An arousing musically-enhanced bird song stimulus mediates circadian rhythm phase advances in dim light

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ABSTRACT

A musically-enhanced bird song stimulus presented in the early subjective night phase delays human circadian rhythms. This study determined the phase-shifting effects of the same stimulus in the early subjective day. Eleven subjects (ages 18-63, mean ± SD, 28.0 ± 16.6 yr) completed two 4-day laboratory sessions in constant dim light (<20 lux). They received 2 consecutive presentations of either a 2-h musically-enhanced bird song or control stimulus from 0600 to 0800 on the second and third mornings while awake. The 4-day sessions employing either the stimulus or the control were counterbalanced. Core body temperature (CBT) was collected throughout the study and salivary melatonin was obtained every 30 min from 1900 to 2330 on the baseline and post-stimulus/post-control nights. Dim light melatonin onset and CBT minimum circadian phase, before and after stimulus or control presentation was assessed. The musically-enhanced bird song stimulus produced significantly larger phase advances of the circadian melatonin (mean ± SD, 0.87 ± 0.36 h vs. 0.24 ± 0.22 h) and CBT (1.08 ± 0.50 h vs. 0.43 ± 0.37 h) rhythms than the control. The stimulus also decreased fatigue and total mood disturbance, suggesting arousing effects. This study shows that a musically-enhanced bird song stimulus presented during the early subjective day phase advances circadian rhythms. However, it remains unclear whether the phase shifts are due directly to the stimulus’ effects on the clock or if they are arousal- or dim-light mediated effects. This nonphotic stimulus mediates circadian resynchronization in either the phase advance or delay direction.

Keywords: Nonphotic; alerting; melatonin; core body temperature; phase shifts
A diverse set of nonphotic stimuli—including locomotor activity, dark pulses, triazolam, and olfactory information—phase shift and entrain circadian rhythms in nonhuman mammals and birds (reviewed in Refs. 21, 50, 52). Furthermore, stimuli containing sounds phase-shift the biological clock in birds and primates. Such stimuli include vocal cues from conspecifics (15, 24, 31, 47, 54, 56) as well as non-conspecific stimuli, such as buzzers, cage rattling, or white noise (26, 45, 54). The human biological clock also shows similar sensitivity: a musically-enhanced bird song stimulus phase delays circadian melatonin and temperature rhythms during the early subjective night (27).

Exercise (3, 6, 11, 13, 22, 51, 64), exogenous melatonin (39, 41, 44, 48, 59, 67), triazolam (10), food intake (40), and the duration and/or timing of sleep episodes (12, 20, 28, 29, 62), also all phase shift human circadian rhythms. Social stimuli also may entrain human circadian rhythms, though these studies are confounded by exposure to other cues such as the light-dark or sleep-wake cycles (2, 33, 35, 36, 65).

By convention, a phase response curve (PRC) describes phase changes produced by a particular stimulus as a function of presentation time. For example, light presented before temperature minimum induces phase delays, but produces phase advances after body temperature minimum in humans (34, 49, 63). Two other well-described PRCs in humans—those for melatonin and exercise—are distinct from the photic PRC and from each other. Exercise produces phase delays when presented before temperature minimum in the early subjective night and day but phase advances rhythms during the daytime or evening (3, 6, 11, 13, 22, 51, 64). By contrast, exogenous melatonin induces phase advances during the early subjective night and phase delays during the early subjective day (41, 44). Thus, PRCs for different nonphotic stimuli may not share common phase advance and/or delay zones.
The present experiment determined whether a musically-enhanced bird song stimulus—which produces phase delays in the early subjective night (27)—would also phase-shift circadian rhythms when presented after CBT minimum, in the early subjective day. To measure putative phase shifts, circadian phase of DLMO and of CBT minimum before and after stimulus or control presentation were assessed. It was predicted that the musically-enhanced bird song stimulus would elicit phase delays in circadian melatonin and CBT rhythms, based on the human exercise PRC.

