

Behavioral and Serotonergic Regulation of Circadian Rhythms

R.E. Mistlberger¹, M.C. Antle¹, J.D. Glass², J.D. Miller³

¹Department of Psychology, Simon Fraser University, Burnaby BC

²Department of Biological Sciences, Kent State University, Kent OH

³Department of Pharmacology, Texas Tech University, Lubbock, TX

ABSTRACT

Endogenous depression is often accompanied by alterations in core parameters of circadian rhythms, and antidepressant treatments, including serotonergic drugs, sleep deprivation and exercise, alter circadian phase or period in humans or animal models. Antidepressants may act in part through the circadian system, and behavioral antidepressants through a common serotonergic path to the clock. This review evaluates the evidence from animal models that serotonin (5-HT) mediates phase-shifting effects of behavioral stimuli on circadian rhythms. In rodents, 'exercise' stimulated during the rest phase of the rest-activity cycle induces large phase shifts of circadian rhythms. These shifts can be mimicked by short-term sleep deprivation without intense activity. During wheel running or sleep deprivation, 5-HT release in the suprachiasmatic nucleus (SCN) circadian clock is significantly elevated. Lesions of 5-HT afferents to the SCN attenuate phase shifts or entrainment induced by activity in response to some stimuli (e.g., triazolam injections in hamsters, treadmill running in mice) but not others (e.g., novel wheel confinement in hamsters). Antagonists selective to 5HT_{1,2} or ₇ receptors do not attenuate shifts induced by wheel running, although 5-HT_{2/7} antagonists do partially block shifts to saline injections. 5-HT agonists (e.g., 8-OH-DPAT) induce large shifts in vitro, but much smaller shifts in vivo, particularly if administered directly to the SCN. Procedures for inducing 5-HT supersensitivity in vivo result in larger shifts to 8-OH-DPAT. 5-HT stimuli may affect the clock by direct and indirect pathways, particularly through the thalamic intergeniculate leaflet, and the role of these pathways may differ across species. At the level of the SCN, 5-HT likely acts through 5-HT₇ receptors on neurons and possibly also glial cells. These receptors may be useful targets for the development of antidepressant drugs. In aggregate, the literature provides mixed support for the hypothesis that exercise or behavioral arousal shift the circadian clock by a 5-HT pathway; the role of indirect pathways, interactions with other transmitters, cellular adaptations to denervation, glial cells, and species differences remain to be more fully clarified. Serotonergic and behavioral stimuli provide an intriguing route to elucidate the circadian clockworks and their possible role in depression.

KEYWORDS: Circadian rhythms, depression, serotonin, exercise, sleep deprivation, microdialysis, neuropeptide Y, animal models.

Address correspondence to: Dr. Ralph Mistlberger, Department of Psychology, Simon Fraser University, 8888 University Drive, Burnaby BC, V5A 1S6 Canada, Tel.: +604-291-3462. Fax: +604-291-3427. E-mail: mistlber@sfu.ca

INTRODUCTION

Depression, serotonin (5-HT) and the circadian timing system are linked by a wide range of clinical and experimental observations, obtained from studies of humans and animal models. In brief, depression is often characterized by atypical circadian timing (Van Cauter & Turek, 1986; Duncan, 1996; Rosenwasser & Wirz-Justice, 1997); antidepressant treatments, including most pharmaceuticals, exercise and sleep deprivation, alter 5-HT activity (Asikainen et al., 1995; Bloom & Kupfer, 1995; Jacobs & Fornal, 1997; Dudley et al., 1998); the primary circadian pacemaker (the suprachiasmatic nucleus, SCN) is richly innervated with 5-HT terminals (Miller et al., 1996); and, virtually all antidepressants alter circadian phase, period or response of the pacemaker to entrainment cues (zeitgebers) in rodents (Duncan, 1996; Rosenwasser & Wirz-Justice, 1997). We do not yet know whether circadian disorder causes depression or whether antidepressants act, even in part, through the circadian system. However, this constellation of findings clearly invites speculation (Wehr & Wirz-Justice, 1982; Rosenwasser & Wirz-Justice, 1997).

In this review, we focus on the link between exercise, 5-HT and circadian timing in animal models. Exercise and many drugs that facilitate 5-HT neurotransmission have antidepressant properties, and are capable of resetting the circadian clock in several species (Martinson, 1990; Duncan, 1996). It is conceivable that exercise and serotonergic drugs affect circadian timing via a common mechanism, and that adjustment of circadian timing plays a role in their therapeutic efficacy. To address this issue, we will assess a specific hypothesis that the effects of exercise on circadian rhythms are mediated by the 5-HT system. We will begin by reviewing our efforts to specify the behavioral state correlates necessary and sufficient for behavioral regulation of circadian timing (Mistlberger laboratory). We will then review the results of our tests of the 5-HT hypothesis, using lesions (Mistlberger laboratory), in vivo neurochemistry (Glass laboratory), in vivo and in vitro pharmacology (Mistlberger, Glass and Miller laboratories) and electrophysiology (Miller laboratory). We intend to emphasize points of convergence and divergence across experimental model systems employed by our labs and those of others, and will attempt to highlight the relative strengths and weaknesses of each approach.

FEEDBACK EFFECTS OF BEHAVIOR ON THE CIRCADIAN SYSTEM

A widely accepted conceptual model of the circadian system postulates three primary components; a circadian pacemaker (the SCN), one or more afferent pathways that function to entrain the pacemaker to zeitgebers such as the LD

cycle (e.g., the retinohypothalamic tract, RHT), and one or more efferent pathways by which the pacemaker confers rhythmicity on neural and endocrine systems. The traditional model did not include feedback coupling among these elements. However, work over the past two decades has revealed that wheel-running activity, which is strongly regulated by the circadian system, can modulate the rate at which the circadian clock oscillates in some nocturnal rodents. For example, in constant lighting conditions, the period (τ) of free-running rhythms is significantly correlated with the amount of wheel running activity in rats (Yamada et al., 1986; Yamada et al., 1988), and with the distribution of wheel running activity within the daily active period (α) in mice (Edgar et al., 1991). The amplitude of circadian rhythms may also be influenced by spontaneous wheel running (Welsh et al., 1988). This implies that wheel running, a clock-controlled process, provides afferent feedback to the clock. Feedback between rhythmic processes and the driving pacemaker may be a significant organizational feature of the circadian system in mammals.

Behavioral feedback regulation of pacemaker τ has implications for interpreting the atypical timing and amplitude of circadian rhythms in depression. There are several possibilities. Changes in the level or distribution of physical activity or sleep-wake states in depression may alter pacemaker τ , and thereby alter the phase angle of entrainment to LD (Aschoff, 1981) or coupling among internal rhythmic processes. These changes may be epiphenomenal to depression, albeit potentially useful as diagnostic markers (e.g., Stampfer, 1997), or they may be part of the depressogenic process (see Fig. 1). Alternatively, changes in activity levels and circadian timing in depression may be independently regulated, or changes in circadian timing and amplitude may cause the changes in activity and sleep-wake.

To establish a stronger empirical basis by which to evaluate these lines of conjecture, we have conducted additional studies of the relationship between spontaneous daily wheel running and the phase and period of circadian rhythms in C57BL/6j mice and Syrian hamsters. The mouse is a current favorite among circadian biologists primarily because of its amenability to molecular genetic approaches to circadian mechanisms. The Syrian hamster is the traditional rodent model of choice for studies requiring accurate assessments of circadian phase, due to the high precision of its wheel running activity rhythms.

In mice, a concentration of wheel running early in α is associated with a shorter τ in constant dark (DD; Edgar et al., 1991; Mistlberger & Holmes, 1999b). Moreover, when running wheels are available, the average number of wheel revolutions/day is negatively correlated with τ (Mistlberger & Holmes, 1999b), a relationship that has also been found for rats (Shioiri et al., 1991). This led us to predict that variations in the phase angle of entrainment to LD across individuals might be associated with variations in the level or distribution of spontaneous

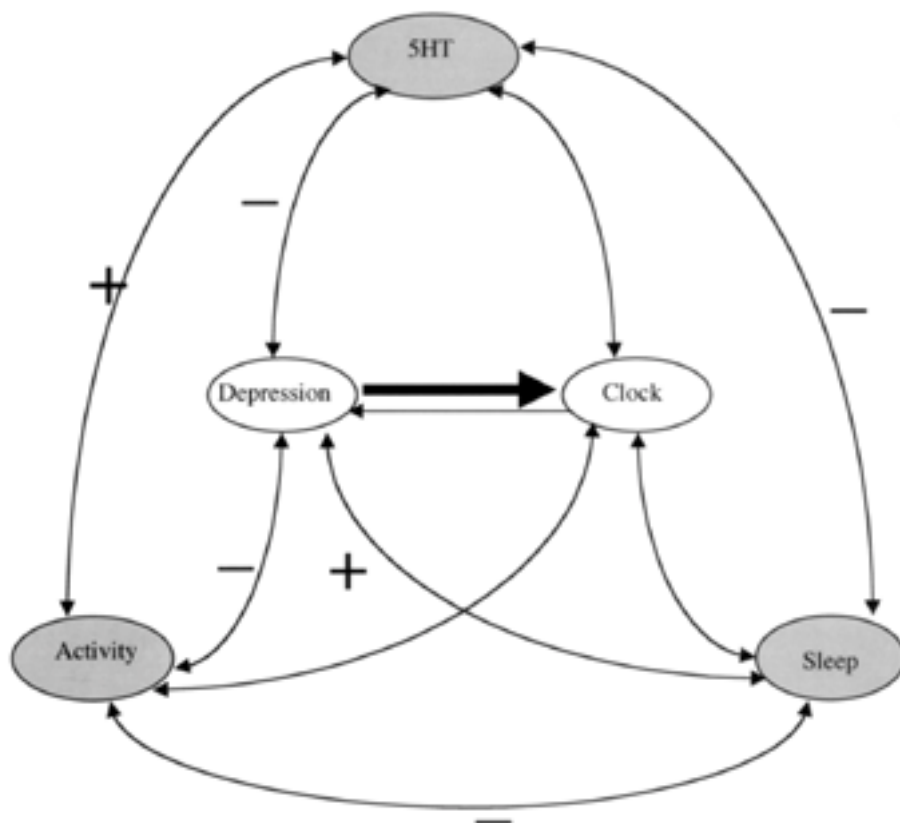


Fig. 1. This figure is in the spirit of a Feynman or “all possible paths” diagram. Instead of sub-atomic particles in this paper we consider certain conceptual constructs from research areas in sleep, circadian rhythms and psychiatry. Briefly, it is obvious that sleep and vigorous conscious motor activity are inversely related (the – sign at the bottom of the diagram). Abundant evidence indicates that motor activity and 5-HT transmission are positively related (Reuter & Jacobs, 1996) whereas 5-HT transmission and sleep are negatively related (Jouvet, 1999). The well-known antidepressant efficacy of selective 5-HT reuptake blockers argues for an inverse relation between 5-HT transmission and clinical depression (Bloom et al., 1995). Similarly, activity levels are generally reduced in depression, but an increase in activity or exercise levels often has an antidepressant effect (Martinson, 1990). Excessive sleep characterizes many forms of endogenous depression, but sleep deprivation has an antidepressant effect (Leibenluft & Wehr, 1992) suggesting a positive relation between sleep and depression. Depression itself is associated with perturbation of circadian rhythmicity (advances or delays in sleep onset; thick line). It is not clear whether severe perturbation of the clock necessarily results in depression, but commonalities of symptoms in jet lag and shift work disorder with depressive symptoms (ennui, fatigue, excessive sleepiness) suggest the possibility (thin line). The circadian clock regulates sleep, activity, and 5-HT transmission (Miller et al., 1996). Interestingly enough, motor activity and 5-HT agonists are also able to phase shift the clock (many studies reviewed herein). The symmetry of this diagram suggests that a sufficiently strong perturbation in sleep might likewise phase shift the clock, a prediction borne out by recent work (Mistlberger et al, 1999).

wheel running within α . Consistent with this prediction, the average amount of activity occurring during the first 2 h of α (circadian times 12-14, where CT12 is the onset of α , by convention) was found to be positively correlated with the timing of activity onset with respect to lights-off; the more activity early in α , the more positive the phase angle of entrainment to the LD cycle (Mistlberger & Holmes, 1999b).

Evidence that spontaneous activity can modulate pacemaker phase and period in Syrian hamsters is less clear. One study reported that access to running wheels was associated with a non-significant trend for lengthening of τ (Aschoff et al., 1971), while two other studies have reported evidence for shorter τ in association with wheel running (Pratt & Goldman, 1986; Mrosovsky, 1999). We found no detectable effect of wheel access on τ in 15 hamsters free-running in DD, using a within-subjects design in which the wheel-open and wheel-locked conditions were counterbalanced for order (Mistlberger et al., unpublished data, 1995).

This apparently weak relation between wheel running activity and τ in hamsters suggests that the circadian pacemaker in this species may be relatively insensitive to feedback effects of activity during α . Alternatively, wheel running expressed during different portions of α may shorten and lengthen τ in equal and opposite directions, resulting in no consistent net change. In a sample of 90 hamsters drawn from archival data, we did find a significant, albeit modest, correlation between the distribution of activity within α and the phase angle of activity onset with respect to the LD cycle (Mistlberger, unpublished, 1997). However, the correlation was in the opposite direction to that evident in the mouse data; hamsters that initiated activity early relative to dark onset tended to express less of their total daily activity during the first 2 h of α . Whatever the explanation for this species difference, the results provide evidence that in at least two mammalian species, changes in the distribution of spontaneous activity within the daily active period are associated with changes in the phase at which this behavioral rhythm entrains to the LD cycle.

Although these results from animal models are consistent with speculation that changes in spontaneous activity with endogenous depression might be causally related to atypical circadian timing in depression, we should note that direct evidence from studies of humans is sparse. Two studies have failed to detect a relation between physical workload and free-running τ in humans in temporal isolation (Wever, 1979; Beersma & Hiddinga, 1998). However, this could be because of a restricted range of workloads (e.g., Mrosovsky, 1999), or because integration across a range of circadian phases produced no net change in τ . To our knowledge, the effects of activity on phase angle in LD, independent of changes in light exposure, have not yet been assessed in humans.

'EXERCISE' PHASE SHIFTS CIRCADIAN RHYTHMS IN MAMMALS

There are two possible mechanisms by which locomotor activity might affect τ and the phase angle of LD entrainment. One possibility is that the level of spontaneous activity or arousal continuously modulates the rate of the clock's oscillation through much or all of its circadian cycle (a so-called parametric or continuous effect, by analogy with hypothesized effects of constant light on τ ; Aschoff, 1960). Another possibility is that activity discretely shifts the pacemaker at only limited pacemaker phases (a non-parametric or discrete effect, by analogy with the phase shifting effects of brief light pulses; Pittendrigh & Daan, 1976). If the mechanism is non-parametric, i.e., phase and period modulation caused by one or two phase shifts each day, then a single bout of activity, of appropriate timing and duration, should be capable of inducing a phase shift.

The induction of phase shifts by single bouts of activity was first demonstrated by Mrosovsky, following chance observations that small phase shifts sometimes occur in hamsters after litter or cage changes (Mrosovsky, 1988). These innocuous procedures awaken the hamsters and often stimulate wheel running. When hamsters were induced to run for 2–3 h by confinement to a novel wheel during their usual rest phase, phase advance shifts were large (averaging close to 3 h; Reeb & Mrosovsky, 1989; Fig 2a,b). Stimulation of running activity during the usual active period induced phase delay shifts of more modest size (averaging less than 1 h late in α , with little or no shifting early in α ; Reeb & Mrosovsky, 1989; Bobrzynska & Mrosovsky, 1998; Figs. 2b, 3).

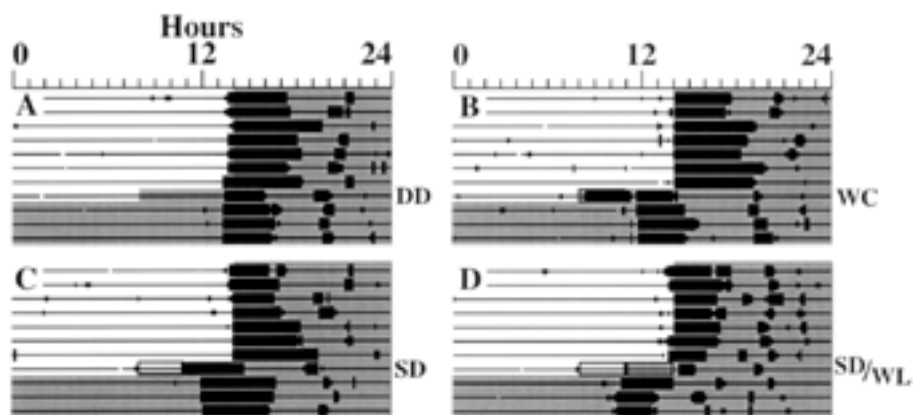


Fig. 2. Actograms of representative phase shifts of Syrian hamster wheel running rhythms in response to (A) DD initiated at ZT6 (6 h before lights off, designated ZT12), (B) 3 h wheel confinement initiated at ZT6, (C) 3 h of sleep deprivation initiated at ZT6, with running wheel locked, and (D) 3 h of sleep deprivation initiated at ZT6, with the home wheel locked for 6 h. Panels A, C, and D present data from the same hamster.

