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The enigma of behavioral inputs to the circadian clock: A test of function using restraint

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Abstract

Wheel running stimulated during the daily rest period can acutely shift circadian rhythms in Syrian hamsters. Spontaneous running, defining the active phase of the circadian rest–activity cycle, can shorten the circadian periodicity in constant light or dark in several nocturnal rodent species. The adaptive significance of these behavioral effects on pacemaker phase and period is unclear. Here we consider a hypothesis that behavioral inputs to the circadian pacemaker serve primarily to enhance the precision of light–dark entrainment and maintain daily activity onset close to lights-off (i.e., dusk) by stabilizing entrainment on a steeper portion of the delay zone of the phase–response curve to light. This hypothesis rests on the evidence that spontaneous activity early in the active period feeds back on the pacemaker to advance its motion. If so, then preventing activity at this time should induce a phase delay shift. Such delay shifts have been reported in Syrian hamsters physically restrained early in the active period. We show here that restraint can induce phase delays but that, using the Aschoff Type 2 procedure for measuring shifts, these delays are very small, are inversely related to behavioral sleep during restraint, and are positively correlated with 'rebound' increases in running following restraint, at a circadian time when stimulated running is known to induce phase delay shifts. Repeated bouts of restraint, to promote habituation, were associated with strong attenuation of 'rebound' running and no significant delay shifts. These results suggest that, in Syrian hamsters, spontaneous activity early at night has little effect on pacemaker motion, and argue against the stated hypothesis.

Keywords: Syrian hamster; Wheel running; Stress; Non-photic entrainment; Suprachiasmatic nucleus

Circadian rhythms in mammals can be synchronized to local environmental time by phase and period (τ) resetting actions of daily light–dark (LD) cycles on a master circadian pacemaker located in the suprachiasmatic nucleus (SCN). Circadian rhythms can also be entrained by non-photic stimuli, in at least two distinct ways. First, daily schedules of food access induce circadian rhythms of food anticipatory activity that in at least some species (e.g., the rat) can readily dissociate from light-entrained rhythms and that, in all species examined so far (i.e., rats, mice and hamsters), persist after ablation of the SCN [1–4]. Feeding-related cues also play a dominant role in synchronizing circadian oscillators in organs such as the liver, heart and lungs, independently of the LD cycle and SCN [5–7]. While the neural mechanisms driving food anticipatory circadian activity rhythms remain to be fully elucidated, the adaptive value of this type of non-photic entrainment is intuitively transparent (behavioral and physiological preparation for expected feeding opportunity).

A second type of non-photic entrainment is mediated by phase resetting actions of behavioral arousal directly on the SCN pacemaker [8,9]. In Syrian hamsters, arousal during the middle or late portion of the daily rest/sleep period (the 'subjective day' in nocturnal animals), stimulated by confinement to a novel wheel or by gentle handling, can induce large phase advance shifts, while the same procedures during the mid-to-late active/wake period (the 'subjective night') or early in the subjective day can induce phase delay shifts [10–14]. More modest effects of arousal on circadian phase or τ have been described in other rodents (e.g., rats and mice), but these are nonetheless sufficient to mediate entrainment of free-running rhythms in constant light or dark, and to modulate the phase angle of entrainment to LD cycles [15–17]. Neural pathways (e.g., originating in the intergeniculate leaflet and midbrain

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raphe nuclei; [18–21]) and possible molecular mechanisms (e.g., suppression of *Per1* and *Per2* gene expression in SCN clock cells; [22,23]) for this type of non-photic entrainment have been identified, but its adaptive significance remains unclear.