MATERIALS AND METHODS

Subjects

Eleven subjects, 3 men and 8 women, ages 18-63 (mean age ± SD, 28.0 ± 16.6 yr) participated in this study. For all subjects, this was the first time they had participated in a laboratory protocol. This study used a similar telephone and in-person screening procedure as that described in a previous paper, with identical inclusionary and exclusionary criteria (27). Subjects maintained a habitual, individualized bedtime and wake time of approximately 8 h for at least 1 wk before each session, verified by sleep logs and daily call-ins at bedtime and upon awakening to an answering machine with time stamp. The prestudy stimulus and control session bedtimes (stimulus: 0004 ± 1.20 h; control: 0017 ± 1.18 h) and wake times (stimulus: 0743 ± 1.05 h; control: 0749 ± 1.23 h) were similar. Wesleyan University’s Institutional Review Board approved the study protocol and all procedures conformed to the Declaration of Helsinki. Subjects received monetary compensation for participation and signed informed consent before study entry.
Procedure

Subjects completed two 4-day laboratory sessions in constant dim light conditions (lighting conditions as described in Ref. 27), maintained throughout all waking periods following study entry, including during exposure to the musically-enhanced bird song and control stimulus. The sessions consisted of one baseline night (night 1), two stimulus/control nights (nights 2 and 3) and one post-stimulus/post-control night (night 4; Fig. 1), and were separated by 3-4 wks. Night 1 began at 1600. Subjects slept in darkness (0 lux) from 2400 to 1200 on nights 1 and 4 and from 2400 to 0545 on nights 2 and 3. They remained in bed in 0 lux if they awakened before the end of the sleep episode (either 1200 or 0545, depending on the session night).

On mornings 2 and 3, at 0545, subjects were awakened and then completed a self-rated mood questionnaire. They received 2 consecutive morning presentations of either the stimulus or control. The 4-day sessions employing either the stimulus or the control were counterbalanced. During the stimulus session, subjects received a 2-h musically-enhanced bird song stimulus (described below) from 0600 to 0800. Presentation conditions were as described in a previous paper (27). EEG was monitored online via polysomnography, with a technician present, on the administration mornings of the protocol. As revealed by EEG, no subject entered into Stage 1 sleep during either the stimulus or control presentations. To assess transient effects of the stimulus and control, subjects completed the Profile of Mood States (described below) at 0550, 0650 and 0750 on mornings 2 and 3. During the protocol, subjects refrained from naps, exercise, alcohol and caffeine, and spent most of the time engaged in recreational activities (e.g., reading, playing cards, watching TV). Subjects completed the study throughout the year.

--Insert Figure 1 about here--
Stimulus

The stimulus was a compact disc of bird song melodies, enhanced with a classical music background (Unison Music, Nashville, TN). Using a standardized player, the compact disc was played continuously for 2 h at 60 dB, intensity equivalent to normal conversation (32). At 60 dB, subjects have rated this particular stimulus as mildly weak to neutral in intensity and more pleasant than bright light using Likert scales (25).

Data Analyses: Circadian Measures

Salivary melatonin. Subjects provided 10 saliva samples at 30-min intervals, with timing of samples enforced by technicians, in dim light from 1900 to 2330 on nights 1 and 4 (Fig. 1). Dinner was completed at least 30 min before sampling began. During sampling, no food was permitted and water was permitted only within 5 min after each sample. Saliva (1.0 to 3.0 ml) was deposited into Salivette tubes (Sarstedt, Nümbrecht, Germany) using absorbent polyester swabs placed in the mouth for 5 min. Salivettes were immediately placed in, and remained stored at, -20°C pending laboratory assay.