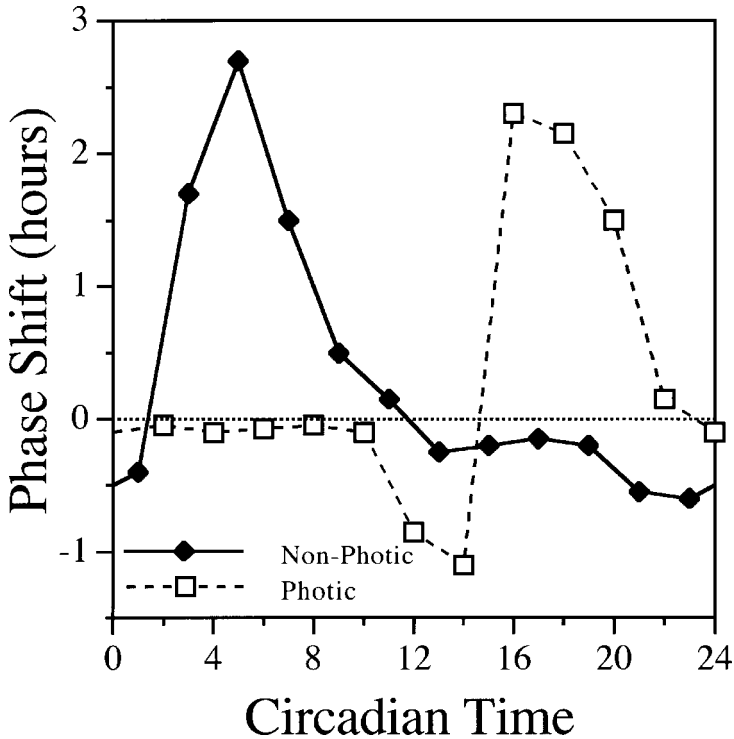


Fig. 3. Non-photic (◆ after Mrosovsky et al., 1992) and photic (□ after Takahashi et al., 1984) phase response curves (PRCs) for hamsters kept in constant dark. The non-photic PRC represents mean phase shift responses to 3 h bouts of novelty-induced wheel running. The photic PRC represents mean phase shift responses to 1 h pulses of saturating light. Points show mean values for two hour bins.

One implication of this discovery was that any stimulus capable of increasing activity or arousal might also be capable of inducing phase shifts, and this prediction was soon confirmed for several stimuli (Reebs et al., 1989; Van Reeth & Turek, 1989). The robustness of these non-photic effects on circadian phase is amply demonstrated by evidence that a daily bout of activity stimulated by behavioral procedures or drugs can entrain free-running rhythms in several rodent species, including hamsters, mice and rats (Rusak et al., 1988; Mrosovsky et al., 1989; Reebs et al., 1989; Van Reeth & Turek, 1989; Edgar & Dement, 1991; Mistlberger, 1991a; Marchant & Mistlberger, 1996; Fig. 4a), and can significantly alter the phase angle of entrainment to LD (Honrado & Mrosovsky, 1991; Mistlberger, 1991b; Mrosovsky & Janik, 1993; Sinclair & Mistlberger, 1997; Fig. 5).

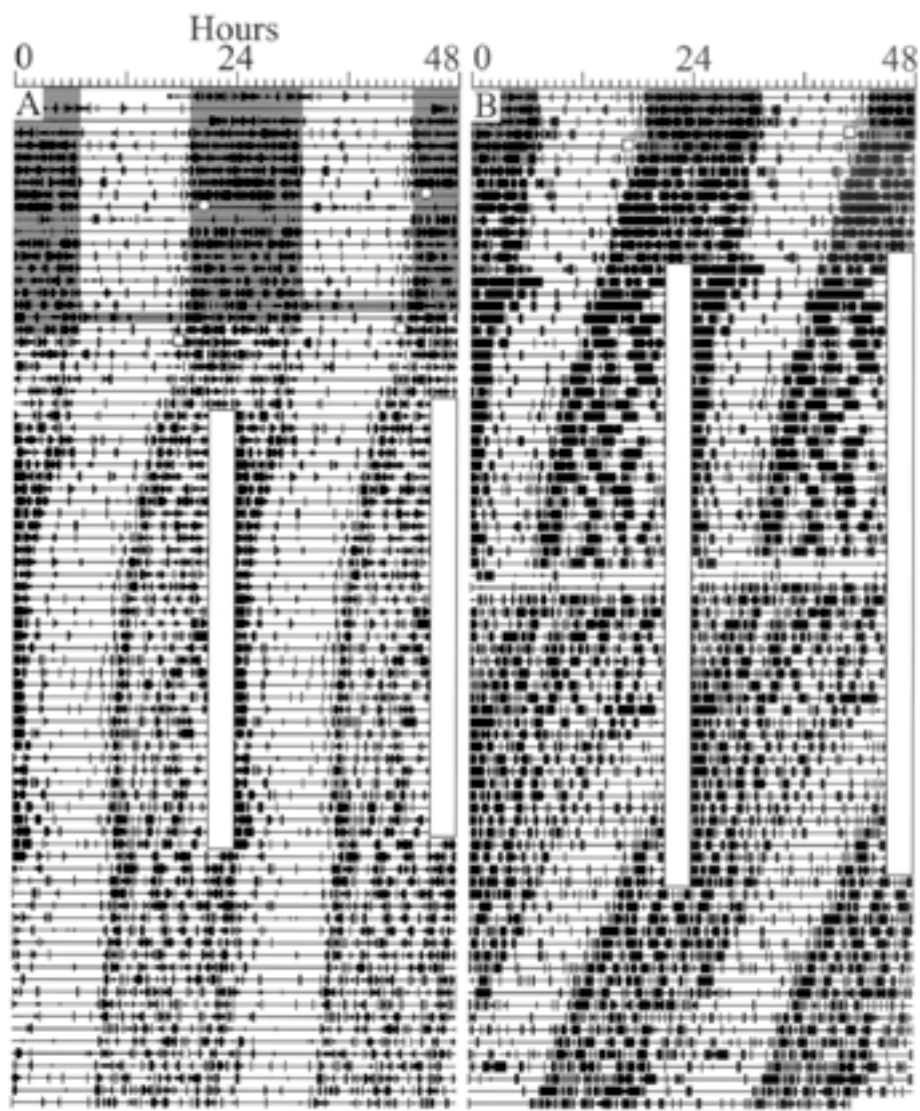


Fig. 4. Double-plotted actograms of drinking activity from enucleated C57BL/6j mice that received the 5-HT neurotoxin 5,7-DHT aimed at the SCN. (A) An incomplete lesion, and (B) a complete lesion of 5-HT input to the SCN. Vertical bar denotes 3 h daily bout of forced running on a treadmill. Panels replotted from Marchant et al. (1997).

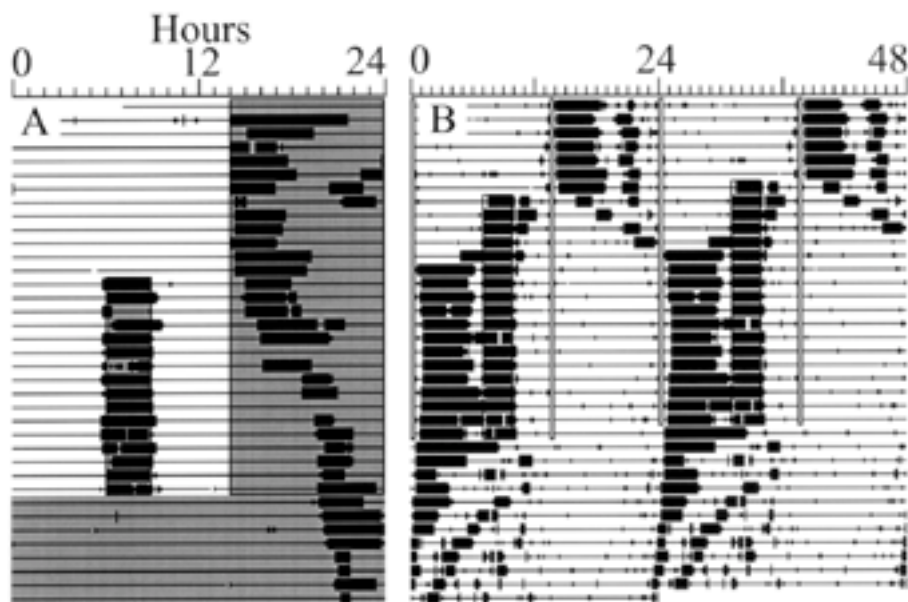


Fig. 5. (A) Activity record from a hamster entrained to a full photoperiod (LD:14:10) and subjected to 3 h of novel-wheel induced running for 17 days beginning at ZT4. DD was initiated at ZT12 after the final wheel confinement. (B) Double plotted activity record from a hamster subjected to scheduled activity at ZT5 in a skeleton photoperiod. Daily 30 min light pulses are indicated by thin vertical bars. Both panels replotted from Sinclair & Mistlberger (1997).

THE NONPHOTIC ZEITGEBER: 'EXERCISE' OR NONSPECIFIC AROUSAL?

Most evidence has implicated high intensity wheel running (what we would call 'exercise' in humans) as necessary for behavioral modulation of circadian parameters. First, τ is shortened by wheel access in rats and mice; when wheel access is blocked and τ lengthens, the animals are not restrained from other cage activity (Yamada et al., 1986; Edgar et al., 1991; Mistlberger & Holmes, 1999b). Second, placing hamsters in a novel wheel induces running and phase shifting, but if running doesn't occur, shifting is very unlikely (e.g., Janik & Mrosovsky, 1993; Mistlberger et al., 1996; Bobrzynska & Mrosovsky, 1998). Third, if hamsters or mice are prevented from running by immobilization in a restraint tube or confinement to a small nest box, they do not exhibit phase shifts in response to dark pulses or injections of triazolam or morphine, drugs that induce activity in these species (Van Reeth & Turek, 1989; Mrosovsky & Salmon, 1990; Marchant & Mistlberger, 1995). These results point to wheel running as the behavioral zeitgeber that causes phase shifting. However, other activities within the home cage may also contribute,

because locking the running wheel during a 3 h dark pulse attenuates phase advances by half, while confining hamsters to a small nest box, thereby reducing all locomotion, blocks phase advances entirely (Reebs et al., 1989).

Dose and phase response studies of shifts induced by wheel running during the daily rest period generally show that large phase shifts have a high probability of occurring if hamsters can be induced to run about 5,000 wheel revolutions in 3 h (Bobrzynska & Mrosovsky, 1998). In one study, it was observed that a few hamsters that failed to run in novel wheels (and thus did not phase shift) could be induced to run by acute exposure to cold, yet still did not exhibit phase shifts, despite generating as many as 10,000 wheel revolutions in 3 h (Mrosovsky & Biello, 1994). This observation was taken to imply that the motivation to run is important, and that running induced by thermal stimuli is an inadequate motivational state for the induction of shifts. However, these particular animals had not been shown to phase shift to any arousing stimulus, so individual differences in pacemaker sensitivity to behavioral stimuli is an equally logical, and interesting, interpretation of that data. The fact that running is rewarding (Kagen & Berkun, 1954; Premack et al., 1964; Collier & Hirsch, 1971) has been taken to suggest that neural correlates of reward, such as mesolimbic dopamine transmission, may be necessary for shifting. But, it would seem that cold-induced running should be especially rewarding, and therefore a stronger, not weaker, zeitgeber.

Another possibility is that motivation *per se* is irrelevant, but that motor patterns differ depending on the stimulus used to induce running. Different patterns of running, measured by such parameters as rate, bout length and interbout interval, may be associated with different patterns of neural activity and transmitter release, which may differentially affect circadian pacemaker phase and period. This idea has yet to be evaluated empirically.

More recent studies cast doubt on the idea that the particular motivation to run matters for nonphotic shifting. First, a modified experimental design was used to demonstrate that hamsters do shift in response to acute cold exposure but not if running wheels are locked (Mistlberger et al., 1996). Other studies showed that forced running on treadmills can induce shifts in mice and entrainment in rats and mice, and that activity associated with re-feeding after food deprivation can induce shifts in hamsters (Mistlberger, 1991a; Marchant & Mistlberger, 1996; Mistlberger et al., 1997b). Finally, it has most recently been demonstrated that 3 h of sleep deprivation during the middle of the rest phase can induce shifts of a magnitude and direction that fully account for the phase shifts that follow 3 h of continuous running (Mistlberger et al., 1999; Fig. 2c,d). During the sleep deprivation, running wheels were locked, and the hamsters were kept awake by gentle touch or air puffs only as necessary. Previous studies did not attempt to measure or rigorously control wake time in response to stimuli used to generate wheel running. However, it now appears likely that hamsters phase shift in response to these

stimuli because they are aroused when they would normally be sleeping, and that intense or continuous locomotor activity per se is not necessary to induce a maximal shift. The amount of wheel running is a robust predictor of phase shifting in response to nonphotic stimuli, but that is probably because running promotes arousal. Animals that don't run or are prevented from running in response to an activating stimulus probably do not shift because they do not stay awake.

Nocturnal animals that are sleep deprived during the subjective day (their sleep phase) would be expected to show a subsequent sleep 'rebound', raising the possibility that phase shifts associated with the deprivation procedure are actually induced by recovery sleep. However, this does not appear to be the case, because hamsters sleep deprived for 3 h showed no behavioral evidence of a sleep rebound, even though the tests ended 3 h before the usual time of lights-out. Rather, the hamsters remained active, and behaved as if their circadian cycle was already advanced by ~ 3 h to the beginning of the subjective night. In fact, when wheels were available, the hamsters generated running counts characteristic of the beginning of the subjective night. This post-deprivation running was not necessary for shifting, because the average phase shift was not attenuated if the wheel was locked for 3 h after the deprivation ended (Mistlberger et al., 1999; Fig. 2d). It is quite likely that the pacemaker is already shifted by the end of the deprivation, and that neither sleep nor running after the deprivation are involved in the resetting response, but this remains to be confirmed.

Our current hypothesis, then, is that intense activity is not necessary for nonphotic clock resetting, at least in hamsters, and that wake time and neural or endocrine correlates of arousal are sufficient to alter the phase of the circadian pacemaker. This hypothesis is intriguing in the context of circadian rhythms and depression because sleep deprivation, without vigorous exercise, is a well-known antidepressant treatment (Leibenluft & Wehr, 1992). Whether circadian phase in humans is shifted by this treatment, independently of changes in light exposure, is, to our knowledge, unknown.

One lab has consistently reported small (~30 min) but significant phase advance shifts in hamsters in response to intraperitoneal injections of saline late in the rest period (Hastings et al., 1995). These phase shifts were associated with cortisol release in response to the injection procedure, suggesting that the shifts might be caused by a stress response. Cortisol release is probably not necessary for phase shifts in response to sleep deprivation in hamsters, because the phase shifts are not affected by pretreatment with the corticosteroid synthesis inhibitor metyrapone (Mistlberger et al., 1999). This result is consistent with an unpublished report that phase shifts in response to activity-inducing stimuli are not prevented by adrenalectomy (cited in Van Reeth et al., 1991).

If nonspecific arousal, rather than 'exercise' per se, accounts for phase shifts that follow 3 h of wheel running in the usual rest phase, then what is the explana-

tion for the association between τ or the phase angle of photic entrainment and the level or distribution of wheel running during the usual wake phase? One possibility is that access to running wheels alters the timing of the sleep-wake states (e.g., wheel running may promote consolidation of sleep and wake states during their respective phases; Welsh et al., 1988) or the level of 'excitation' (arousal) during the wake state (as first suggested by Aschoff, 1960). These interpretations remain to be directly tested.

In the remainder of this paper, we will review our studies of circadian clock resetting by serotonergic stimuli, and our tests of the hypothesis that 5-HT mediates the clock resetting effects of behavioral activity induced by confinement to novel wheels or treadmill devices. We have only begun to extend these tests to the sleep deprivation model of nonphotic phase resetting, but expect that this model will prove informative in further evaluating the relation between 5-HT transmission and behavioral regulation of circadian phase.

FUNCTIONAL CORRELATES OF SEROTONIN ACTIVITY: *IN VIVO* NEUROCHEMISTRY

In vivo electrophysiological and neurochemical assessments support a role for raphe 5-HT in the regulation of circadian rhythms by activity or arousal. A variety of studies have demonstrated a strong relationship between behavioral state and activity of the central 5-HT system (Schwartz et al., 1989; Wilkinson et al., 1991; Linthorst et al., 1995; Mendlin et al., 1996; Rueter & Jacobs, 1996; Jouvet, 1999). These studies have revealed that, in rats, neuronal 5-HT release in many brain regions is increased during arousal associated with alert waking, locomotion, postural adjustments, feeding and stressors. In several areas innervated by the dorsal raphe (DRN) or median raphe (MRN), the magnitude of the 5-HT release in aroused animals appears to be independent of the specific behaviors elicited during the test (Rueter & Jacobs, 1996). However, within the rat SCN, one study reported that 5-HT levels are positively correlated with locomotor activity, suggesting that processing of information within the SCN may be influenced by the intensity of physical activity (Shioiri et al., 1991). Other studies in nocturnal rodents have revealed pronounced circadian fluctuations in central 5-HT release in the cerebellum (Mendlin et al., 1996), hippocampus, striatum, amygdala, frontal cortex (Rueter & Jacobs, 1996) and SCN (Shioiri et al., 1991; Glass et al., 1992; Dudley et al., 1998), with highest levels associated with the time of the subjective LD transition, when locomotor activity is usually maximal.

