

Activity-induced circadian clock resetting in the Syrian hamster: effects of melatonin

Michael C. Antle¹, Sacha Ludgate, Ralph E. Mistlberger*

Department of Psychology, Simon Fraser University, 8888 University Drive, Burnaby, British Columbia, Canada V5A 1S6

Received 9 July 2001; received in revised form 11 October 2001; accepted 12 October 2001

Abstract

Circadian rhythms in the Syrian hamster can be phase advanced by arousal during the mid-rest period. Similar phase shifts are induced by 5-HT₇ receptor activation in vivo and in vitro. Shifts in vitro are dependent on mobilization of intracellular cyclic AMP (cAMP), and can be blocked by melatonin, which opposes cAMP accumulation. If phase shifts to arousal in vivo are also dependent on cAMP, then these shifts may also be attenuated by melatonin. Hamsters were confined to a novel running wheel for 1.5 or 3 h in the mid-rest period. Melatonin (1 mg/kg i.p.) as a single bolus did not induce phase shifts, and single or multiple doses did not affect shifts to arousal. These data suggest that stimulation of cAMP by 5-HT₇ receptor activation is not necessary for clock resetting by behavioral arousal. © 2002 Elsevier Science Ireland Ltd. All rights reserved.

Keywords: Circadian rhythms; Non-photic phase shifts; Golden hamster; Serotonin; 5-HT₇ receptor; Cyclic adenosine monophosphate; Wheel running

Circadian rhythms in the Syrian hamster can be phase advanced by behavioral arousal in the mid-subjective day (the rest-phase in nocturnal animals; [1,18]). Similar phase shifts can be induced by the 5-HT_{1A/7} receptor agonist, 8-OH-DPAT, applied systemically to hamsters in vivo (e.g. [20]), or to the rat suprachiasmatic nucleus (SCN; the site of the circadian pacemaker) in vitro [17]. Shifts to 8-OH-DPAT are mediated by activation of 5-HT₇ receptors, and mobilization of intracellular cyclic AMP (cAMP) by positive receptor coupling to adenylate cyclase [12]. Inhibitors of adenylate cyclase and cAMP significantly attenuate 8-hydroxy-dipropylaminotetratin-(8-OH-DPAT)-induced phase shifts in vitro [17].

Given the similarity of phase shifts induced by behavioral arousal and 5-HT_{1A/7} agonists, and the positive association between arousal and serotonin (5-HT) release in the SCN [10], it has been hypothesized that 5-HT mediates phase shifts to behavioral stimuli. However, results from other studies have failed to support this view (for detailed review, see [15]). In particular, receptor antagonists that block phase shifts to 8-OH-DPAT in vitro fail to even partially attenuate

phase shifts to locomotor activity in vivo [2]. The more recent observation that melatonin inhibits serotonergic phase resetting in vitro provides an additional, and potentially more powerful, pharmacological tool to test the hypothesis [16]. Although the melatonin 1A receptor number declines precipitously early in development in the Syrian hamster SCN [9], a significant percentage of adult SCN neurons are inhibited by melatonin [21]. Melatonin is negatively coupled to adenylate cyclase, does not compete with endogenous 5-HT for binding sites, and is not subject to barriers that may constrain access of exogenous antagonists to 5-HT receptors interior to the synapse [22]. Melatonin is of additional interest because of its potential efficacy as a chronobiotic in humans [4]. Presently, there are no data available on how this hormone might interact with non-photic ‘zeitgebers’, such as exercise and arousal, in behaving animals.

Male Syrian hamsters ($N = 10$, 75–100 g; Charles River, Montreal, Quebec) were recorded in plastic cages with wire floors, housed in ventilated isolation cabinets. Each cage was equipped with a 17 cm running wheel monitored continuously by microcomputer. Wheel revolutions were detected by microswitch closures, and were stored to disc at 10 min intervals. Food and water were available ad libitum.