The dim light melatonin onset (DLMO), a reliable marker of circadian rhythm phase (43), was defined as the first interpolated point (derived from between two points) at 3.0 pg/ml on the rising curve of melatonin concentration. Concentration levels remained above this threshold thereafter. A double-antibody radioimmunoassay was used (Bühlmann Laboratories AG, Allschwil, Switzerland). Samples of 200 µl were run in duplicate. The intra- and interassay coefficients of variation were <5% and <9%, at quality control levels of 1.6 pg/ml and 16 pg/ml, respectively. Absolute recovery was >95% with a lowest detectable quantity of 0.5 pg/ml.
Core body temperature. Core body temperature (CBT) was collected and stored in 2-min intervals throughout the study using disposable rectal thermistors (YSI Precision 400 Series, Yellow Springs Instruments, Co., Yellow Springs, OH) attached to Mini-loggers (Mini-Mitter, Co, Inc., Sunriver, OR). The thermistors were inserted at a 10-cm depth and secured with tape. A complex cosine fit, with a 12-h harmonic determined the phase of CBT minimum on nights 1 and 4. An $r^2$ value of $\geq 0.90$ was used as a goodness-of-fit criterion (variance accounted for). The data were not mathematically demasked, since CBT minimum occurred during each subject’s sleep cycle on nights 1 and 4 and thus was not obscured by waking activity (3, 4). Temperature demasking also increases the variability of CBT minimum estimates and reduces correlations between CBT minimum and other phase markers such as DLMO (7).

Mood measures

Profile of Mood States Questionnaire. The Profile of Mood States Questionnaire (POMS; 46) is a 65-item self-report scale that assesses transient affective states in response to various stimuli including sound-based cues (27, 60). The POMS has been validated in repeated measures designs (see review, Ref. 57) and tested with repeated acute sampling periods (e.g., 3 min; 46). Each item is rated on a scale from 0 to 4 (0, not at all; 4, extremely), on one of six factors: depression-dejection (Depression), tension-anxiety (Tension), anger-hostility (Anger), confusion-bewilderment (Confusion), vigor-activity (Vigor), fatigue-inertia (Fatigue). The total score for each factor is calculated by adding together the respective set of adjectives corresponding to that factor. The total mood disturbance score, a global estimate of affective state, derives from summing the factors together, with vigor-activity weighted negatively.

Statistical Analyses
Baseline circadian phase and phase shift data did not show normal distributions; therefore, the Wilcoxon nonparametric test (z) assessed DLMO and CBT minimum phase shifts at baseline (night 1) and after exposure (night 4) between the stimulus and control sessions and determined baseline differences in phase markers between sessions. Repeated-measures ANOVA examined differences in POMS scores for mornings 2 and 3 between sessions. Pearson product-moment correlation coefficient analyses (r) quantified the various relationships between DLMO and CBT minimum phases for the stimulus and control nights. The magnitude of session differences in phase shifts and POMS scores was expressed as effect size, d, the standardized difference between means (d = 0.3, small; 0.5, medium; 0.8, large; 17). Data are presented as means ± SD; P ≤ 0.05 was considered significant for all statistical analyses.

RESULTS

Circadian Rhythm Phase Shifts

Prestimulus. On night 1, the stimulus and control session DLMOs (2125 ± 0.80 h vs. 2138 ± 0.84 h; Z = -1.07, P = 0.29; r = 0.76, P < 0.01, n = 11) and CBT minima (0435 ± 1.29 h vs. 0450 ± 1.11 h; Z = -0.89, P = 0.37; r = 0.74, P < 0.05, n = 11) did not differ significantly and were significantly related. The mean timing of the musically-enhanced bird song and control stimuli did not differ significantly in relation to hours after baseline DLMO (9.58 ± 0.80 h vs. 9.36 ± 0.84 h; Z = -1.07, P = 0.29) or CBT minimum (2.42 ± 1.29 h vs. 2.16 ± 1.11 h; Z = -0.89, P = 0.37).