More recent studies in hamsters have revealed that 5-HT release in the SCN is elevated by ~50% during 3 h of wheel running or 3 h of sleep deprivation, without running, in the middle of the usual rest phase (Dudley et al., 1998; Glass, unpub-

lished observations, 1999; Fig. 6). This indicates that the contribution of running to 5-HT release in the SCN is probably secondary to arousal. Sleep deprivation in rats has also been shown to increase the activity of midbrain raphe 5-HT neurons, at least in part by desensitization of inhibitory somatodendritic 5-HT_{1A} autoreceptors (Maudhuit et al., 1996; Prévot et al., 1996; Gardner et al., 1997; Schreiber et al., 1997). In hamsters, 6 h of sleep deprivation can significantly attenuate the phase shifting effects of light pulses early in the night (Mistlberger et al., 1997a), a result that is consistent with other work, discussed further below, showing that 5-HT inhibits circadian responses to light (e.g., Selim et al., 1993; Glass et al., 1994; Rea et al., 1994). These studies, in aggregate, provide a solid foundation of correlational evidence consistent with the 5-HT hypothesis of behaviorally-induced phase shifting.

A causal relationship between behavioral state and the circadian 5-HT rhythm has long been a matter of speculation; i.e., whether the 5-HT rhythm is driven by behavior or vice versa. Another alternative is that behavioral state and 5-HT

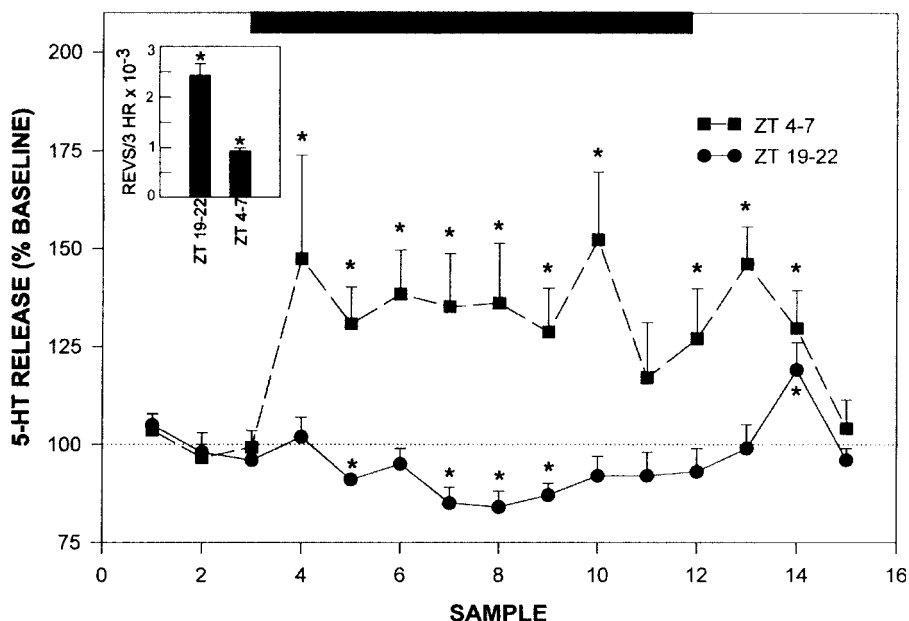


Fig. 6. Profiles of novel wheel-running-induced alterations in 5-HT release from the SCN region during midday (ZT4-7) and during the late dark phase (ZT19-22). Solid bar denotes the 3 h period of confinement in the wheel. * $p < 0.05$ relative to pre-running baseline. Data from all animals in each group are included irrespective of running intensity. Inset: Bars represent total number of revolutions over the 3 hr wheel-running period for both groups. * $p < 0.05$ between groups. Figure re-plotted from Dudley et al. (1998).

activity are both driven by and in turn modulate the circadian clock in the SCN (see Fig. 1). Data derived from studies of cats suggest that the firing rate of neurons in the DRN is determined primarily by behavioral state, and not the LD cycle (Trulson & Jacobs, 1983). On the other hand, diurnal changes in somatodendritic 5-HT_{1A} autoreceptor-mediated response may also be important in regulating the circadian changes in 5-HT tonus, at least in the SCN (Dudley et al., 1998). This contention follows from the finding that systemic application of the mixed autoreceptor agonist, BMY7378, has a substantially greater suppressive effect of on SCN 5-HT release during the night, compared to the day.

In that study the existence of a pronounced time-of-day effect of behavioral state on the activity of the serotonergic system was also demonstrated. Novelty-induced wheel running during the day significantly enhanced SCN 5-HT release, whereas running at night caused a small suppression of 5-HT release (Fig. 6). From these observations, it can be concluded that the normal daily rhythm of central 5-HT release, which is strongly influenced by behavior in a phase-dependent manner, is also organized by an underlying circadian variation in raphe somatodendritic autoreceptor responsiveness. Our recent findings that the respective inhibitory and stimulatory effects of the 5-HT_{1A} autoreceptor agonist, 8-hydroxy-2-(di-n-propylamino)tetralin (8-OH-DPAT), and antagonist WAY100635, on 5-HT release in the SCN are significantly greater when microinjected into the MRN, compared to the DRN, indicate that 5-HT_{1A} autoreceptors on MRN neurons provide the predominant raphe autoregulation of 5-HT output in the SCN (Dudley et al., 1999).

These results have therapeutic implications, in that the effects of behavioral or other related approaches to manipulating the serotonergic pathway to the SCN may be highly phase-dependent. With regard to involvement of 5-HT in circadian phase-shifting, as discussed below in this review, it is intriguing that the timing of the greatest stimulatory effect of wheel-running on 5-HT release (mid-subjective day, ~ZT 4-7) overlaps with the phase of greatest phase-advancing action of induced activity. Conversely, the phase of activity-induced suppression of 5-HT release corresponds to the time when activity reportedly induces small phase-delays (Mrosovsky et al., 1989; Bobrzynska & Mrosovsky, 1998). This temporal correlation strengthens the association between raphe activity and nonphotic phase shifts, but also demonstrates that enhanced release of 5-HT within the SCN may not be necessary for nonphotic shifts at some circadian phases. These results suggest that phase advances and delays induced by behavioral activity may be mediated, at least in part, by different mechanisms.

SEROTONIN AND NONPHOTIC PHASE RESETTING: LESION AND DEPLETION STUDIES

The relationship between raphe 5-HT neuronal activity and behavior provides strong circumstantial evidence supporting a role for 5-HT in the regulation of circadian phase and τ by locomotor activity or arousal. If 5-HT release in the SCN pacemaker is necessary for inducing nonphotic phase shifting, then procedures that reduce 5-HT levels in the SCN should attenuate or prevent phase and τ responses to activity. The effects of a reduction of 5-HT on nonphotic phase shifting have now been assessed in two species, using three different procedures for inducing shifts and four different procedures for reducing 5-HT levels. In hamsters, the phase shifting effects of triazolam injections are dependent on the expression of locomotor activity, and these shifts are attenuated or blocked by depletion of forebrain 5-HT with the 5-HT neurotoxins 5,7-dihydroxytryptamine (5,7-DHT) or p-chloroamphetamine (Cutrera et al., 1994a; Penev et al., 1995a; Meyer-Bernstein & Morin, 1998). However, hypothalamic 5,7-DHT lesions do not affect phase shifts induced by running in a novel wheel (Bobrzynska et al., 1996; Meyer-Bernstein & Morin, 1998). Another recent study used a tryptophan-free diet to reduce 5-HT release (Antle & Mistlberger, unpublished, 1999). This diet potentiated phase delay shifts to light pulses early in the subjective night, a result consistent with reduced 5-HT release in the SCN, but the diet did not affect phase shifts induced by 3 h of sleep deprivation in the mid-subjective day. However, the 5-HT synthesis inhibitor p-CPA, combined with the 5-HT_{2/7} antagonist ritanserlin (discussed further below), did significantly attenuate phase shifts to 3 h of SD (Antle & Mistlberger, unpublished observations, 1999).

In mice, two studies have reported that hypothalamic 5,7-DHT lesions eliminate entrainment to either restricted daily access to a home cage wheel or forced running in a treadmill (Edgar et al., 1997; Marchant et al., 1997; Fig. 4b). Hypothalamic 5,7-DHT lesions also eliminated the τ -shortening effect of spontaneous wheel activity in free-running mice (Mistlberger et al., 1998).

The interpretation of lesion studies can be complicated by the ability of neurochemical systems to compensate for extensive damage if even just a fraction of the normal complement of cells or terminals survive. Catecholamine systems are a well-known case in point; lesions often have to be near-complete before behavioral output is impaired. One indication that this may also be true of 5-HT in the circadian system comes from studies showing that 5-HT lesions of the SCN, induced by forebrain or midbrain 5,7-DHT infusions, cause a phase advance of activity onset in LD, but only if the loss of 5-HT fibers in the SCN is virtually complete (Morin, 1992; Meyer-Bernstein et al., 1997). A phase advance of nocturnal activity onset may therefore represent a behavioral marker for complete SCN 5-HT denervation. If so, then the results of the Bobrzynska et al. (1996)

study need to be interpreted with caution, because hamsters treated with 5,7-DHT in that study did not exhibit a phase advance of activity onset in LD. On the other hand, the null effect of these lesions on phase shifts to novelty-induced running is consistent with the results of Meyer-Bernstein & Morin (1998), a study in which 5,7-DHT lesions did phase advance activity onset in LD and also eliminated phase shifts to triazolam injections.

A related pitfall with the lesion method is recovery of function over time. The 5-HT system is well known to adapt to experimental perturbations, by pre- and post-synaptic mechanisms (e.g., Azmitia et al., 1978; Blier & de Montigny, 1985; Berendsen et al., 1991; Morin, 1992). However, this is not necessarily associated with recovery of circadian function (e.g., Meyer-Bernstein et al., 1997). In addition, 5,7-DHT lesions that eliminate source neurons in the brainstem are associated with little if any regrowth of fibers into denervated targets (Meyer-Bernstein & Morin, 1996). By scheduling behavioral tests relatively soon after lesions are made, the likelihood of functional recovery is further minimized (Morin, 1992).

A third interpretive issue associated with the 5-HT lesion and depletion approaches concerns the effects of these interventions on the locomotor or arousal response to the experimental activating stimulus. In 5-HT depletion studies that utilized triazolam to stimulate activity, examination of the published material suggests that loss of phase shifting could be secondary to a reduced locomotor response to the drug (Cutrera et al., 1994a; Penev et al., 1995a). Similarly, we found that hamsters treated with p-CPA in combination with ritanserin were very difficult to keep awake (Antle & Mistlberger, unpublished observations, 1999). The attenuating effects of these drugs on phase shifts to sleep deprivation could thus be secondary to an impaired arousal response.

The two mouse 5,7-DHT studies are spared these methodological concerns, as both reported loss of entrainment following 5-HT lesions, and in neither study did the lesions reduce the amount of activity expressed at the scheduled activity time (Edgar et al., 1997; Marchant et al., 1997). Indeed, the treadmill method was expressly adopted to circumvent this potential confound. However, in at least one of the studies, there is evidence that τ of the free-running rhythm was modulated by the daily running schedule, suggestive of a weak residual zeitgeber effect (Marchant et al., 1997). This could be due to incomplete SCN 5-HT denervation, although the very high dose of 5,7-DHT employed and the complete absence of 5-HT immunoreactivity (IR) in the SCN and surrounding anterior hypothalamus in these mice make this an unlikely interpretation.

Serotonin depletion does not reduce the average level of spontaneous wheel running in mice (Edgar et al., 1997; Marchant et al., 1997). However, in our study of τ modulation by wheel access, mice with 5,7-DHT lesions exhibited an altered distribution of activity within α , with more activity concentrated late in the animal's subjective night, compared to intact mice (Mistlberger et al., 1998). When

the distribution of activity is skewed in this direction in intact mice, τ tends to be longer (Edgar et al., 1991). Logically, this altered distribution of activity could obscure the τ -shortening effect normally associated with wheel access in mice. Thus, it is conceivable that 5,7-DHT lesions prevented τ -shortening in response to wheel access by altering α waveform, rather than by interrupting a 5-HT pathway hypothesized to mediate feedback from behavior to the SCN pacemaker.

Taken together, the weight of evidence from lesion studies provides qualified support for the hypothesis that 5-HT plays a role in the regulation of circadian phase and τ by behavioral activity. However, the results are short of definitive. The two mouse studies provide consistent findings, while results from hamster studies employing different activity-inducing stimuli are much more variable.

One way to organize this corpus of data is by species. The data imply that 5-HT may be more critical for behaviorally-induced phase resetting in mice than in hamsters. However, even in the mouse, 5-HT release within the SCN pacemaker is not sufficient to mediate behaviorally-induced shifts, because lesions of the intergeniculate leaflet (IGL), which eliminate neuropeptide Y (NPY) -IR but not 5-HT-IR within the SCN, also eliminate entrainment to daily treadmill schedules in mice (Marchant et al., 1997). It would appear that both of these afferent systems are necessary for nonphotic entrainment in mice. The role of NPY in nonphotic phase resetting, and possible interactions with 5-HT, are considered further below and in a companion review by Prosser (this issue).

Another way to organize the results from 5-HT ablation studies is by the stimulus used to induce activity. Phase shifts induced by triazolam are consistently blocked by 5-HT lesions, while shifts induced by confinement to novel wheels have not been blocked by these lesions. Lesions of the superior colliculus have also been reported to block phase shifts to triazolam but not to running in novel wheels (Marchant & Morin, 1998), providing additional support for the idea that, at least in hamsters, different nonphotic stimuli may access the circadian system via at least partially distinct pathways. In the interest of preserving parsimony in our physiological model of nonphotic phase resetting, it will be important to understand more clearly the basis for this differential effect of 5-HT and collicular lesions on phase shifts to different locomotor-activating stimuli.

SEROTONIN PHARMACOLOGY: ANTAGONISTS

Drugs that block post-synaptic 5-HT receptors provide another means to evaluate the contribution of 5-HT to activity-induced phase shifts. Several pharmacological antagonists have been screened in the novel wheel confinement model of nonphotic phase shifting. Drugs tested to date include 1. WAY100135 (10 & 15 mg/kg s.c.) and NAN190 (5 & 10 mg/kg i.p.), both of which block postsynaptic 5-

HT_{1A} receptors and reduce 5-HT release by activating presynaptic 5-HT_{1A} autoreceptors, 2. metergoline (2 mg/kg i.p.), which blocks 5-HT_{1/7} receptors and, 3. ritanserin (2 & 4 mg/kg i.p., and 1, 2, 8, 16 & 32 nmol intra-SCN), which blocks 5-HT_{2/7} receptors. None of these functional antagonists significantly attenuated phase shifts induced by wheel running during the middle of the subjective day, and none were found to induce phase shifts (Antle et al., 1998b). In one other study, the 5-HT_{2/7} antagonists ketanserin and ritanserin both significantly attenuated the small phase advances observed following saline injections, but neither drug eliminated these shifts at any of the doses tested (Sumova et al., 1996). To our knowledge, there are no published reports on the effects of 5-HT receptor antagonists on the phase shift response to triazolam.

These results fail to support the hypothesis that 5-HT transmission mediates activity-induced phase shifts in hamsters. One possibility is that the tested drugs target the wrong 5-HT receptors. However, as discussed below and in a companion review (Prosser, this issue), activation of 5-HT receptors in the SCN induces phase advance shifts of the circadian rhythm of SCN neuronal activity *in vitro*, with a phase dependency that roughly matches shifts induced by activity *in vivo*. These advance shifts are blocked by antagonists of the 5-HT₇ receptor (including ritanserin and metergoline; Lovenberg et al., 1993). Thus, if phase advance shifts induced by behavioral activity are mediated by 5-HT receptors in the SCN, then the most likely target receptor is the 5-HT₇ subtype, and both ritanserin and metergoline, in that order of potency, should be effective antagonists. Phase delay shifts induced by 5-HT agonists *in vitro* appear to involve a different 5-HT receptor (Lovenberg et al., 1993), but 5-HT antagonists have not yet been tested against the phase delay portion of the nonphotic phase-response curve (PRC) in hamsters.

Assuming that the selection of drugs was appropriate, another methodological issue is whether an adequate range of doses was tested. Relative to other studies that have used these drugs to demonstrate serotonergic mediation of specific brain functions in rodents, the doses used ranged from moderate to very high (for references, see Antle et al., 1998b). The use of even higher doses is made problematic by possible nonspecific receptor binding and by attenuation of the behavioral response to the stimulus used to induce activity or arousal.

A related concern is accessibility of the drug to SCN synapses. Post-synaptic receptors are, by definition, interior to the synapse, and the terminal may be further "insulated" by surrounding astrocytes. In contrast, non-synaptic receptors such as metabotropic glutamate receptors, melatonin receptors, peptide receptors, and some terminal presynaptic receptors may be much more accessible to locally administered agents (Zoli et al., 1999). Receptor accessibility is also influenced by the lipophilicity of the administered agent. Peptides, for example, are much more lipophilic than 5-HT. Thus, it may be that some drugs, even when microinjected into parenchyma close to the SCN, do not gain ready access to the relevant

5-HT receptors within the SCN. SCN neurons *in vitro* are readily activated by iontophoresed 5-HT, which would seem to argue against this notion (e.g., Miller & Fuller, 1990), but it should be remembered that iontophoresis is performed only when a piece of neuronal membrane is adjacent to the recording pipette. In the typical multibarrel pipette, the drug administration barrel is within a micron of the recording tip. If a neuron is unresponsive to drug, the driving current is usually increased until a response is observed. Thus the iontophoretic technique selects for those neurons that are responsive to a given drug, sometimes requiring much higher local concentrations than are reached even with an intra-SCN cannula.