A modified Aschoff type 2 design was used to measure the phase shifting effects of melatonin and locomotor activ-

* Corresponding author. Tel.: +1-604-291-3462; fax: +1-604-291-3427.

E-mail address: mistlber@sfu.ca (R.E. Mistlberger).

¹ Present address: Dept. of Psychology, Columbia University, 1190 Amsterdam Ave, New York, NY, 10027.

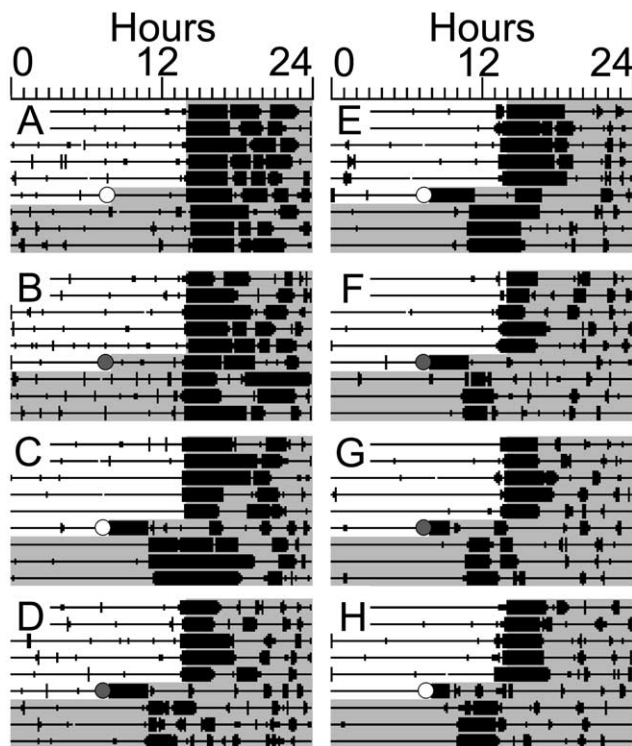


Fig. 1. Wheel running activity of a representative hamster that received s.c. injections of melatonin (shaded circles; B, D, F and G) or vehicle (open circles; A, C, E and H). (A,B) Injection at ZT5.5 with DD at ZT6; (C,D), injection at ZT5.5 with DD and a 3 h wheel confinement (WC) starting at ZT6; (E,F), DD and a 3 h WC starting at ZT6 with injections at ZT5.5, 6, 7, and 8; (G,H) DD and a 1.5 h WC starting at ZT6 with injections at ZT5.5, 6, 6.5, and 7. Each line of the chart represents 24 h, plotted in 10 min bins from left to right. Vertical deflections on each line indicate bins during which wheel counts were registered. Lights-out is indicated by shading.

ity. The following four melatonin treatment conditions were tested, each counterbalanced for order with a vehicle control treatment, using a within-subjects design: (1), single injections of melatonin (Research Biochemicals International; 1 mg/kg s.c.) or its vehicle (1% ethanol in phosphate-buffered saline (pH 7.4)), at zeitgeber time (ZT) 5.5 (6.5 h before the normal time of lights-out, defined as ZT12 by convention); (2), single injections of melatonin or vehicle at ZT5.5, followed by confinement to a novel Wahmann running wheel for 3 h, beginning at ZT6 — this procedure elicits more or less continuous running in a majority of hamsters, and induces phase advance shifts of 2–3 h in magnitude [18]; (3), injections of melatonin (1 mg/kg) or vehicle at ZT5.5, 6, 7 and 8, with wheel confinement from ZT6–9; (4), injections of melatonin or vehicle at ZT5.5, 6, 6.5 and 7, with wheel confinement from ZT6–7.5. During each test, the lights were turned off at ZT6, and remained off for 3 days. Phase shifts were measured by comparing the time at which spontaneous home cage wheel running began on the second day after the experimental treatments with the average time of activity onset during the 5 days before the treat-

ments. The hamsters were re-entrained to LD for at least 10 days between tests.