Poststimulus. The stimulus produced significantly larger mean phase advances than the control in DLMO (0.87 ± 0.36 h vs. 0.24 ± 0.22 h; Z = -2.93, P = 0.003; d = 2.12) and CBT minimum (1.08 ± 0.50 h vs. 0.43 ± 0.37 h; Z = -2.94, P = 0.003; d = 1.51). Individually, the stimulus
induced phase advances in CBT minimum and DLMO in 10 out of 11 subjects, while the control produced phase advances in 9 out of 11 subjects (Fig. 2). In addition, the phase change for DLMO and CBT minimum from night 1 to night 4 was significant for both the stimulus and control sessions (all P’s < 0.05). The timing of CBT minimum or DLMO and the magnitude of phase shifts were not significantly related in either session (control: CBT: $r = -0.22$, $P = 0.51$, $n = 11$; DLMO: $r = 0.19$, $P = 0.57$, $n = 11$; stimulus: CBT: $r = -0.29$, $P = 0.39$, $n = 11$; DLMO: $r = 0.02$, $P = 0.96$, $n = 11$).

**Pre- and Poststimulus relationships.** Within each session, night 1 and 4 DLMOs (control: $r = 0.97$, $P < 0.001$, $n = 11$; stimulus: $r = 0.91$, $P < 0.001$, $n = 11$) and CBT minima (control: $r = 0.96$, $P < 0.001$, $n = 11$; stimulus: $r = 0.92$, $P < 0.001$, $n = 11$) were significantly related.

---Insert Figure 2 about here---

**Relationships between circadian phase markers.** The stimulus and control sessions showed similar mean timing differences between DLMO and CBT minimum on night 1, with phase shifts of these markers correlating significantly (control: $r = 0.81$, $P < 0.01$, $n = 11$; stimulus: $r = 0.80$, $P < 0.01$, $n = 11$). These measures also related positively and significantly on nights 1 and 4 for both the control (night 1: $r = 0.91$, $P < 0.001$, $n = 11$; night 4: $r = 0.91$, $P < 0.001$, $n = 11$) and stimulus sessions (night 1: $r = 0.74$, $P < 0.01$, $n = 11$; night 4: $r = 0.61$, $P < 0.05$, $n = 11$).

**Mood Measures**

On morning 2, the stimulus decreased fatigue and total mood disturbance (TMD; a measure of overall affective state) compared with the control (fatigue: $1.23 \pm 1.70$ vs. $2.63 \pm$
DISCUSSION

A musically-enhanced bird song stimulus produced phase advances in DLMO and CBT minimum, with large effect sizes, compared with the control, when presented during the early subjective day after body temperature minimum. The stimulus was arousing and mood elevating, as evidenced by decreased fatigue and total mood disturbance scores. These data—along with a previous report of phase delays during the early subjective night (27)—indicate this particular stimulus may be an effective nonphotic phase-shifting cue in humans.

The present results extend findings from a previous study (27), in which early subjective night presentation of the musically-enhanced bird song stimulus phase delayed circadian melatonin and temperature rhythms. The data are consistent with studies showing phase-shifting effects of conspecific and nonconspecific sound-containing stimuli on circadian rhythms in birds and monkeys (15, 24, 26, 31, 45, 47, 54, 56). Notably, the reported phase advances are comparable in relative size to those produced by other nonphotic stimuli such as food intake, exercise and triazolam (3, 6, 10, 11, 13, 40, 64).

Since the stimulus produced phase advances, in contrast with the original prediction, the delay portion of the musically-enhanced bird song stimulus PRC does not extend from the early subjective night (27) through the early subjective day, as has been documented for exercise (13). The melatonin PRC (41, 44) differs from that for exercise and for this stimulus, indicating variability across human nonphotic PRCs. The phase advances, however, are consistent in direction with those produced by light (34, 49, 63). Thus, despite its similarity to both light and
exercise in the early subjective night with respect to phase delays (27), the musically-enhanced bird song stimulus PRC may be more similar to that of light. To determine whether this is the case, description of a complete human musically-enhanced bird song stimulus PRC, particularly at points 6-8 h after DLMO, when a putative crossover point may occur, must be described in future studies.

The musically-enhanced bird song stimulus may be arousing since on morning 2 it decreased fatigue by 1.4 points (by 53%) and total mood disturbance by 5.6 points (by 44%) relative to the control, though all scores fell within the average range for these measures. These results concur with another study in which stimulus presentation from 0100 to 0300 also decreased fatigue compared with the same control (27). An alerting stimulus could significantly increase cutaneous sympathetic nerve activity and produce vasoconstriction, which could lead to phase shifts (Refs. 5, 38). Other nonphotic stimuli also induce elevated activity/arousal that relate to circadian phase shifts (reviewed in Refs. 50 and 52).