Receptor accessibility is also influenced by competition between exogenous drug and endogenous 5-HT; if the 5-HT system is driven at high levels during a bout of wheel running (Dudley et al., 1998), it may be difficult for competitive antagonists like metergoline and ritanserin to block enough receptors to prevent 5-HT-mediated effects. This concern does not apply to NAN190 or WAY100135, both of which mimic the autoinhibitory effects of endogenous 5-HT on 5-HT release, or to tryptophan depletion, which decreases endogenous 5-HT synthesis and release. Both NAN190 (Rea et al., 1995) and tryptophan depletion have been shown to potentiate phase shifts to light pulses (Antle & Mistlberger, unpublished, 1999), which confirms that these procedures cause functionally important changes in 5-HT release in the SCN, where this transmitter normally inhibits neuronal responses to light (e.g., Selim et al., 1993; Glass et al., 1994; Rea et al., 1994; Glass et al., 1995). Thus, regardless of methodological issues associated with the other antagonists, the failure of NAN190 and tryptophan depletion to attenuate nonphotic phase shifts in hamsters complicates any straightforward attribution of these shifts to 5-HT release in the SCN.

SEROTONIN PHARMACOLOGY: AGONISTS

If 5-HT mediates phase shifts induced by exercise or arousal, then 5-HT agonists should mimic these shifts. In hamsters, systemic injections of the 5-HT_{1A/7} agonist 8-OH-DPAT during the middle of the subjective day do induce phase advance shifts (Tominaga et al., 1992; Cutrera et al., 1994b; Bobrzynska et al., 1996; Cutrera et al., 1996; Penev et al., 1997). These shifts, averaging about 60–90 min across studies, are considerably smaller than those induced by 3 h bouts of locomotor activity or sleep deprivation, but are not far off the range of shifts induced by 1 h bouts of wheel running in hamsters housed without access to home cage wheels (Wickland & Turek, 1991), and are somewhat larger than those induced by saline injections (Hastings et al., 1995).

Studies utilizing intracerebral 8-OH-DPAT injections have had more variable outcomes across laboratories. One study reported significant, albeit small (20–30 min) phase advances in response to bilateral 8-OH-DPAT injections just dorsal to

the SCN (Challet et al., 1998), while another study reported no significant shifts following unilateral 8-OH-DPAT injections into the SCN area (Mintz et al., 1997). In one study, we also failed to detect significant shifts in response to peri-SCN injections of 8-OH-DPAT (Ehlen et al., 1999). However, we were able to significantly attenuate light-induced phase shifts by 8-OH-DPAT injections through the same cannula. This indicates that the drug did have functional effects bilaterally in the SCN. Nonetheless, these effects may have been at presynaptic sites on RHT terminals, which may be more accessible than postsynaptic receptors on putative clock cells.

Autoradiographic analysis of the distribution of labeled 8-OH-DPAT confirms that the drug spreads throughout both SCN following unilateral injections just dorsal to the SCN (Weber et al., 1998). Therefore, it is unlikely that different results across labs are attributable to the use of unilateral as opposed to bilateral infusion procedures. In fact, in two of the three studies, the mean shifts were nearly identical (Mintz et al., 1997; Challet et al., 1998); significance was obtained in one study, despite smaller group sizes ($n = 4 - 6$), because of lower variance in the data. Complicating matters, in an ongoing follow-up study, we have observed some substantial phase advance shifts (as large as 98 min) following single, unilateral peri-SCN injections of 8-OH-DPAT at doses that previously did not produce mean phase shifts significantly greater than vehicle injections (Antle & Mistlberger, unpublished observations, 1999). This raises concerns that the two studies known to us that failed to detect phase shifts in response to peri-SCN injections of 8-OH-DPAT may have been compromised by inadequate statistical power.

In the SCN slice *in vitro*, 8-OH-DPAT administered in the projected mid-subjective day consistently induces very large phase advances that exceed in magnitude even those typically induced by behavioral activation *in vivo* (see Prosser, this issue). Failure of either systemic or peri-SCN injections of 8-OH-DPAT to induce similarly large shifts *in vivo* could result from one or more of several factors. One potential factor is the duration of 5-HT receptor stimulation. Single injections of 8-OH-DPAT administered *in vivo* may result in a shorter period of receptor activation, compared to the 1 h drug applications typically used in the *in vitro* slice preparation and the 3 h of activity typically used in behavioral studies. However, even 5 min applications of 5-HT *in vitro* are sufficient to induce large shifts of SCN neuronal activity. In addition, injections of 8-OH-DPAT can block phase shifts to a light pulse that follows as much as 1 h later (Antle & Mistlberger, unpublished, 1998). This suggests that the duration of stimulation is probably not the primary explanation for differences in the size of serotonergic phase shifts *in vivo* and *in vitro*.

Another possibility is that deafferentation of the SCN during slice preparation alters the sensitivity of the SCN to 8-OH-DPAT, due to rapid 5-HT receptor upregulation or loss of non-serotonergic inputs that might normally inhibit SCN

responses to 5-HT (e.g., glutamate and NPY; see Biello et al., 1997; Prosser, 1998 and this issue). A third possibility is that slice preparation alters the accessibility of 5-HT receptors to exogenous drug, due to loss of innervating fibers.

Direct tests of these potential explanations are lacking. One recent study has reported that phase shifts to systemic 8-OH-DPAT are reduced in hamsters following chronic exposure to the monoamine oxidase inhibitor clorgyline (Duncan et al., 1999). This result is consistent with the idea that the number or affinity of 5-HT receptors that influence circadian function might be altered by changes in the level of endogenous serotonergic activity. We are further testing these hypotheses in two ways.

First, to circumvent the issue of receptor accessibility, we have used procedures to enhance release of endogenous 5-HT. Systemic and intra-raphé injections of the selective 5-HT_{1A} antagonist WAY100635 stimulate 5-HT release by blocking raphe 5-HT_{1A} inhibitory autoreceptors (Mundey et al., 1996). We found using microdialysis that systemic doses of .45 or 4.5 mg/kg raise SCN 5-HT release by ~50% for 20–40 min, a magnitude comparable to that induced by wheel running during the mid-subjective day (Antle et al., 1998a; Dudley et al., 1999). However, WAY100635 injections at this phase, at doses up to 10.0 mg/kg, did not induce significant phase shifts or potentiate phase shifts induced by running in a novel wheel (Antle et al., 1998a). The drug also blocks postsynaptic 5-HT_{1A} receptors, but *in vitro* studies, discussed further below, clearly show that these are not involved in serotonergic phase shifts, so postsynaptic receptor antagonism is unlikely to be a confound (Lovenberg et al., 1993).

Serotonin release can also be enhanced by the 5-HT precursor L-tryptophan (Fernstrom, 1981; Wurtman & Wurtman, 1995). A single bolus injection of tryptophan (150 mg/kg, *i.p.*) in the mid-subjective day in hamsters can significantly increase SCN 5-HT, after a delay of ~1 h, by $205 \pm 30\%$ of baseline for ~1 h, while single injections in the subjective night can significantly attenuate phase shifts to light pulses (Glass et al., 1995). However, 150 mg/kg L-tryptophan injections in the mid-subjective day did not induce significant phase shifts, with or without chronic (10 day) treatment with the 5-HT reuptake inhibitor fluoxetine (10 mg/kg), and did not potentiate phase shifts induced by running in novel wheels, in those hamsters that did run (few animals ran following tryptophan injections; Mistlberger et al, unpublished, 1997). Injections of fluoxetine (10 mg/kg) alone, repeated for 10 days, also did not induce shifts or potentiate shifts induced by wheel running in these hamsters.

The lack of effect of chronic treatments in hamsters may be a result of compensatory changes in the state of 5-HT receptors in the SCN or elsewhere, a possibility that needs to be tested given recent evidence that a variety of antidepressants, including fluoxetine, downregulate the 5-HT₇ receptor in the SCN (Mullins et al., 1999).

In contrast to these null effects in hamsters, there is one report that chronic fluoxetine can shorten τ in mice with free access to a running wheel (Possidente et al., 1992). This result is consistent with other evidence noted above that 5-HT transmission mediates the τ -shortening effect of wheel running in mice (Mistlberger et al., 1998). Clorgyline, another antidepressant that increases 5-HT levels also alters τ in hamsters, although this MAO inhibitor is not selective to 5-HT and is thus less informative about mechanism (Duncan et al., 1988).

The magnitude and duration of increased 5-HT release in the SCN in response to a single systemic injection of WAY100635 or tryptophan is comparable to that induced by 20-30 min of electrical stimulation of the MRN or DRN (Dudley et al., 1999). The effect of DRN stimulation on SCN 5-HT release is blocked by metergoline; this has been interpreted as evidence that the DRN can influence SCN 5-HT transmission by activation of 5-HT receptors in the MRN, either on 5-HT cells or on inhibitory interneurons. Electrical stimulation of the MRN using comparable stimulation parameters can induce small phase advance shifts in the subjective day in hamsters (Meyer-Bernstein & Morin, 1999). These drug and electrical stimulation results, taken together, indicate that the small phase shifts induced by electrical stimulation of the raphe may not be due to 5-HT release in the SCN. Shifts may instead depend on activation of fibers of passage or non-serotonergic raphe afferents, which account for about 50% of the total raphe projection to the SCN (R.Y. Moore, personal communication). GABA projections from the MRN to the SCN are one candidate.

To address the issue of duration of receptor stimulation, we are currently examining the effects of intra-MRN injections of WAY100635, repeated at 20 min intervals for 3 h during the mid-subjective day. This procedure does induce phase advance shifts, averaging ~80 min. However, we also observed ~70 min phase advance shifts to injections of the saline vehicle ($p > .1$). In addition, the size of the shifts induced by drug or vehicle injections correlated significantly with the amount of time that the animals were awake during the 3 h procedure, and in two animals, sham injections (no drug or vehicle) also produced ~70 min phase shifts. These results suggest that this procedure induces phase shifts by partial sleep deprivation. If phase shifts in response to sleep deprivation are mediated by 5-HT, then these shifts should be potentiated by drugs, like the autoreceptor antagonist WAY100635, that potentiate endogenous 5-HT. Thus, the failure of WAY100635 to induce shifts larger than those induced by saline or sham injections supports arguments against the 5-HT hypothesis of nonphotic phase shifting.

One caveat is that partial sleep deprivation induced by the drug injection procedure may result in maximal 5-HT release and receptor occupancy in the SCN, preventing any additional response to WAY100635. However, such a ceiling effect seems unlikely, given that the phase shifts induced by the partial sleep

deprivation were only half maximal, and that WAY100635 can significantly potentiate the equally substantial release of 5-HT induced by selective 5-HT reuptake inhibitors (Romero et al., 1996; Hjorth et al., 1997).

To address the possibility of 5-HT receptor supersensitivity in the deafferented SCN slice *in vitro*, the effects of intra-SCN infusions of 8-OH-DPAT, delivered for 3 h by reverse microdialysis during the mid-subjective day, are being examined in hamsters pretreated with the 5-HT synthesis inhibitor p-CPA. This procedure induces a mean phase advance shift of 105 ± 17 min, which, unlike the intraraphe WAY100635 injections, is statistically significant by comparison with saline infusions (-4 ± 14 min) and is not associated with significant behavioral arousal (Ehlen et al., 1999). In hamsters that were not pretreated with PCPA, 3 h 8-OH-DPAT perfusions produced phase-advances averaging only 22 ± 9 min ($p < 0.05$ vs. P-CPA+8-OH-DPAT group). These results provide the first empirical support for the idea that rapid induction of supersensitivity may contribute to the large phase shifts associated with 5-HT stimulation of the SCN slice *in vitro*.

In a parallel test of supersensitivity, the phase shifting effects of single systemic injections of 8-OH-DPAT are being examined in hamsters deprived of dietary tryptophan for 7 days (Mistlberger & Antle, unpublished, 1999). These animals exhibit an advance of the phase angle of photic entrainment, and potentiation of phase delay shifts to light pulses early in the subjective night. Both of these effects are characteristic of rodents sustaining reductions of SCN 5-HT induced by 5,7-DHT (Morin, 1992; Bradbury et al., 1997), suggesting that the diet does cause a functionally significant reduction in SCN 5-HT. However, no significant change in the size of phase advance shifts to systemic 8-OH-DPAT could be detected, suggesting that the dietary deprivation procedure was not sufficient to supersensitize the relevant 5-HT receptor.

The brainstem 5-HT system can influence SCN function directly, via the MRN, and indirectly, via DRN projections to the IGL and possibly other areas that project to the SCN (Meyer-Bernstein & Morin, 1996). Several recent studies provide convergent evidence that the indirect pathway may be involved in serotonergic effects on the circadian system. First, injections of 8-OH-DPAT directly into the DRN in the mid-subjective day can induce phase advance shifts that mimic those induced by systemic injections of the drug (Mintz et al., 1997). Second, phase shifts induced by 8-OH-DPAT are attenuated in aged hamsters (Penev et al., 1995b), and this correlates with a reduction in the number of 5-HT₇ receptors in the DRN (Duncan et al., 1999). No age-related changes in 5-HT₇ receptors were detected in other regions of the circadian system, including the SCN and the IGL, and no changes in 5-HT_{1A} receptors were detected in any area examined. In contrast, substantial changes in the density of 5HT7 and 5HT1A receptors in the aged rat SCN have been reported (Miller et al., 1997), suggesting once again that species differences may be important. Third, unlike 8-OH-DPAT,

which activates both 5-HT_{1A} and 5-HT₇ receptors, systemic injections of the selective 5-HT_{1A} agonist MKC-242 during the subjective night do not induce phase shifts (Moriya et al., 1998). Finally, electrical stimulation of the DRN in the subjective day induces small phase shifts and raises SCN 5-HT, and both effects are blocked by the 5-HT_{2/7} antagonists ritanserin and ketanserin, but not by the selective 5-HT_{1A} antagonist WAY100635 (DiNardo & Glass, 1999). Taken together, these results imply that phase shifts induced by systemic 8-OH-DPAT are mediated at least in part by a 5-HT_{2/7} receptor in the DRN or DRN efferent pathway rather than the SCN. Activation of this receptor may result in modulation of activity in areas that project to the SCN, such as the MRN and the IGL. This model predicts that phase shifts to systemic 8-OH-DPAT would be blocked by systemic or intra-MRN injections of ritanserin or ketanserin, but not by intra-SCN injections. This model also suggests that endogenous 5-HT release in the hamster SCN has relatively little direct effect on pacemaker phase (apart from modulation of RHT input to the clock), unless SCN 5-HT receptors are upregulated in some fashion. Whether or not this general model is correct, the results do underscore the possibility that exogenous serotonergic drugs may alter circadian phase by indirect, polysynaptic routes to the SCN pacemaker.

The pharmacological approach to elucidating the role of 5-HT within the circadian system is further complicated by evidence that there may be significant differences in responses to serotonergic drugs across species or different strains of the same species. One study utilizing Wistar rats bred in the U.S.A. reported that administration of 8-OH-DPAT or the general 5-HT agonist quipazine in the mid-subjective day induces phase advances of activity rhythms (Edgar et al., 1993). However, another study utilizing ACI, BH, and LEW rats bred in Europe reported that quipazine mimics the phase shifting effects of light on activity rhythms (Kohler et al., 1999; Wollnik, this issue), and several other studies using rats bred in Australia have reported that quipazine and agonists active at 5-HT_{2c} receptors mimic the phase shifting and acute suppressing effects of light on plasma melatonin levels (Kennaway et al., 1996; Kennaway & Moyer, 1998). In hamsters, systemic quipazine does not induce phase shifts (Bobrzynska et al., 1996). Thus, there is a surprising degree of variability in circadian responses to serotonergics when different agonists, species and strains are compared. In vitro studies might help to clarify the basis for these apparent species and strain differences, but so far virtually all of the in vitro work has utilized SCN slices from rats, and comparisons of different strains have not been made within any lab. This issue is discussed further by Wollnik (this issue).

Another notable limitation of the literature on serotonergics and circadian function is the lack of studies using primates. In a recent study using a diurnal primate, the common marmoset, phase shifts were assessed in response to systemic administration of 8-OH-DPAT or 2 h of activity and arousal by exposure to a

novel cage. Both treatments induced large phase delays when scheduled early in the subjective night, and large advances when scheduled late in the subjective night (Ney et al., 1998). These two-point PRC's thus mimic the phase shifting effects of light. Because marmosets cannot be studied in constant dark, light contamination during the nonphotic manipulation is a concern, but preliminary assessments suggest that the shifts are not caused by increased light exposure. These results are intriguing for two reasons. First, they suggest that arousal and serotonergic drugs have chronobiotic potential for primates. Second, they raise the possibility that in diurnal animals, light might shift the clock, in part, because it induces arousal.