Activity onsets were identified by computer algorithm as the first 10 min bin that exceeded 100 revolutions following an interval of 4 h, during which counts in each bin were below this level. Phase shift results from the first three conditions were analyzed using two-way repeated measures analysis of variance and Student–Newman–Keuls post-hoc analysis. Results of the fourth condition were evaluated using a paired *t*-test. Paired *t*-tests were also used to determine if melatonin affected the amount of running during wheel confinements.

There was a main effect of wheel confinement ($F_{2,18} = 25.95$, $P < 0.0001$), but no effect of drug treatment ($F_{1,18} = 0.079$, $P = 0.785$) and no significant interaction ($F_{2,18} = 0.773$, $P = 0.48$). Wheel confinement produced much larger phase shifts than did the control procedure (i.e. constant dark with no wheel confinement; Figs. 1 and 2). These shifts were not affected by single or multiple injections of melatonin. In the 90 min activity test, there was a trend for larger shifts following melatonin treatment ($t_{(5)} = -1.66$, $P = 0.08$). Melatonin did not affect the amount of activity during wheel confinement (paired *t*-tests, $P > 0.4$ by comparison with vehicle, in all cases; Fig. 3).

These data clearly demonstrate that melatonin does not affect phase shifts in response to behavioral arousal in the mid-subjective day. The dose used was well within the range (0.25–2.5 mg/kg) demonstrated to significantly increase extracellular melatonin levels in the anterior hypothalamus for up to 60 min [8]. Melatonin at these

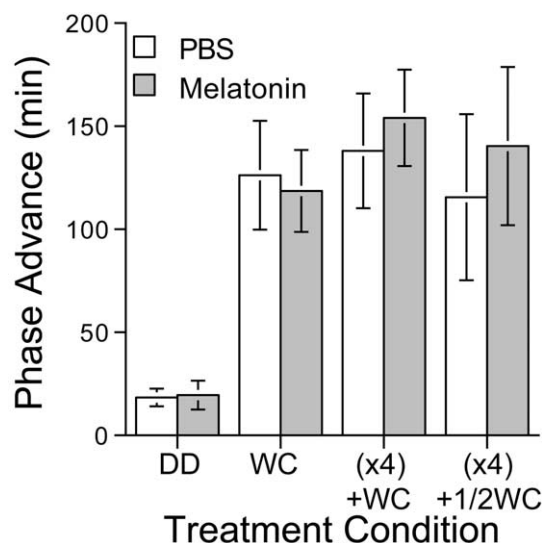


Fig. 2. Mean ($\pm$ SEM) phase shifts to each treatment condition. Open bar represents vehicle treatments; shaded bars represent melatonin (1 mg/kg). DD, injection alone at ZT5.5, with DD starting at ZT6; WC, injection at ZT5.5 with a 3 h wheel confinement (WC) at ZT6; (x4) + WC, injections at ZT5.5, 6, 7, and 8 with a 3 h WC at ZT6; (x4) + 1/2 WC, injections at ZT5.5, 6, 6.5 and 7 with a 1.5 h WC at ZT6.