The effects of behavioral activity and arousal on circadian phase and τ suggest two possible adaptive functions. One hypothesis is that acute phase shifts to arousal serve to rapidly adjust the timing of the daily rest–activity cycle in response to significant events occurring outside of the usual active phase. According to the phase–response curve (PRC) to arousal described above, Syrian hamsters aroused by a significant stimulus in the latter part of the subjective day will advance their daily 'wake-up', so that the next day they are more likely to be spontaneously awake at this time. This would seem to be adaptive if the arousing stimulus is rewarding, such as a potential mate or food. Indeed, small phase advances have been observed in male hamsters exposed to the sight, sound and smell of estrous females approximately 1 h prior to the usual onset of nocturnal activity [24]. However, surprisingly, the effects of estrous females in some paradigms for measuring phase shifts are absent if the hamsters are actually allowed to mate [25]. Similarly, hamsters deprived of food for 27 h, and then provided with food in the mid-to-late subjective day do not exhibit phase shifts unless they engage in wheel running prior to or at the time that the food is made available [26]. Evidently, neural or metabolic correlates of natural food reward are not sufficient to reset the SCN circadian pacemaker. Moreover, large phase advance shifts can be induced in Syrian hamsters by aversive, hostile interactions between a dominant resident and a subordinate intruder in the mid-subjective day, in both the dominant and the subordinate animals [27]. Shifts have also been reported to brief foot shock [28], although another stressful stimulus, physical restraint combined with intermittent noxious compressed air stimulation, does not induce phase shifts [27]. Thus, the circadian clock in Syrian hamsters may fail to shift to align the wake phase of the rest–activity cycle with a positively rewarding stimulus, and yet may be strongly phase advanced by an aversive stimulus encountered at this time of day. Intuitively, one might predict that interactions with rewarding stimuli would promote temporal synchrony, whereas interactions with aversive stimuli would promote temporal segregation, as, for example, between dominant alpha males and non-dominant males in some social species (reviewed in [9]).

An alternative hypothesis is that the primary function of behavioral inputs to the circadian pacemaker is related to the stability and precision of LD entrainment. The circadian clock is phase delayed by light exposure early in the subjective night (i.e., near dusk), and advanced by light late in the subjective night (near dawn). In rats and mice free-running in constant dark, access to a running wheel shortens τ (as measured by general activity or drinking behavior) [29–32], and the degree of shortening is positively correlated with the amount of running [32,33] or with the proportion of total daily running concentrated early in the active period [30]. Similarly, mice that concentrate activity early in the active period tend to have a more positive phase angle of entrainment to a LD cycle [34]. These data suggest that spontaneous, clock-controlled activity during the early subjective night feeds back on the pacemaker to phase advance its motion, at a time of day when light exposure induces shifts in the opposite direction. This may create a dynamic tension between delaying actions of light and advancing actions of arousal, which could serve to stabilize photic entrainment on a steeper portion of the phase delay zone of the PRC to light. According to the non-parametric theory of entrainment developed from work on nocturnal rodents, this should enhance the precision of the daily rest–activity cycle, and may assist in maintaining a stable phase of activity onset close to (i.e., tracking) dusk [35]. By this hypothesis, the large phase shifts induced by behavioral arousal stimulated during the mid-rest period are construed as incidental to the primary function of behavioral inputs to the clock.

This 'precision of photic entrainment' hypothesis of behavioral inputs to the clock rests primarily on the inference from the relation between τ and spontaneous wheel running that activity or arousal early in the subjective night phase advances the clock. However, this portion of the non-photic PRC has been characterized as a non-responsive ('dead') zone, because stimulating activity at this time, e.g., by confining hamsters to a novel wheel, does not induce phase shifts [10,12]. As hamsters are spontaneously awake and active at this phase under the control condition (whether or not they have free-access to a wheel), the absence of a detectable shift in response to stimulated running may represent a ceiling effect, i.e., the circadian pacemaker may already be maximally advanced by spontaneous arousal and activity in the baseline condition. If so, then inhibition of activity or induction of sleep at this phase should induce a phase delay shift by attenuating the usual phase advancing effect of spontaneous activity or arousal. Evidence consistent with this prediction is provided by studies of physical restraint imposed on Syrian hamsters near the beginning of their subjective night. Hamsters restrained for 3–6 h at this time, using a restraint tube, small jar or nest box, exhibit small phase delays, averaging ~40 min [11,36,37].

Several issues need to be clarified to substantiate the interpretation that phase delay shifts in response to restraint early in the subjective night are caused by removal of phase advancing feedback from clock-controlled activity or arousal. First, animals in these studies were maintained in constant light, raising the possibility that shifts were mediated in whole or in part by a photic stimulus. Restraint is associated with sympathetic activation and pupil dilation, and this may increase light input, resulting in a light-induced phase delay. Second, mice prevented from wheel running during the first half of the night exhibit a significant increase in running when their wheels are actuated during the second half of the night [34]. If hamsters are prone to a similar 'rebound' running response, then it is conceivable that phase delays to 3 h or 6 h of restraint early in the subjective night may be caused not by the absence of running at that time, but by increased running later in the subjective night. Third, when hamsters are confined to restraint tubes for 3 h during the subjective day, they go to sleep within the first hour [27]. Whether hamsters sleep when restrained early in the subjective night is not certain, thus it is an open