The results of pharmacological studies clearly do not yet form a cohesive story. In hamsters, drug procedures that enhance endogenous 5-HT release have not yet been demonstrated to induce phase shifts, while procedures that deliver a 5-HT_{1A/7} agonist directly to the SCN typically induce only small or statistically unreliable shifts unless the 5-HT system is first sensitized by 5-HT depletion. In rats, 5-HT_{1A/7} agonists consistently induce 'nonphotic' type phase shifts of SCN neural activity rhythms *in vitro*, but the results from *in vivo* studies have differed widely across labs using different rat strains or species, with some labs reporting 'nonphotic' PRCs, and others reporting 'photic' PRCs to the same classes of drug. Initial studies in a diurnal primate model suggest a 'photic' PRC for 5-HT agonists. Considerably more work is needed to elucidate the bases for these surprisingly diverse results.

SEROTONIN AND BEHAVIORAL ZEITGEBERS: POSSIBLE CELLULAR MECHANISMS

Behavioral and *in vivo* pharmacological studies raise many questions about the role of endogenous 5-HT release in the SCN. Further insights may be gained by analysis of the intracellular transduction mechanisms that mediate the actions of 5-HT and behavioral zeitgebers on SCN clock cells. Although relatively little work has been done on transduction mechanisms mediating the effects of behavioral zeitgebers, considerable progress has been made with respect to 5-HT. The *in vitro* slice preparation has been the model of choice for this analysis. Given the preliminary evidence that supersensitivity, at least in some species, may be necessary to see significant phase shifts to 5-HT agonists *in vivo*, the slice preparation, due to its deafferented state, may be criticized as overly 'non-physiological'. However, there may be circumstances *in vivo* where 5-HT supersensitivity does occur, and thus the slice preparation may be a good model for these conditions. In addition, supersensitivity *in vitro* allows strong perturbation of intracellular transduction pathways, resulting in large, easily detected pacemaker responses. The

method of strong perturbation can be very useful for determining the basic operating parameters of an uncharacterized system. The assumption of strong perturbation studies is that the response is linearly related to the stimulus strength; weaker and more physiological stimuli will have qualitatively similar effects, but those effects will necessarily be more difficult to detect. Of course, this assumption must be tested, but at first approximation, strong perturbation can reveal many properties of a physical or biological system.

The earliest electrophysiological studies of cellular responses to 5-HT in the SCN were conducted *in vivo* using anesthetized rats. These studies showed that many SCN neurons are responsive to both light and 5-HT agonists, e.g., quipazine (Miller & Fuller, 1990). The sign and magnitude of these single unit responses were not correlated, and the photic responses were not blocked by the general 5-HT antagonist metergoline, at least when administered in the subjective day. These results indicate that 5-HT does not mediate neuronal responses to photic input in the SCN in the subjective day. Pharmacological studies have since shown that 5-HT input to the SCN potentially inhibits retinohypothalamic activation of SCN neurons, in part via a presynaptic 5-HT_{1B} heteroreceptor on the RHT terminals (Selim et al., 1993; Pickard & Rea, 1997). A role for the 5-HT₇ receptor in modulating the response of SCN neurons to light is also probable, although it is not clear whether 5-HT₇ effects are entirely postsynaptic or also involve a presynaptic component (Pickard & Rea, 1997; Ying & Rusak, 1997).

Subsequent studies using the slice preparation have shown that 5-HT can reset the clock independently of photic inputs. This evidence is reviewed briefly here, and in more detail in Prosser (1999, this issue). The general agonist quipazine phase advances the circadian rhythm of SCN neural activity when applied to the slice in the subjective day, and phase delays the rhythm when applied in the subjective night (Prosser et al., 1990). The advances can be mimicked by agonists known to be active at the 5-HT_{1A} receptor (8-OH-DPAT, RU24969, buspirone) and blocked by the general 5-HT antagonist metergoline and the selective 5-HT_{1A} antagonist, NAN-190, suggesting mediation by the 5-HT_{1A} receptor subtype. Although autoradiographic studies show 5-HT_{1A} receptors in the SCN (Prosser et al., 1993), 5-HT_{1A} mRNA is extremely sparse (Roca et al., 1993). Furthermore, phase advances induced by 8-OH-DPAT are qualitatively similar to phase advances produced by cAMP derivatives, whereas the classical 5-HT_{1A} receptor is known to lower cAMP.

The subsequent discovery of the 5-HT₇ receptor resolved this seeming paradox (Erlander et al., 1993). This receptor is abundant in the SCN at the mRNA level (M.A. Rea, personal communication, 1996) and at the protein level. Furthermore, 5-HT₇ has sufficient affinity for 8-OH-DPAT, metergoline, NAN-190 and buspirone to explain the phase advances observed (Lovenberg et al., 1993), and potentially elevates adenylate cyclase activity and cAMP in contrast to 5-HT_{1A}. But

most importantly, 8-OH-DPAT phase advances in the SCN slice are potently antagonized by the 5-HT_{2/7} antagonist, ritanserin, and not by pindolol, a 5-HT_{1A} antagonist with little or no affinity for 5-HT₇. Thus, the available data suggest that the serotonergic phase advance in the SCN slice is solely mediated by the 5-HT₇ receptor.

Interestingly, while metergoline administered alone did not produce phase shifts in mid-subjective day, the drug did produce phase delays when administered at CT12 (the beginning of the subjective night), suggesting considerable 5-HT tonus at that time, even in the deafferented slice. However, at other times in the subjective night, 5-HT receptor-specific agonists currently available do not generate phase delays. It is not known what type of 5-HT receptor is responsible for the phase delays that can be induced by the general agonist quipazine.

One of the continuing difficulties in this work is the lack of truly selective 5-HT₇ agonists and antagonists. However, recently two new 5-HT₇ antagonists have been developed (DR4004, SB258719; Forbes et al., 1998; Thomas et al., 1998; Kikuchi et al., 1999). Both compounds potently antagonize adenylate cyclase activity elevated via the 5-HT₇ receptor and DR4004 blocks serotonergic phase shifts *in vivo* (Miller, unpublished, 1999). Another promising approach is the application of 5-HT₇ receptor antisense which may be used to "knock down" the expression of the 5-HT₇ receptor and prevent 5-HT₇-mediated elevations in cAMP (Shimizu et al., 1996). It will be interesting to evaluate these methods in the behavioral phase shifting paradigms.

Regarding signal transduction, there is general agreement that the 5-HT₇ receptor elevates cAMP in SCN neurons. The 5-HT₇-mediated phase advance *in vitro* is blocked by inhibitors of cAMP (RpcAMPS) or cAMP-dependent protein kinase A (Prosser et al., 1994). This suggests that some phosphorylation substrate is essential for the 5-HT₇ phase advance. One such candidate is the calcium/cAMP response element binding protein (CREB). This transcription factor is

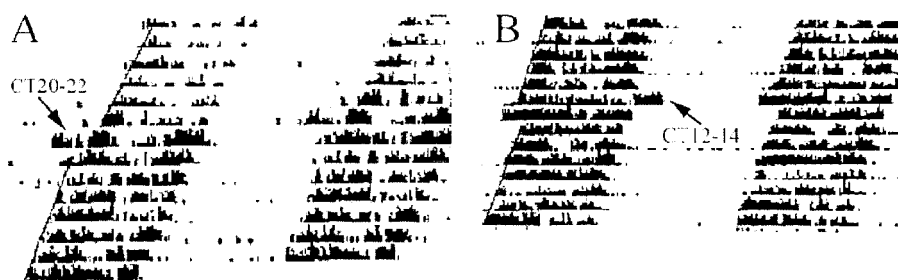


Fig. 7. Locomotor activity of marmosets in constant light, measured by telemetry. A 2 h bout of activity stimulated by confinement to a novel cage from CT20-22 induced a phase advance shift (A), while a 2 h bout from CT12-14 induced a phase delay shift (B).

translocated into the nucleus following phosphorylation by kinases such as PKA and CaM Kinase. However, to date there is no evidence that CREB is phosphorylated following 8-OH-DPAT administration in the middle of the subjective day. Furthermore, the downstream transcription factors *c-fos* and *junB*, which are thought to be activated by P-CREB, are actually reduced by serotonergic stimulation in the SCN slice (Prosser et al., 1994b). Finally, basal *c-fos* levels are low at CT15 in the SCN, a phase when substantial amounts of endogenous 5-HT are being released in the SCN. These data argue against an important role for P-CREB, *c-fos*, and *junB* in the 5-HT₇ signal transduction pathway. The same may also be true for phase shifts induced by activity, given that running in a novel wheel is associated with 5-HT release in the SCN and with a reduction in *fos* protein in the SCN (Mikkelsen et al., 1998). Delineation of the actual 5-HT₇-associated phosphorylation substrates will clarify the function of the 5-HT₇ receptor as well as fill in some of the gaps between receptor activation and modulation of the endogenous clock proteins thought to be at the heart of the circadian oscillator in the SCN.

It is interesting to note that an activator of adenylate cyclase (forskolin) and an inhibitor of the phosphodiesterase that breaks down cAMP (rolipram) are both antidepressants. Forskolin is also known to phase shift single unit rhythms in the SCN slice. Clinical studies show that simultaneous administration of a 5-HT_{1A} antagonist (pindolol) accelerates the therapeutic action of antidepressants (Blier et al., 1997). This may be because pindolol blocks raphe autoreceptors, thereby permitting antidepressants to significantly increase 5-HT release in the forebrain. However, pindolol should also block the SCN 5-HT_{1A} receptors that ordinarily depress cAMP. This would enable the antidepressant-induced elevation of synaptic 5-HT to act more selectively on 5-HT receptors known to elevate cAMP. Such an interpretation suggests that a selective and lipophilic 5-HT₇ agonist might be a particularly potent and fast-acting antidepressant.

Blockade of potassium channels with BaCl₂, apamin, and charybdotoxin antagonizes 5-HT₇-mediated phase advances in the SCN slice. BaCl₂ and apamin also block cAMP phase advances and a potassium channel ionophore, valinomyacin, itself produces phase advances (Prosser et al., 1994). These data suggest that the opening of a potassium channel, probably a calcium-dependent potassium channel, is essential for the 5-HT₇ phase advance. Indeed, in cultured SCN neurons, we have recently shown that a calcium-dependent potassium conductance is activated by 5-HT₇ receptor occupation (Miller, unpublished, 1999; Figure 8). An important qualifier here is that both cAMP and potassium channel opening appear to be essential, because cAMP block prevents the phase shift seen with the potassium ionophore. One possible explanation of this dual requirement may be that the hyperpolarization produced by a potassium channel opener prevents the activity of calcium-dependent phosphatases on substrates phosphorylated by endog-

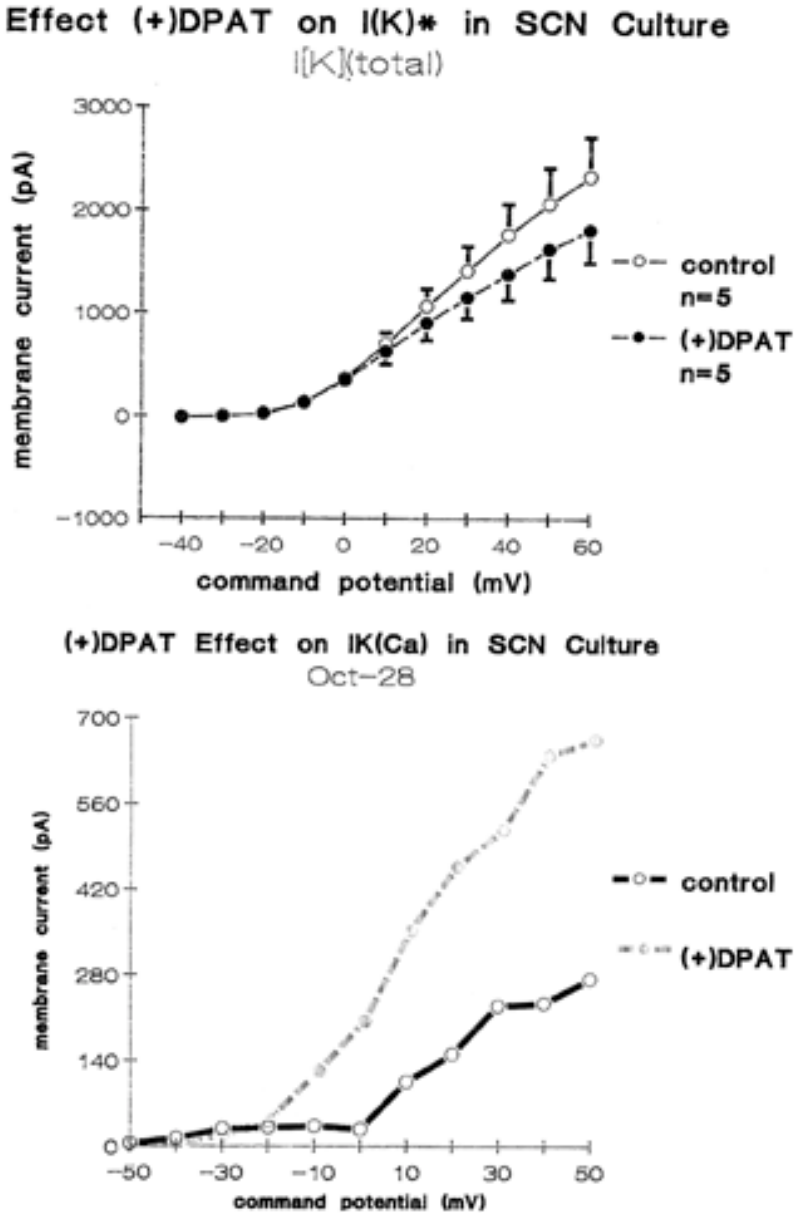


Fig. 8. The top panel of this figure demonstrates that (+) DPAT (5 μ M) administered to cultured SCN neurons under whole cell voltage clamp conditions produces a consistent but non-significant reduction in total potassium current at relatively depolarized membrane potentials. In contrast, the bottom panel shows that the calcium-sensitive portion of the total potassium current (isolated by replacing normal medium with zero calcium medium or calcium channel blockade) is greatly elevated by application of (+) DPAT in the medium. This effect has been seen repeatedly in cultured SCN neurons and in 5HT7-transfected CHO cells.

enous PKA. Similarly, potassium channel block will depolarize neurons and facilitate calcium entry and calcium-dependent phosphatase activity actions, which will antagonize the effect of PKA-dependent phosphorylation. The implication of such a model is that 5-HT₇ phase advances are strongly associated with membrane hyperpolarization.

In at least some hypothalamic neurons, 8-OH-DPAT can transiently elevate intracellular calcium (Baker et al., 1998). Calcium elevation may be essential for the opening of the potassium channels discussed above, as well as activation of calcium-dependent adenylyl cyclases (Type 1 & 8). However, to date only calcium-independent adenylyl cyclase has been observed in the SCN (Type 2; Cagampang et al., 1998). This could be a means of isolating the 5-HT₇/adenylate cyclase system from the light/glutamate transduction system, since photic stimulation at CT14 will ultimately cause a large elevation in intracellular calcium in the SCN, presumably with a corresponding activation of any calcium-dependent adenylyl cyclase isoforms present.

SEROTONIN IN THE SCN: A ROLE FOR GLIAL CELLS?

Serotonergic phase advances of SCN neuronal activity in the slice can occur in the presence of agents that disrupt synaptic transmission, such as the sodium channel blocker tetrodotoxin (TTX) or the calcium channel blocker magnesium (Prosser et al., 1992). These results suggest that the effects of 5-HT are mediated by 5-HT₇ receptors on clock neurons. However, it is very clear in such studies that virtually the entire population of SCN neurons must be phase shifted simultaneously, otherwise the shift would not be detected by random sampling of single units from a population of cells. Yet, apparent shifting of the entire population occurs in TTX-treated slices in spite of the fact that the 5-HT innervation of the SCN is anatomically restricted and 5-HT₇ receptors occur on only a subpopulation of SCN neurons. It therefore appears that the shift must be propagated by some form of non-synaptic transmission. One non-synaptic means of transmission is via release of a membrane diffusible messenger such as nitric oxide. In fact, nitric oxide antagonists do prevent serotonergic phase shifts in the SCN slice (Starkey, 1996).

Another mechanism for non-synaptic propagation of serotonergic phase shifting might be via astrocytes. Despite the relatively small ratio of astrocytes to neurons in the SCN, these cells provide extensive neuronal somal coverage and enclose synaptic junctions in this nucleus (Van den Pol, 1980; Elliott & Nunez, 1994). They perform critical metabolic functions in neural microclimate regulation, including glutamate/glutamine cycling, ionic homeostasis and glycogen storage (Bigami, 1991; Sorg & Magistretti, 1991; Swanson, 1992). Several studies suggest that astrocytes may be essential for circadian rhythmicity. In the hamster

SCN *in vivo*, the astrocytic structural element glial fibrillary acidic protein (GFAP) exhibits a circadian variation in phase with the daily rhythm of glucose utilization (Lavialle & Serviere, 1993). In the SCN slice, agents that block gap junctions or inhibit glial metabolism disrupt the circadian rhythm of neural activity (Prosser et al., 1994a). In organotypic SCN cultures, circadian rhythms of VIP and VP release become uncoupled when glial cells are lost by antimitotic treatment (Shinohara et al., 1995). Finally, SCN neurons in cultures likewise treated with antimitotics to prevent glial proliferation have unsynchronized circadian rhythms of electrical activity, despite abundant synapses (Welsh et al., 1995).