At present, it remains unclear whether musically-enhanced bird song information directly affects the biological clock or whether it mediates phase shifts via increases in nonspecific arousal. Moreover, further studies must determine to what degree arousal occurs, using other measures, such as response latency tests, and other comparison controls, including various types of auditory stimuli. In addition, although arousal is one possible phase-shifting component of the stimulus, it cannot be ruled out that other stimulus components produce the observed circadian phase shifts. Since in other species sound-based stimuli without ecological content can phase shift circadian rhythms (45,54), individual or combined sound components of the stimulus (e.g., the bird song, the background music), rather than arousal, may be the salient cue.

It is possible, though unlikely, that pupillary dilation resulting from the musically-
enhanced bird song stimulus underlies the phase-shifting differences between the stimulus and control sessions. Although various sound-containing stimuli induce pupillary dilation, effects are nominal (19) or very brief (1), and show rapid habituation (1, 19, 61). Notably, one study failed to detect dilation at decibels comparable with the one used in this study (53). To investigate this question further, subsequent studies could examine the musically-enhanced bird song stimulus’ effects in subjects maintained under near darkness conditions (6, 20) or in the totally blind (42), as has been done for other nonphotic stimuli, or test subjects in the paradigm used by Cajochen et al. (16).

Circadian melatonin and CBT rhythms showed small phase advances with the dim light control. These advances are likely due to the protocol itself: on 2 mornings sleep termination occurred at 0545, thereby exposing subjects to dim light during the phase advance portion of the photic PRC (34, 49, 63). Indeed, early morning sleep termination has been shown to phase advance circadian rhythms in dim light (55). However, other experimental factors, such as inactivity or boredom also may underlie the phase advances observed in the control session.

At baseline, CBT minimum and DLMO were closely related in both sessions and also with each other, indicating internal phase marker stability and a consistent within-subject temporal relationship between markers (3, 7, 11, 39, 58, 63, 66). The direction and magnitude of DLMO and CBT minimum phase shifts also were positively related, demonstrating a direct effect on the circadian timing system, similar to other studies (8, 18, 20, 23, 30, 39, 58). DLMO occurred a little over 7 h before CBT minimum, at baseline and following exposure, with a positive relationship between the two markers, as has been reported previously (7, 9, 14, 23).

The musically-enhanced bird song stimulus potentially has important applications. Because it is easily administered, it may be advantageous in situations where light is improperly
timed or insufficient. It also may be useful for subjects who cannot tolerate bright light, such as the elderly, since it is effective in this population (this study; 27). Moreover, the stimulus may be a viable adjunctive to light during the early subjective day, a time of apparent overlap of the photic and musically-enhanced bird song stimulus PRCs.
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FIGURE LEGENDS

**Fig. 1.** Schematic representation of the study protocol. The 4-day sessions employing either the stimulus or the control were counterbalanced. Solid bars represent sleep time (0 lux) and open bars represent wake time in dim light (<20 lux). The checkered bars represent melatonin sampling (sampling occurred in 30-min intervals). The arrows indicate the 2-h administration time of the musically-enhanced bird song stimulus or control.

**Fig. 2.** Phase shifts as measured by (A) CBT minimum and (B) DLMO to two consecutive days of a 2-h presentation of the musically-enhanced bird song stimulus or control. Data points connected by lines represent the observed phase shift for individual subjects (numbered 1-11) in response to the stimulus or control. As per convention, advances are designated by positive numbers and delays by negative numbers on the ordinate.
Figure 1

![Image of a clock time diagram showing nights and days with specific times marked.](image-url)
Figure 2

A

CBT Phase Shift (h)

1 2 3 4 5 7 8 9 10 11

Stimulus
Control

B

DLMO Phase Shift (h)

1 2 3 4 5 6 7 9 10 11