replication in larger sample sizes. However, they indicate that light may be a valuable treatment option for this condition.

In the third session, entitled "Clinical and Physiological Facets of SAD", J.M. Eagles et al. reported a high demand upon health care services in patients with SAD. R.W.Lam proposed that SAD may not only be a categorical diagnosis, but that seasonality is a dimensional trait superimposed on psychiatric disorders. L.Sher and coworkers from NIMH focussed on the potential role of the hypothalamic-pituitary-thyroid axis in the pathogenesis of SAD. They were followed by T.T. Postolache et al. who examined the olfactory performance of SAD patients in a phenylethyl alcohol monorhinal detection threshold test.

The fourth oral session was opened by A. Neumeister presenting a β -CIT study with healthy female controls supporting previous findings which suggest seasonal variations in brain serotonergic functioning. R.L. Levitan and colleagues suggested that a variant of the tryptophan hydroxylase gene may play a role for susceptibility to SAD. Finally, E. Hilger and coworkers presented preliminary data of drug surveillance with the selective noradrenaline reuptake inhibitor Reboxetine, providing further evidence that, in addition to serotonergic systems, catecholaminergic pathways might be involved in the pathophysiology of SAD.

The SLTBR Young Investigator Award was given to Marc Herbert from the Biological Rhythms Research Laboratory of Chicago for his work on the potential effects of light and melatonin administration on retinal cone and rod sensitivity, as measured by electroretinogram.

This year's Distinguished Lecturer Presentation was given by Charlotte E. Reme from the University Hospital of Zurich. In her thrilling talk she presented data on the emerging role of rhodopsin and retinal gene expression for the mechanisms of light-induced retinal cell death. As there has been shown to be a spectral dependence of the mechanism of apoptosis, she suggested a minimization of the blue component in therapeutic lamps.

One afternoon during the meeting was dedicated to a symposium in honour of Norman E. Rosenthal's career at the NIMH. The symposium included speakers from all over the world. F.K. Goodwin, S. Kasper, B. Kjellman, M. Okawa, S. Swedo, T.A. Wehr and A. Wirz-Justice gave talks addressing Seasonality around the world: Research updates and reflections on the contributions of the NIMH Clinical Psychobiology Branch. The speakers themselves and the high quality of their presentations were excellent evidence for the important and stimulating role Norm Rosenthal played in their scientific lives. Thanks go to Norman E. Rosenthal and his group at the NIMH who have been stimulating research in this area during the past years.

This summary must not be concluded without expressing thanks to the organizers both for the organization of the scientific program and the intimate atmosphere they created throughout the whole meeting. As in the previous years, many excellent and fascinating presentations addressed a wide variety of questions. Through the lively discussions of data, plus opportunities to establish and renew friendships, the Annual Meeting again proved to be a primary means of communication for the SLTBR. We look forward to meeting again at the 12th Annual Meeting of the Society in 2000.

Meeting summary submitted by:

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ANNOUNCEMENTS

WORKSHOP OF THE EUROPEAN SOCIETY FOR CHRONOBIOLOGY - News from the Plant Chronobiology Research Markgrafeneheide, 15.-17.9.2000 *'To us self-important humans, it is rather startling to realize that animals and plants can tell time without the aid of wristwatches'* (Sweeney, 1987) If you are interested please contact Local organizer: Prof. Dr. Birgit Piechulla, University of Rostock, Dept. of Molecular Physiology and Biotechnology, Gertrudenstr. 11a, 18051 Rostock, Phone: 0381/4942245, Fax: 0381/4942243, Email: bpiechu@imppm.bio4.uni-rostock.de

POSTDOCTORAL POSITION - Post doctoral associate with prior post-doc experience and publications required. Tumor biology/metastatic potential as affected by circadian and reproductive cycles. We offer a clear path to independent funding of common projects. Send c.v. and description of achievements and goals to Dr. William J.M. Hrushesky, (111-C), Stratton VAMC, Albany, NY 12208. (www.rpi.edu/~hrushw)

POSTDOCTORAL POSITION (IIA BAT) - Candidates should have a Ph.D. and training in neuroscience, preferably with a circadian/behavioral background. Experience with analysis of gene expression and signal transduction cascades and/or neuroanatomical techniques is desirable. Candidates with good publication records and postdoctoral experience will be given preference. Applicants are expected to develop a funded, independent research program in the general field of mammalian circadian rhythms and to participate in undergraduate and graduate teaching (Animal Physiology, Behavioral Neuroscience, Chronobiology). Habilitation is possible and expected. The position is for a period of three years (with a possible extension of two more years) starting October-December 1999. The University of Stuttgart particularly welcomes applications from women in order to increase the proportion of female staff. Send applications in German or English (CV, statement of research interests, two letters of recommendation) to: Prof. Dr. Franziska Wollnik University of Stuttgart Biological Institute Pfaffenwaldring 57 70550 Stuttgart. E-mail: Franziska.Wollnik@po.uni-stuttgart

POSTDOCTORAL POSITION - Postdoctoral position available on an NIH-funded project investigating the cellular and molecular mechanisms whereby aging disrupts circadian rhythms. Research focuses on pre- and post-synaptic changes in the serotonergic system and the NPY system. Techniques include receptor autoradiography, computerized image analysis, in situ hybridization, immunohistochemistry, stereotaxic surgery, monitoring of locomotor activity rhythms and *in vivo* microdialysis. A Ph.D. degree and experience in one or more of these techniques is desirable. Send CV, summary of research experience, and three letters of reference to: Dr. M. J. Duncan, Molecular Endocrinology Focus Group, Dept. of Anatomy and Neurobiology, University of Kentucky Medical Center, 800 Rose St., Lexington, KY 40536-0084. E-mail: mjdunc0@pop.uky.edu.

POSTDOCTORAL POSITION - A post doctoral research position oriented toward basic and functional neuroanatomy of the mammalian circadian rhythm system is currently open in the laboratory of Dr. Larry Morin in the Department of Psychiatry and Graduate Program in Neurobiology and Behavior, Stony Brook University. Interested applicants should contact Dr. Morin by phone (516-444-1613) or by email (lmorin@epo.som.sunysb.edu).

TEXTBOOK - A potential textbook for courses on biological rhythms will be published this September by CRC Press. *Circadian Physiology*, by Roberto Refinetti, highlights the basic processes and latest research findings in circadian biology and describes how this knowledge is applied in business and health care. Targeted at life scientists who are not specialists in biological rhythms, the book is also accessible to general readers and to graduate and undergraduate students. For more information, visit www.circadian.org/book.html. To order a 30-day examination copy, call CRC Press at (800) 272-7737.

MEETING ANNOUNCEMENT - The V Latin American Symposium of Chronobiology will take place in Buenos Aires, Argentina, from September 30th to October 3rd, 1999. Previous meetings were held every two years alternatively in Brazilian and Mexican locations, and have attracted not only a growing community of Latin American chronobiologists, but also several scientists from North America and Europe. The current meeting will include a series of lectures, workshops, symposia and poster presentations. Prior to the meeting, a short graduate course and invited lectures will be held at the University of Buenos Aires and the University of Quilmes. Since lodging is limited, we would appreciate potential participants to contact the organizers as soon as possible. Abstract preparation instructions, the preliminary program and registration information are all posted in our web site: www.unq.edu.ar/5lasc. For more information, please contact Dr. Diego Golombek at 5lasc@unq.edu.ar or at fax number (54-11) 4365-7132.