The dominant astrocytic 5-HT receptor in the CNS appears to be the 5-HT₇ receptor (Hösli & Hösli, 1993; Hirst et al., 1998), and it has been demonstrated immunocytochemically that these receptors are abundant on SCN astrocytes (Miller, unpublished, 1999, antibody kindly provided by Mike Brownstein; Figure 9). A specific role for SCN astrocytes in mediating serotonergic effects on the SCN is suggested by evidence that cultured SCN astrocytes exhibit calcium waves in response to 5-HT (van den Pol et al., 1992), and that systemic injections of 8-OH-DPAT can alter the contents and distribution of GFAP in the SCN of Syrian hamsters and C57BL/6j mice (Glass, unpublished, 1999). Administration of 8-OH-DPAT during midday caused a significant reduction in immunoreactive GFAP content within 1 h of injection, compared to vehicle controls. This effect was not evident 3 h after drug injection. Treatment with 8-OH-DPAT late in the dark-phase had little effect on GFAP content. The 8-OH-DPAT-induced reduction in GFAP content evident at midday was blocked partially by pretreatment with the 5-HT_{2/7} receptor antagonist, ritanserin, and more substantially by pretreatment with the 5-HT_{1A/7} receptor antagonist, NAN-190. Treatment with 8-OH-DPAT also caused a significant redistribution of immunoreactive GFAP, such that the dense mesh-like appearance seen in vehicle controls was significantly reduced. Immunoblot assessments of GFAP contents 2 h prior to, and 1 h after lights-off revealed a significant time-of-day difference in GFAP expression, with lowest levels occurring at the latter time point, associated with maximal endogenous 5-HT release in the SCN.

It is interesting to speculate that circadian fluctuation in astrocytic activity could be a response to the daily rhythm in 5-HT release in the SCN. The phase-dependent nature of the suppressive effect of 8-OH-DPAT on GFAP content suggests further that there may be a daily rhythm in response of SCN astrocytes to 5-HT. Results from the 5-HT antagonist trials implicate the 5-HT₇ and possibly the 5-HT_{1A} receptor subtypes in regulating changes in SCN GFAP activity. Definitive proof of such receptor involvement would require application of more specific antagonists, e.g., DR4004 for 5-HT₇ and WAY100635 for 5-HT_{1A}.

Pending further work, the role of SCN astroglia in the regulation of circadian timekeeping functions remains a matter of speculation. The existence of numer-

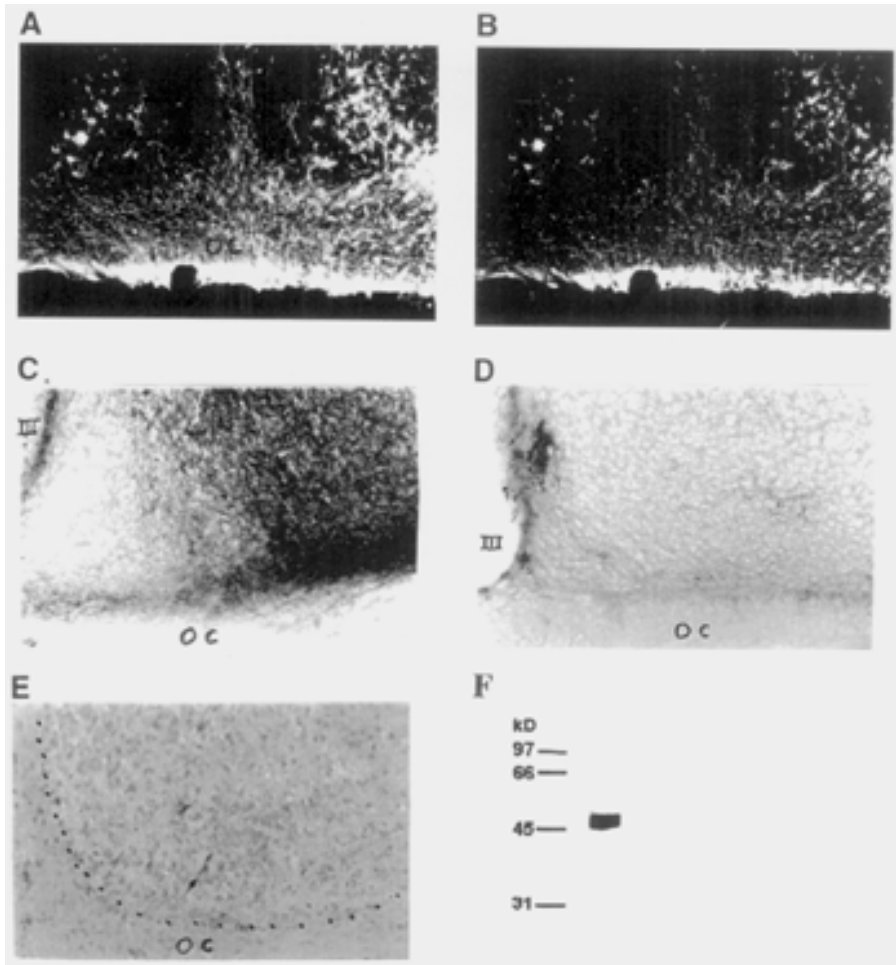


Fig. 9. Panel A (10X) is a 40 μ m section through the SCN immunolabelled for the astrocyte marker, GFAP (conjugated to FITC fluorophore). Panel B (10X) is the same section as in A, but visualized for a second antibody, raised against the 5HT7 receptor (courtesy of Mark Brownfield) and conjugated to a second fluorophore (FITC). Note colocalization of GFAP and 5HT7 in ventral and lateral SCN, presumably in GFAP-containing astrocytes. This region is roughly coincident with the termination of 5HTergic afferents in the rat SCN. Panel C (20X) is 5HT7 immunostaining alone (DAB chromogen). Panel D (20X) is a section from the same AP level as panel C, but with the primary antibody deleted. Immunoneutralization controls (not shown) also eliminate all 5HT7 immunostaining. Panel E (20X) is a Nissl-stained section indicating the ventrolateral extent of the SCN (dotted line) in the same AP plane as the other panels. Panel F is a Western blot (courtesy of Mark Brownfield) from whole brain showing that the 5HT7 antibody labels only one protein. OC = optic chiasm, III = third ventricle.

ous anatomical interactions between astrocytes and neurons in the SCN (Van den Pol, 1980; Shen et al., 1997) suggest that they may participate in the regulation of neuron-neuron communication, possibly serving to synchronize multiple oscillators. It is possible that 5-HT₇ receptors on astrocytes entrain astrocytic sequestration of extracellular potassium in the vicinity of SCN neurons. Control of extracellular potassium regulates the neuronal resting membrane potential and the probability of action potential production by neurons. Thus synchronized astrocytic activity could synchronize neuronal activity in the SCN. One possible synchronizing influence on the astrocytes could be 5-HT-induced propagation of intercellular calcium waves (Van den Pol et al., 1992).

OTHER TRANSMITTER SYSTEMS: NPY

While this review has focused on possible serotonergic mediation of nonphotic inputs to the SCN pacemaker, another body of evidence has accumulated indicating that NPY plays a vital role in this process. These data have recently been reviewed elsewhere (e.g., Hastings et al, 1998; Harrington & Mistlberger, 1999), but the core observations are as follows: 1. NPY injected into the SCN area induces phase shifts similar in timing and magnitude to those induced by wheel running (Albers & Ferris, 1984; Biello et al., 1994). 2. Antibody to NPY injected into the SCN blocks phase shifts to wheel running (Biello et al., 1994). 3. IGL lesions that eliminate NPY in the hamster SCN prevent phase shifts in response to triazolam injections (Johnson et al., 1988), saline injections (Maywood et al., 1997) and wheel running (Janik & Mrosovsky, 1994; Wickland & Turek, 1994). 4. IGL lesions that eliminate NPY in the mouse SCN prevent entrainment to forced or voluntary running schedules (Marchant et al., 1997) 5. NPY-containing neurons in the IGL are activated during running in a novel wheel, as indicated by increased fos expression (Janik et al., 1995; Mikkelsen et al., 1998).

Note that while these findings constitute strong evidence that NPY is necessary and sufficient for nonphotic shifting in the hamster, data from other species is very sparse. In addition, another set of observations suggests that 5-HT and NPY almost certainly interact within the circadian system. 1. As noted above, the DRN projects directly to the IGL (Meyer-Bernstein & Morin, 1996). 2. 5-HT and NPY containing terminals are often found in close apposition in the SCN, or synapse on the same SCN neurons (Bosler, 1989; François-Bellan & Bosler, 1992). 3. IGL-NPY neurons and MRN- 5-HT neurons are activated when hamsters are behaviorally active, based on studies of fos expression in the IGL and 5-HT release in the SCN (Janik et al., 1995; Dudley et al., 1998). Given that NPY and 5-HT have similar nonphotic PRCs, converge anatomically within the SCN and are both activated during behavioral arousal, a reasonable prediction would be that 5-HT

and NPY combine, in series or in parallel, to generate nonphotic phase resetting of the clock. However, postsynaptically, these two molecules have opposite effects on cAMP signal transduction pathways (Aakerlund et al., 1990; Lovenberg et al., 1993; Larhammar, 1996; see discussion above and Prosser, this issue). Moreover, in the rat SCN *in vitro*, NPY actually blocks phase shifts induced by 5-HT (Prosser, 1998). One possibility is that *in vivo*, NPY is primarily responsible for nonphotic phase resetting, while 5-HT is primarily responsible for modulation of photic inputs to the clock. *In vitro*, phase resetting responses to 5-HT may be disinhibited by loss of NPY afferents. This model predicts that phase shifts to 5-HT *in vivo* would be enhanced by NPY lesions, antibodies or receptor antagonists. However, additional information on the interactions between photic and behavioral zeitgebers, discussed next, suggests that the functions of 5-HT and NPY within the circadian system are not so easily segregated.

INTERACTIONS BETWEEN LIGHT AND BEHAVIORAL ZEITGEBERS

Few mammalian organisms live in constant dark environments, thus the contribution of nonphotic stimuli to circadian timing must also be evaluated 'in the light of day'. As noted above, scheduled daily activity and even a single bout of induced activity can modulate the phase angle of entrainment to LD cycles (Honrado & Mrosovsky, 1991; Mistlberger, 1991b; Ralph & Mrosovsky, 1992; Sinclair & Mistlberger, 1997). When photic zeitgebers are weaker, e.g., in a skeleton photoperiod, the phase angle of entrainment to the LD cycle can be greatly modified to the point of complete inversion by a daily, 3 h bout of wheel running scheduled in the mid-subjective day (Sinclair & Mistlberger, 1997). Some of these effects are predictable from the respective phase response curves for light and activity pulses. However, other studies have revealed apparently non-linear interactions between light and activity. Light pulses immediately following a scheduled bout of activity during the subjective day, when light alone has little or no phase shifting effect, can block or attenuate the phase shift response to the activity pulse (Mrosovsky, 1991; Biello & Mrosovsky, 1995). Light pulses can also significantly attenuate phase shifts to systemic injections of 8-OH-DPAT (Penev et al., 1997; Mistlberger & Landry, unpublished observations, 1997) and intra-SCN injections of NPY (Biello & Mrosovsky, 1995). Finally, we have recently observed that phase shifting effects of sleep deprivation in the mid-subjective day can be attenuated by concurrent light exposure (Antle & Mistlberger, unpublished, 1999).

Other studies indicate that the inhibitory relationship between light and activity is mutual. If hamsters and mice are induced to run before and during a light pulse in the subjective night, the magnitude of the shift to light is significantly

attenuated, although this amount of running alone is not sufficient to induce a phase shift (Ralph & Mrosovsky, 1992; Mistlberger & Antle, 1998; Mistlberger & Holmes, 1999). In both species, this effect seems to be more robust late in the subjective night (when light induces phase advances), than early in the subjective night (when light induces phase delays). In hamsters, the effect can be blocked with the general 5-HT_{1/7} antagonist metergoline (Mistlberger & Antle, 1998).

One interpretation of these results is that locomotor activity induces 5-HT release in the SCN, and that 5-HT release inhibits photic input to SCN pacemaker cells in a phase dependent manner. This interpretation provides a behavioral context for the by now well-documented role of 5-HT as an anti-photic signal within the hamster circadian system. However, this model is complicated by the evidence reviewed above that running late in the subjective night actually decreases 5-HT release within the SCN of Syrian hamsters (Dudley et al., 1998). In that study, wheel running was stimulated from ZT19-22, or about 1.5 h later than the scheduling of activity in the light-inhibition study, so it is possible that this difference in circadian time accounts for the lack of convergence of the results of these two studies.

Alternatively, a reduction of 5-HT release during bouts of wheel running stimulated late in the subjective night might disinhibit 5-HT-recipient IGL neurons that release NPY in the SCN, and increased NPY might be the antiphotic signal at this phase. Metergoline might prevent disinhibition by blocking raphe autoreceptors. Consistent with this idea, NPY injections can inhibit phase shifts induced by light (Weber & Rea, 1997), while injections of anti-NPY can potentiate photic shifts (Biello, 1995). In the SCN slice *in vitro*, glutamate (the presumed photic signal) blocks phase shifts to NPY (Biello et al., 1997). This model assumes that the relevant 5-HT receptor in the IGL is not blocked by metergoline.

As if further complications are needed, it should also be recalled that the IGL is retinorecipient, and that IGL lesions actually attenuate phase advance shifts, although not phase delay shifts, to light (Harrington & Rusak, 1986; Pickard et al., 1987). This seems paradoxical, given the evidence that NPY is antiphotic within the SCN; if anything, larger shifts to light would be predicted following IGL ablation. However, a change in the shape of the photic PRC could be secondary to alterations in the nonphotic afferent stream to the SCN pacemaker. The nonphotic stream could be directly interrupted by loss of IGL cells, or could be weakened by the low levels of wheel running characteristic of IGL-ablated hamsters. Changes in the afferent stream to the SCN pacemaker might alter coupling among its component oscillators, thereby altering the integrated response of the ensemble to photic inputs.

CONCLUSIONS

The neurobiological basis of depression in humans is not well understood. Many procedures that modulate monoamine transmission, including drugs, exercise and sleep deprivation, have antidepressant actions, and most of these procedures have been shown to modulate circadian rhythms in one or more mammalian species. The literature linking exercise, monoamine transmission and circadian phase control is growing in size, but is in many respects distressingly complex and incomplete. There are certainly many results that generally support the idea that behavioral activity or arousal may regulate circadian phase via a serotonergic pathway to the circadian pacemaker. However, a good deal more work will be necessary to clarify the nature of the inconsistencies that have arisen from the variety of experimental approaches that have been brought to bear on the issue. In particular, we need to better understand the differences between the SCN *in vivo* and *in vitro*, and to determine with more certainty whether different nonphotic zeitgebers use different pathways to the pacemaker, whether phase delays and advances involve different mechanisms, whether nonphotic input pathways utilize co-factors, and whether variability across strains and species reflects substantive physiological differences or technical limitations of our methods. The relevance of all this to human depression also remains tentative at this time. But, if there is an etiological link between circadian function and mood disorders, then elucidating the pathways that link antidepressant treatments to the molecular machinery of the circadian pacemaker may have great heuristic value for designing the best possible behavioral and pharmacological therapies.

ACKNOWLEDGEMENTS

Supported by grants from NSERC, Canada (R.E.M, M.C.A), NIA AG11084 (J.D.M) and NIH NS35229 and MH57034 (J.D.G.).

REFERENCES

- Aakerlund L, Gether U, Fuhlendorff J, Schwartz TW, Thastrup O (1990): Y1 receptors for neuropeptide Y are coupled to mobilization of intracellular calcium and inhibition of adenylate cyclase. *FEBS Letters* 260(1): 73–8.
- Albers HE, Ferris CF (1984): Neuropeptide Y: role in light-dark cycle entrainment of hamster circadian rhythms. *Neurosci Lett* 50(1–3): 163–8.
- Antle MC, Dudley T, Glass JD, Mistlberger RE (1998a): The silent 5HT_{1A} antagonist WAY100635 increases endogenous 5HT release in hamster SCN: Effects on circadian phase and response to a nonphotic zeitgeber. *Soc Res Biol Rhythms* 6: 75.
- Antle MC, Marchant EG, Niel L, Mistlberger RE (1998b): Serotonin antagonists do not attenuate activity-induced phase shifts of circadian rhythms in the Syrian hamster. *Brain Res* 813(1): 139–49.