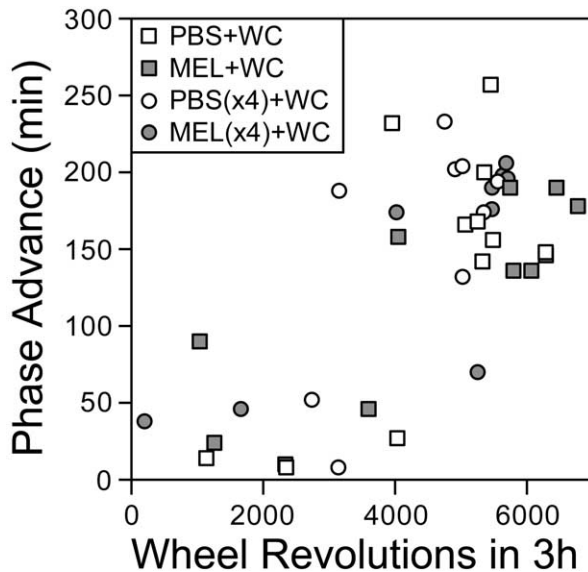


Fig. 3. Scatter plot depicting the relationship between activity levels during the 3 h wheel confinement (WC) and the resulting phase shift. Shaded symbols represent melatonin; open symbols represent vehicle; squares represent injection at ZT5.5 with WC; circles represent injections at ZT5.5, 6, 7, and 8 with WC.

supraphysiological levels has significant effects on hamster reproductive function [19]. To control for a short plasma melatonin half-life (<20 min), multiple injections at 30 or 60 min intervals were also utilized, and these were similarly without effect, even against a half-maximal (90 min) behavioral stimulus.

Melatonin was used here as a tool to further test the role of endogenous 5-HT in phase shifting to behavioral stimuli. Circadian rhythms of neural activity in the SCN slice *in vitro* are phase shifted during the mid-subjective day by the 5-HT_{1A/7} agonist, 8-OH-DPAT, an effect that is blocked by melatonin and other inhibitors of cAMP [16]. If serotonergic activation of cAMP in SCN neurons was necessary for the induction of phase shifts to behavioral arousal in the subjective day, then melatonin should attenuate these shifts. Failure of melatonin to have this effect is consistent with the results of other studies suggesting that 5-HT is neither necessary nor sufficient for non-photic clock resetting in hamsters. First, 5-HT receptor antagonists and neurotoxic lesions also fail to prevent non-photic clock resetting in hamsters *in vivo* [2,5,13]. Other studies employing drugs or neurotoxins to deplete forebrain 5-HT did report attenuation of phase shifting induced by activity in the mid-subjective day, but the results of these studies may be confounded by a treatment-induced reduction in the magnitude of the activity stimulus (for review, see [15]). Second, while 5-HT_{1A/7} agonists do induce shifts when administered systemically in hamsters (e.g. [20]), these shifts are either very small (~30 min; [6]) or absent when drugs are microinjected into the SCN area *in vivo* [14,15]. Third, significant

shifts are induced by microinjection of 8-OH-DPAT into the median raphe, a procedure that inhibits the release of 5-HT into the SCN [14], and no shifts are observed after injections of the autoreceptor antagonist, WAY100635, a procedure that induces 5-HT release into the hamster SCN [3]. These results, taken together with those of the present study, argue against serotonergic mediation of non-photic phase shifts in the hamster *in vivo*.

Large shifts to 5-HT *in vitro* remain to be fully explained. One possibility is a species difference. The rat SCN may be more responsive to 5-HT than the hamster SCN. Complicating this hypothesis is a growing corpus of data indicating that in at least some rat strains, 5-HT shifts the clock in the subjective night, but not in the subjective day (for reviews, see [11,15]). Another possibility is that the SCN slice is supersensitive to 5-HT or is lacking afferent inputs that normally constrain serotonergic shifting. Support for the former hypothesis is provided by the recent observation that reverse dialysis of 8-OH-DPAT into the SCN area for 3 h induces large phase shifts in hamsters pretreated for 2 days with the 5-HT synthesis inhibitor, pCPA [7]. Presumably, the 5-HT deafferented hamster SCN slice would also respond with phase shifts to 8-OH-DPAT.

Melatonin has demonstrated utility for entraining or modifying the phase of circadian rhythms in humans with entrainment disorders, and has also some efficacy in enhancing the rate of adaptation of circadian rhythms following transmeridian jet travel or shift rotation [4]. The results reported here suggest that these actions are not likely to be mediated by effects of melatonin on behavioral inputs to the clock.

Supported by operating grants to R.E.M. and graduate fellowships to M.C.A. from NSERC, Canada.

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