- Aschoff J (1960): Exogenous and endogenous components in circadian rhythms. *Cold Spring Harbor Symposium on Quantitative Biology*. XXV: 11–28.
- Aschoff J (1981): Free running and entrained rhythms. In *Handbook of Neurobiology*: Vol 4. Biological Rhythms, Aschoff J (ed.), Plenum Press, New York, pp. 81–94.
- Aschoff J, Fatransk $\ddot{a}$ M, Giedke H, Doerr P, Stamm D, Wisser H (1971): Human circadian rhythms in continuous darkness: entrainment by social cues. *Science* 171(967): 213–5.
- Asikainen M, Deboer T, Porkka-Heiskanen T, Stenberg D, Tobler I (1995): Sleep deprivation increases brain serotonin turnover in the Djungarian hamster. *Neurosci Lett* 198(1): 21–4.
- Azmitia EC, Buchan AM, Williams JH (1978): Structural and functional restoration by collateral sprouting of hippocampal 5-HT axons. *Nature* 274(5669): 374–6.
- Baker LP, Nielsen MD, Impey S, Metcalf MA, Poser SW, Chan G, Obrietan K, Hamblin MW, Storm DR (1998): Stimulation of type 1 and type 8 Ca²⁺/calmodulin-sensitive adenylyl cyclases by the Gs-coupled 5-hydroxytryptamine subtype 5-HT_{7A} receptor. *J Biol Chem* 273(28): 17469–76.
- Beersma DG, Hiddinga AE (1998): No impact of physical activity on the period of the circadian pacemaker in humans. *Chronobiol Int* 15(1): 49–57.
- Berendsen HH, Broekkamp CL, van Delft AM (1991): Depletion of brain serotonin differently affects behaviors induced by 5HT_{1A}, 5HT_{1C}, and 5HT₂ receptor activation in rats. *Behav Neural Biol* 55(2): 214–26.
- Biello SM (1995): Enhanced photic phase shifting after treatment with antiserum to neuropeptide Y. *Brain Res* 673(1):25–9.
- Biello SM, Golombek DA, Harrington ME (1997): Neuropeptide Y and glutamate block each other's phase shifts in the suprachiasmatic nucleus in vitro. *Neuroscience* 77(4): 1049–57.
- Biello SM, Janik D, Mrosovsky N (1994): Neuropeptide Y and behaviorally induced phase shifts. *Neuroscience* 62(1): 273–9.
- Biello SM, Mrosovsky N (1995): Blocking the phase-shifting effect of neuropeptide Y with light. *Proc R Soc Lond B Biol Sci* 259(1355): 179–87.
- Bigami A (1991): Glial cells in the central nervous system: *Discussions in Neuroscience* 8(1): Amsterdam, Elsevier.
- Blier P, Bergeron R, de Montigny C (1997): Selective activation of postsynaptic 5-HT_{1A} receptors induces rapid antidepressant response. *Neuropsychopharmacology* 16(5): 333–8.
- Blier P, de Montigny C (1985): Serotonergic but not noradrenergic neurons in rat central nervous system adapt to long-term treatment with monoamine oxidase inhibitors. *Neuroscience* 16(4): 949–55.
- Bloom FE, Kupfer D, Eds. (1995): *Psychopharmacology: The fourth generation of progress*. New York, Raven Press.
- Bobrzynska KJ, Godfrey MH, Mrosovsky N (1996): Serotonergic stimulation and nonphotic phase-shifting in hamsters. *Physiol Behav* 59(2): 221–30.
- Bobrzynska KJ, Mrosovsky N (1998): Phase shifting by novelty-induced running: activity dose-response curves at different circadian times. *J Comp Physiol [A]* 182(2): 251–8.
- Bosler O (1989): Ultrastructural relationships of serotonin and GABA terminals in the rat suprachiasmatic nucleus. Evidence for a close interconnection between the two afferent systems. *J Neurocytol* 18(1): 105–13.
- Bradbury MJ, Dement WC, Edgar DM (1997): Serotonin-containing fibers in the suprachiasmatic hypothalamus attenuate light-induced phase delays in mice. *Brain Res* 768(1–2): 125–34.
- Cagampang FR, Antoni FA, Smith SM, Piggins HD, Coen CW (1998): Circadian changes of type II adenylyl cyclase mRNA in the rat suprachiasmatic nuclei. *Brain Res* 810(1–2): 279–82.
- Challet E, Scarbrough K, Penev PD, Turek FW (1998): Roles of suprachiasmatic nuclei and intergeniculate leaflets in mediating the phase-shifting effects of a serotonergic agonist and their photic modulation during subjective day. *J Biol Rhythms* 13(5): 410–21.
- Collier G, Hirsch E (1971): Reinforcing properties of spontaneous activity in the rat. *J Comp Physiol Psychol* 77(1): 155–60.
- Cutrer $\acute{a}$ RA, Kalsbeek A, P $_vet$ P (1994a): Specific destruction of the serotonergic afferents to the suprachiasmatic nuclei prevents triazolam-induced phase advances of hamster activity rhythms. *Behav Brain Res* 62(1): 21–8.

- Cutrer RA, Ouarour A, Pévet P (1994b): Effects of the 5-HT_{1A} receptor agonist 8-OH-DPAT and other non-photic stimuli on the circadian rhythm of wheel-running activity in hamsters under different constant conditions. *Neurosci Lett* 172(1–2): 27–30.
- Cutrer RA, Saboureau M, Pévet P (1996): Phase-shifting effect of 8-OH-DPAT, a 5-HT_{1A}/5-HT₇ receptor agonist, on locomotor activity in golden hamster in constant darkness. *Neurosci Lett* 210(1): 1–4.
- Dudley TE, DiNardo LA, Glass JD (1998): Endogenous regulation of serotonin release in the hamster suprachiasmatic nucleus. *J Neurosci* 18(13): 5045–52.
- Dudley TE, Dinardo LA, Glass JD (1999): In vivo assessment of the midbrain raphe nuclear regulation of serotonin release in the hamster suprachiasmatic nucleus. *J Neurophysiol* 81(4): 1469–77.
- Duncan WCJ (1996): Circadian rhythms and the pharmacology of affective illness. *Pharmacol Ther* 71(3): 253–312.
- Duncan WCJ, Johnson KA, Wehr TA (1999): 5-HT agonist-induced phase-advances of the circadian pacemaker are diminished by chronic antidepressant drug treatment. *Brain Res* 815(1): 126–30.
- Duncan WCJ, Tamarkin L, Sokolove PG, Wehr TA (1988): Chronic clorgyline treatment of Syrian hamsters: An analysis of effects on the circadian pacemaker. *J Biol Rhythms* 3: 305–322.
- Edgar DM, Dement WC (1991): Regularly scheduled voluntary exercise synchronizes the mouse circadian clock. *Am J Physiol* 261(4 Pt 2): R928–33.
- Edgar DM, Martin CE, Dement WC (1991): Activity feedback to the mammalian circadian pacemaker: influence on observed measures of rhythm period length. *J Biol Rhythms* 6(3): 185–99.
- Edgar DM, Miller JD, Prosser RA, Dean RR, Dement WC (1993): Serotonin and the mammalian circadian system: II. Phase-shifting rat behavioral rhythms with serotonergic agonists. *J Biol Rhythms* 8(1): 17–31.
- Edgar DM, Reid MS, Dement WC (1997): Serotonergic afferents mediate activity-dependent entrainment of the mouse circadian clock. *Am J Physiol* 273(1 Pt 2): R265–9.
- Ehlen JC, Antle M, Mistlberger RE, Glass JD (1999): Phase shifting effects of timed in vivo microdialysis perfusion of the SCN with 8-OH-DPAT. *Soc Neurosci Abstr* 25.
- Elliott AS, Nunez AA (1994): An ultrastructural study of somal appositions in the suprachiasmatic nucleus and anterior hypothalamus of the rat. *Brain Res* 662(1–2): 278–82.
- Erlander MG, Lovenberg TW, Baron BM, de Lecea L, Danielson PE, Racke M, Slone AL, Siegel BW, Foye PE, Cannon K, Burns JE, Sutcliffe JG (1993): Two members of a distinct subfamily of 5-hydroxytryptamine receptors differentially expressed in rat brain. *Proc Natl Acad Sci U S A* 90(8): 3452–6.
- Fernstrom JD (1981): Dietary precursors and brain neurotransmitter formation. *Ann Rev Med* 32: 413–25.
- Forbes IT, Dabbs S, Duckworth DM, Jennings AJ, King FD, Lovell PJ, Brown AM, Collin L, Hagan JJ, Middlemiss DN, Riley GJ, Thomas DR, Upton N (1998): (R)-3,N-dimethyl-N-[1-methyl-3-(4-methyl-piperidin-1-yl)propyl]benzenesulfonamide: the first selective 5-HT₇ receptor antagonist. *J Med Chem* 41(5): 655–7.
- François-Bellan AM, Bosler O (1992): Convergent serotonin and GABA innervation of VIP neurons in the suprachiasmatic nucleus demonstrated by triple labeling in the rat. *Brain Res* 595(1): 149–53.
- Gardner JP, Fornal CA, Jacobs BL (1997): Effects of sleep deprivation on serotonergic neuronal activity in the dorsal raphe nucleus of the freely moving cat. *Neuropsychopharmacology* 17(2): 72–81.
- Glass JD, Randolph WW, Ferreira SA, Rea MA, Hauser UE, Blank JL, De Vries MJ (1992): Diurnal variation in 5-hydroxyindole-acetic acid output in the suprachiasmatic region of the Siberian hamster assessed by in vivo microdialysis: evidence for nocturnal activation of serotonin release. *Neuroendocrinology* 56(4): 582–90.
- Glass JD, Selim M, Rea MA (1994): Modulation of light-induced C-Fos expression in the suprachiasmatic nuclei by 5-HT_{1A} receptor agonists. *Brain Res* 638(1–2): 235–42.
- Glass JD, Selim M, Srkalovic G, Rea MA (1995): Tryptophan loading modulates light-induced responses in the mammalian circadian system. *J Biol Rhythms* 10(1): 80–90.
- Harrington ME, Rusak B (1986): Lesions of the thalamic intergeniculate leaflet alter hamster circadian rhythms. *J Biol Rhythms* 1(4): 309–25.

- Harrington ME, Mistlberger RE (1999): Anatomy and physiology of the mammalian circadian system. In: *Principles and Practice of Sleep Medicine*, Third Edition, MH Kryger, T Roth, WC Dement (eds), Philadelphia, W.B. Saunders Co, in press.
- Hastings MH, Ebling FJ, Grosse J, Herbert J, Maywood ES, Mikkelsen JD, Sumova A (1995): Immediate-early genes and the neural bases of photic and non-photic entrainment. *Ciba Found Symp* 183: 175–89; discussion 190–7.
- Hastings MH, Duffield GE, Smith EJ, Maywood ES, Ebling FJ (1998): Entrainment of the circadian system of mammals by nonphotic cues. *Chronobiol Int* 15: 425–45.
- Hirst WD, Cheung NY, Rattray M, Price GW, Wilkin GP (1998): Cultured astrocytes express messenger RNA for multiple serotonin receptor subtypes, without functional coupling of 5-HT1 receptor subtypes to adenylyl cyclase. *Mol Brain Res* 61(1–2): 90–9.
- Hjorth S, Westlin D, Bengtsson HJ (1997): WAY100635-induced augmentation of the 5-HT-elevating action of citalopram: relative importance of the dose of the 5-HT1A (auto)receptor blocker versus that of the 5-HT reuptake inhibitor. *Neuropharmacology* 36(4–5): 461–5.
- Holmes MM, Mistlberger RE, (1999, submitted): Food anticipation activity and photic entrainment in food restricted balb/c mice.
- Honrado GI, Mrosovsky N (1991): Interaction between periodic socio-sexual cues and light-dark cycles in controlling the phasing of activity rhythms in golden hamsters. *Ethol Ecol Evol* 3(3): 221–232.
- Hösli E, Hösli L (1993): Receptors for neurotransmitters on astrocytes in the mammalian central nervous system. *Prog Neurobiol* 40(4): 477–506.
- Jacobs BL, Fornal CA (1997): Serotonin and motor activity. *Curr Opin Neurobiol* 7(6): 820–5.
- Janik D, Mikkelsen JD, Mrosovsky N (1995): Cellular colocalization of Fos and neuropeptide Y in the intergeniculate leaflet after nonphotic phase-shifting events. *Brain Res* 698(1–2): 137–45.
- Janik D, Mrosovsky N (1993): Nonphotically induced phase shifts of circadian rhythms in the golden hamster: activity-response curves at different ambient temperatures. *Physiol Behav* 53(3): 431–6.
- Janik D, Mrosovsky N (1994): Intergeniculate leaflet lesions and behaviorally-induced shifts of circadian rhythms. *Brain Res* 651(1–2): 174–82.
- Johnson RF, Smale L, Moore RY, Morin LP (1988): Lateral geniculate lesions block circadian phase-shift responses to a benzodiazepine. *Proc Natl Acad Sci U S A* 85(14): 5301–4.
- Jouvet M (1999): Sleep and serotonin: an unfinished story. *Neuropsychopharmacology* 21(2 Suppl): 24S–27S.
- Kagen J, Berkun M (1954): The reward value of running activity. *J Comp Physiol Psychol* 47: 108.
- Kennaway DJ, Moyer RW (1998): Serotonin 5-HT2C agonists mimic the effect of light pulses on circadian rhythms. *Brain Res* 806(2): 257–70.
- Kennaway DJ, Rowe SA, Ferguson SA (1996): Serotonin agonists mimic the phase shifting effects of light on the melatonin rhythm in rats. *Brain Res* 737(1–2): 301–7.
- Kikuchi C, Nagaso H, Hiranuma T, Koyama M (1999): Tetrahydrobenzindoles: selective antagonists of the 5-HT7 receptor. *J Med Chem* 42(4): 533–5.
- Kohler M, Kalkowski A, Wollnik F (1999): Serotonin agonist quipazine induces photic-like phase shifts of the circadian activity rhythm and c-Fos expression in the rat suprachiasmatic nucleus. *J Biol Rhythms* 14(2): 131–40.
- Larhammar D (1996): Evolution of neuropeptide Y, peptide YY and pancreatic polypeptide. *Regul Pept* 62(1): 1–11.
- Lavialle M, Serviere J (1993) Circadian fluctuations in GFAP distribution in the Syrian hamster suprachiasmatic nucleus. *Neuroreport* 4:1243–6.
- Leibenluft E, Wehr TA (1992): Is sleep deprivation useful in the treatment of depression? *Am J Psychiatry* 149(2): 159–68.
- Linthorst AC, Flachskamm C, M'Yller-Preuss P, Holsboer F, Reul JM (1995): Effect of bacterial endotoxin and interleukin-1 beta on hippocampal serotonergic neurotransmission, behavioral activity, and free corticosterone levels: an in vivo microdialysis study. *J Neurosci* 15(4): 2920–34.
- Lovenberg TW, Baron BM, de Lecea L, Miller JD, Prosser RA, Rea MA, Foye PE, Racke M, Slone AL, Siegel BW, Danielson PE, Sutcliffe JG, Erlander MG (1993): A novel adenylyl cyclase-activating serotonin receptor (5-HT7) implicated in the regulation of mammalian circadian rhythms. *Neuron* 11(3): 449–58.

- Marchant EG, Mistlberger RE (1995): Morphine phase-shifts circadian rhythms in mice: role of behavioural activation. *Neuroreport* 7(1): 209–12.
- Marchant EG, Mistlberger RE (1996): Entrainment and phase shifting of circadian rhythms in mice by forced treadmill running. *Physiol Behav* 60(2): 657–63.
- Marchant EG, Morin LP (1998): Superior colliculus mediates triazolam-induced phase shifts of hamster circadian rhythms. *Soc Neurosci* 24: 1919.
- Marchant EG, Watson NV, Mistlberger RE (1997): Both neuropeptide Y and serotonin are necessary for entrainment of circadian rhythms in mice by daily treadmill running schedules. *J Neurosci* 17(20): 7974–87.
- Martinsen EW (1990): Benefits of exercise for the treatment of depression. *Sports Med* 9(6):380–9.
- Maudhuit C, Jolas T, Chastanet M, Hamon M, Adrien J (1996): Reduced inhibitory potency of serotonin reuptake blockers on central serotonergic neurons in rats selectively deprived of rapid eye movement sleep. *Biol Psychiatry* 40(10): 1000–7.
- Maywood ES, Smith E, Hall SJ, Hastings MH (1997): A thalamic contribution to arousal-induced, non-photic entrainment of the circadian clock of the Syrian hamster. *Eur J Neurosci* 9(8): 1739–47.
- Medanic M, Gillette MU (1992) Serotonin regulates the phase of the rat suprachiasmatic circadian pacemaker in vitro only during the subjective day. *J Physiol (Lond)* 450:629–42.
- Mendlin A, Mart'n FJ, Rueter LE, Jacobs BL (1996): Neuronal release of serotonin in the cerebellum of behaving rats: an in vivo microdialysis study. *J Neurochem* 67(2): 617–22.
- Meyer-Bernstein EL, Blanchard JH, Morin LP (1997): The serotonergic projection from the median raphe nucleus to the suprachiasmatic nucleus modulates activity phase onset, but not other circadian rhythm parameters. *Brain Res* 755(1): 112–20.
- Meyer-Bernstein EL, Morin LP (1996): Differential serotonergic innervation of the suprachiasmatic nucleus and the intergeniculate leaflet and its role in circadian rhythm modulation. *J Neurosci* 16(6): 2097–111.
- Meyer-Bernstein EL, Blanchard JH, Morin LP (1997): The serotonergic projection from the median raphe nucleus to the suprachiasmatic nucleus modulates activity phase onset, but not other circadian rhythm parameters. *Brain Res* 755(1):112–20.
- Meyer-Bernstein EL, Morin LP (1998): Destruction of serotonergic neurons in the median raphe nucleus blocks circadian rhythm phase shifts to triazolam but not to novel wheel access. *J Biol Rhythms* 13(6): 494–505.
- Meyer-Bernstein EL, Morin LP (1999): Electrical stimulation of the median or dorsal raphe nuclei reduces light-induced FOS protein in the suprachiasmatic nucleus and causes circadian activity rhythm phase shifts. *Neuroscience* 92(1): 267–279.
- Mikkelsen JD, Vrang N, Mrosovsky N (1998): Expression of Fos in the circadian system following nonphotic stimulation. *Brain Res Bull* 47(4): 367–76.
- Miller JD, Edgar DM, Rea MA (1997) 5HT receptors in the aged SCN. *Soc Neurosci Abstr*.
- Miller JD, Fuller CA (1990): The response of suprachiasmatic neurons of the rat hypothalamus to photic and serotonergic stimulation. *Brain Res* 515(1–2): 155–62.
- Miller JD, Morin LP, Schwartz WJ, Moore RY (1996): New insights into the mammalian circadian clock. *Sleep* 19(8): 641–67.
- Mintz EM, Gillespie CF, Marvel CL, Huhman KL, Albers HE (1997): Serotonergic regulation of circadian rhythms in Syrian hamsters. *Neuroscience* 79(2): 563–9.
- Mistlberger RE (1991a): Effects of daily schedules of forced activity on free-running rhythms in the rat. *J Biol Rhythms* 6(1): 71–80.
- Mistlberger RE (1991b): Scheduled daily exercise or feeding alters the phase of photic entrainment in Syrian hamsters. *Physiol Behav* 50(6): 1257–60.
- Mistlberger RE, Antle MC (1998): Behavioral inhibition of light-induced circadian phase resetting is phase and serotonin dependent. *Brain Res* 786(1–2): 31–8.
- Mistlberger RE, Antle MC, Dane L, Wood A (1999): Sleep deprivation phase shifts circadian rhythms in hamsters. *Soc Neurosci Abs* 25.
- Mistlberger RE, Bossert JM, Holmes MM, Marchant EG (1998): Serotonin and feedback effects of behavioral activity on circadian rhythms in mice. *Behav Brain Res* 96(1–2): 93–9.
- Mistlberger RE, Holmes MM (1999): Morphine-induced activity attenuates phase shifts to light in C57BL/6J mice. *Brain Res* 829: 113–119.

- Mistlberger RE, Holmes MM (1999b, submitted): Behavioral modulation of circadian rhythms in the light of day: photic entrainment and PRC.
- Mistlberger RE, Landry GJ, Marchant EG (1997a): Sleep deprivation can attenuate light-induced phase shifts of circadian rhythms in hamsters. *Neurosci Lett* 238(1–2): 5–8.
- Mistlberger RE, Marchant EG, Sinclair SV (1996): Nonphotic phase-shifting and the motivation to run: cold exposure reexamined. *J Biol Rhythms* 11(3): 208–15.
- Mistlberger RE, Sinclair SV, Marchant EG, Neil L (1997b): Phase shifts to refeeding in the Syrian hamster mediated by running activity. *Physiol Behav* 61(2): 273–8.
- Morin LP (1992): Serotonergic reinnervation of the hamster suprachiasmatic nucleus and intergeniculate leaflet without functional circadian rhythm recovery. *Brain Res* 599(1): 98–104.
- Moriya T, Yoshinobu Y, Ikeda M, Yokota S, Akiyama M, Shibata S (1998): Potentiating action of MKC-242, a selective 5-HT_{1A} receptor agonist, on the photic entrainment of the circadian activity rhythm in hamsters. *Br J Pharmacol* 125(6): 1281–7.
- Mrosovsky N (1988): Phase response curves for social entrainment. *J Comp Physiol A*, 162(1): 35–46.
- Mrosovsky N (1991): Double-pulse experiments with nonphotic and photic phase-shifting stimuli. *J Biol Rhythms* 6(2): 167–79.
- Mrosovsky N (1995): A non-photic gateway to the circadian clock of hamsters. *Ciba Found Symp* 183: 154–67.
- Mrosovsky N (1999): Further experiments on the relationship between the period of circadian rhythms and locomotor activity levels in hamsters. *Physiol Behav* 66(5): 797–801.
- Mrosovsky N, Biello SM (1994): Nonphotic phase shifting in the old and the cold. *Chronobiol Int* 11(4): 232–52.
- Mrosovsky N, Janik D (1993): Behavioral decoupling of circadian rhythms. *J Biol Rhythms* 8(1): 57–65.
- Mrosovsky N, Reeb SG, Honrado GI, Salmon PA (1989): Behavioural entrainment of circadian rhythms. *Experientia* 45(8): 696–702.
- Mrosovsky N, Salmon PA (1990): Triazolam and phase-shifting acceleration re-evaluated. *Chronobiol Int* 7(1): 35–41.
- Mrosovsky N, Salmon PA, Vrang N (1998): Revolutionary science: an improved running wheel for hamsters. *Chronobiol Int* 15(2): 147–58.
- Mullins UL, Gianutsos G, Eison AS (1999): Effects of antidepressants on 5-HT₇ receptor regulation in the rat hypothalamus. *Neuropsychopharmacology* 21: 352–367.
- Mundey MK, Fletcher A, Marsden CA (1996): Effects of 8-OHDPAT and 5-HT_{1A} antagonists WAY100135 and WAY100635, on guinea-pig behaviour and dorsal raphe 5-HT neurone firing. *Br J Pharmacol* 117(4): 750–6.
- Ney V, Avramut M, Blank JL, Tardif SD, Glass JD (1998): Behavioral and serotonergic circadian clock resetting in a diurnal monkey, the common marmoset. *Soc Res Biol Rhythms* 6: 132.
- Penev PD, Turek FW, Zee PC (1995a): A serotonin neurotoxin attenuates the phase-shifting effects of triazolam on the circadian clock in hamsters. *Brain Res* 669: 207–16.
- Penev PD, Zee PC, Turek FW (1997): Serotonin in the spotlight. *Nature* 385(6612): 123.
- Penev PD, Zee PC, Wallen EP, Turek FW (1995b): Aging alters the phase-resetting properties of a serotonin agonist on hamster circadian rhythmicity. *Am J Physiol* 268(1 Pt 2): R293–8.
- Pickard GE, Ralph MR, Menaker M (1987): The intergeniculate leaflet partially mediates effects of light on circadian rhythms. *J Biol Rhythms* 2(1): 35–56.
- Pickard GE, Rea MA (1997): TFMPP, a 5HT_{1B} receptor agonist, inhibits light-induced phase shifts of the circadian activity rhythm and c-Fos expression in the mouse suprachiasmatic nucleus. *Neurosci Lett* 231(2): 95–8.
- Pittendrigh CS, Daan S (1976): A functional analysis of circadian parameters in nocturnal rodents. IV. Entrainment: Pacemaker as clock. *J Comp Physiol A* 106: 291–331.
- Possidente B, Lumia AR, McEldowney S, Rapp M (1992): Fluoxetine shortens circadian period for wheel running activity in mice. *Brain Res Bull* 28(4): 629–31.
- Pratt BL, Goldman BD (1986): Environmental influences upon circadian periodicity of Syrian hamsters. *Physiol Behav* 36(1): 91–5.
- Premack D, Schaeffer RW, Hundt A (1964): Reinforcement for drinking by running: Effect of fixed ratio and reinforcement time. *J Exp Anal Behav* 7: 91–96.
- Prévoit E, Maudhuiet C, Le Poul E, Hamon M, Adrien J (1996): Sleep deprivation reduces the citalo-

- pram-induced inhibition of serotonergic neuronal firing in the nucleus raphe dorsalis of the rat. *J Sleep Res* 5(4): 238–45.
- Prosser RA (1998): Neuropeptide Y blocks serotonergic phase shifts of the suprachiasmatic circadian clock in vitro. *Brain Res* 808(1): 31–41.
- Prosser RA, Dean RR, Edgar DM, Heller HC, Miller JD (1993): Serotonin and the mammalian circadian system: I. In vitro phase shifts by serotonergic agonists and antagonists. *J Biol Rhythms* 8(1): 1–16.
- Prosser RA, Edgar DM, Heller HC, Miller JD (1994a): A possible glial role in the mammalian circadian clock. *Brain Res* 643(1–2): 296–301.
- Prosser RA, Heller HC, Miller JD (1992): Serotonergic phase shifts of the mammalian circadian clock: effects of tetrodotoxin and high Mg^{2+} . *Brain Res* 573(2): 336–40.
- Prosser RA, Macdonald ES, Heller HC (1994b): c-fos mRNA in the suprachiasmatic nuclei in vitro shows a circadian rhythm and responds to a serotonergic agonist. *Mol Brain Res* 25(1–2): 151–6.
- Prosser RA, Miller JD, Heller HC (1990): A serotonin agonist phase-shifts the circadian clock in the suprachiasmatic nuclei in vitro. *Brain Res* 534(1–2): 336–9.
- Ralph MR, Mrosovsky N (1992): Behavioral inhibition of circadian responses to light. *J Biol Rhythms* 7(4): 353–9.
- Rea MA, Barrera J, Glass JD, Gannon RL (1995): Serotonergic potentiation of photic phase shifts of the circadian activity rhythm. *Neuroreport* 6(10): 1417–20.
- Rea MA, Glass JD, Colwell CS (1994): Serotonin modulates photic responses in the hamster suprachiasmatic nuclei. *J Neurosci* 14(6): 3635–42.
- Reebs SG, Lavery RJ, Mrosovsky N (1989): Running activity mediates the phase-advancing effects of dark pulses on hamster circadian rhythms. *J Comp Physiol [A]* 165(6): 811–8.
- Reebs SG, Mrosovsky N (1989): Effects of induced wheel running on the circadian activity rhythms of Syrian hamsters: entrainment and phase response curve. *J Biol Rhythms* 4(1): 39–48.
- Roca AL, Weaver DR, Reppert SM (1993): Serotonin receptor gene expression in the rat suprachiasmatic nuclei. *Brain Res* 608(1): 159–65.
- Romero L, Hervás I, Artigas F (1996): The 5-HT_{1A} antagonist WAY-100635 selectively potentiates the presynaptic effects of serotonergic antidepressants in rat brain. *Neurosci Lett* 219(2): 123–6.
- Rosenwasser AM, Wirz-Justice A (1997). Circadian Rhythms and Depression: Clinical and Experimental Models: *Handbook of Experimental Pharmacology: Physiology and Pharmacology of Biological Rhythms*. Redfern PH, Lemmer B. Berlin, Springer-Verlag. 125: 457–486.
- Rueter LE, Jacobs BL (1996): A microdialysis examination of serotonin release in the rat forebrain induced by behavioral/environmental manipulations. *Brain Res* 739: 57–69.
- Rusak B, Mistlberger RE, Losier B, Jones CH (1988): Daily hoarding opportunity entrains the pace-maker for hamster activity rhythms. *J Comp Physiol [A]* 164(2): 165–71.
- Schreiber W, Opper C, Dickhaus B, Heiser P, Wesemann W, Krieg JC (1997): Alterations of blood platelet MAO-B activity and LSD-binding in humans after sleep deprivation and recovery sleep. *J Psychiatr Res* 31(3): 323–31.
- Schwartz DH, McClane S, Hernandez L, Hoebel BG (1989): Feeding increases extracellular serotonin in the lateral hypothalamus of the rat as measured by microdialysis. *Brain Res* 479(2): 349–54.
- Selim M, Glass JD, Hauser UE, Rea MA (1993): Serotonergic inhibition of light-induced fos protein expression and extracellular glutamate in the suprachiasmatic nuclei. *Brain Res* 621(2): 181–8.
- Shen H, Watanabe M, Tomasiewicz H, Rutishauser U, Magnuson T, Glass JD (1997): Role of neural cell adhesion molecule and polysialic acid in mouse circadian clock function. *J Neurosci* 17(13): 5221–9.
- Shimizu M, Nishida A, Zensho H, Yamawaki S (1996): Chronic antidepressant exposure enhances 5-hydroxytryptamine₇ receptor-mediated cyclic adenosine monophosphate accumulation in rat frontocortical astrocytes. *J Pharmacol Exp Ther* 279(3): 1551–8.
- Shinohara K, Honma S, Katsuno Y, Abe H, Honma K (1995): Two distinct oscillators in the rat suprachiasmatic nucleus in vitro. *Proc Natl Acad Sci U S A* 92(16): 7396–400.

- Shioiri T, Takahashi K, Yamada N, Takahashi S (1991): Motor activity correlates negatively with free-running period, while positively with serotonin contents in SCN in free-running rats. *Physiol Behav* 49(4): 779–86.
- Sinclair SV, Mistlberger RE (1997): Scheduled activity reorganizes circadian phase of Syrian hamsters under full and skeleton photoperiods. *Behav Brain Res* 87(2): 127–37.
- Sorg O, Magistretti PJ (1991): Characterization of the glycogenolysis elicited by vasoactive intestinal peptide, noradrenaline and adenosine in primary cultures of mouse cerebral cortical astrocytes. *Brain Res* 563(1–2): 227–33.
- Stampfer HG (1998): The relationship between psychiatric illness and the circadian pattern of heart rate. *Aust N Z J Psychiatry* 32(2): 187–98.
- Starkey SJ (1996): Melatonin and 5-hydroxytryptamine phase-advance the rat circadian clock by activation of nitric oxide synthesis. *Neurosci Lett* 211(3): 199–202.
- Sumova A, Maywood ES, Selva D, Ebling FJ, Hastings MH (1996): Serotonergic antagonists impair arousal-induced phase shifts of the circadian system of the syrian hamster. *Brain Res* 709(1): 88–96.
- Swanson RA (1992): Astrocyte glutamate uptake during chemical hypoxia in vitro. *Neurosci Lett* 147(2): 143–6.
- Thomas DR, Gittins SA, Collin LL, Middlemiss DN, Riley G, Hagan J, Gloger I, Ellis CE, Forbes IT, Brown AM (1998): Functional characterisation of the human cloned 5-HT7 receptor (long form); antagonist profile of SB-258719. *Br J Pharmacol* 124: 1300–6.
- Tominaga K, Shibata S, Ueki S, Watanabe S (1992): Effects of 5-HT1A receptor agonists on the circadian rhythm of wheel-running activity in hamsters. *Eur J Pharmacol* 214(1): 79–84.
- Trulson ME, Jacobs BL (1983): Raphe unit activity in freely moving cats: lack of diurnal variation. *Neurosci Lett* 36(3): 285–90.
- Van Cauter E, Turek FW (1986): Depression: a disorder of timekeeping? *Perspect Biol Med* 29(4): 510–9.
- Van den Pol AN (1980): The hypothalamic suprachiasmatic nucleus of rat: intrinsic anatomy. *J Comp Neurol* 191(4): 661–702.
- Van den Pol AN, Finkbeiner SM, Cornell-Bell AH (1992): Calcium excitability and oscillations in suprachiasmatic nucleus neurons and glia in vitro. *J Neurosci* 12(7): 2648–64.
- Van Reeth O, Hinch D, Tecco JM, Turek FW (1991): The effects of short periods of immobilization on the hamster circadian clock. *Brain Res* 545(1–2): 208–14.
- Van Reeth O, Turek FW (1989): Stimulated activity mediates phase shifts in the hamster circadian clock induced by dark pulses or benzodiazepines. *Nature* 339(6219): 49–51.
- Weber ET, Rea MA (1997): Neuropeptide Y blocks light-induced phase advances but not delays of the circadian activity rhythm in hamsters. *Neurosci Lett* 231: 159–62.
- Weber ET, Gannon RL, Rea MA (1998): Local administration of serotonin agonists blocks light-induced phase advances of the circadian activity rhythm in the hamster. *J Biol Rhythms* 13(3): 209–18.
- Wehr TA, Wirz-Justice A (1982): Circadian rhythm mechanisms in affective illness and in antidepressant drug action. *Pharmacopsychiatry* 15: 31–39.
- Welsh D, Richardson GS, Dement WC (1988): Effect of running wheel availability on circadian patterns of sleep and wakefulness in mice. *Physiol Behav* 43(6): 771–77.
- Welsh DK, Logothetis DE, Meister M, Reppert SM (1995): Individual neurons dissociated from rat suprachiasmatic nucleus express independently phased circadian firing rhythms. *Neuron* 14(4): 697–706.
- Wever RA (1979): Influence of physical workload on freerunning circadian rhythms of man. *Pflugers Arch* 381(2): 119–26.
- Wickland C, Turek FW (1994): Lesions of the thalamic intergeniculate leaflet block activity-induced phase shifts in the circadian activity rhythm of the golden hamster. *Brain Res* 660(2): 293–300.
- Wickland CR, Turek FW (1991): Phase-shifting effects of acute increases in activity on circadian locomotor rhythms in hamsters. *Am J Physiol* 261(5 Pt 2): R1109–17.
- Wilkinson LO, Auerbach SB, Jacobs BL (1991): Extracellular serotonin levels change with behavioral state but not with pyrogen-induced hyperthermia. *J Neurosci* 11: 2732–41.

- Wurtman RJ, Wurtman JJ (1995): Brain serotonin, carbohydrate-craving, obesity and depression. *Obesity Research* 3(Suppl 4): 477S–480S.
- Yamada N, Shimoda K, Ohi K, Takahashi S, Takahashi K (1988): Free-access to a running wheel shortens the period of free-running rhythm in blinded rats. *Physiol Behav* 42(1): 87–91.
- Yamada N, Shimoda K, Takahashi K, Takahashi S (1986): Change in period of free-running rhythms determined by two different tools in blinded rats. *Physiol Behav* 36(2): 357–62.
- Ying SW, Rusak B (1997): 5-HT₇ receptors mediate serotonergic effects on light-sensitive suprachiasmatic nucleus neurons. *Brain Res* 755(2): 246–54.
- Zoli M, Jansson A, Syková E, Agnati LF, Fuxe K (1999): Volume transmission in the CNS and its relevance for neuropsychopharmacology. *Trends Pharmacol Sci* 20:142–50.