Late-night presentation of an auditory stimulus phase delays human circadian rhythms

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Goel, Namni. Late-night presentation of an auditory stimulus phase delays human circadian rhythms. Am J Physiol Regul Integr Comp Physiol 289: R209–R216, 2005.—Although light is considered the primary entrainer of circadian rhythms in humans, nonphotic stimuli, including exercise and melatonin also phase shift the biological clock. Furthermore, in birds and nonhuman mammals, auditory stimuli are effective zeitgebers. This study investigated whether a nonphotic auditory stimulus phase shifts human circadian rhythms. Ten subjects (5 men and 5 women, ages 18–72, mean age 44.7 ± 21.4 yr) completed two 4-day laboratory sessions in constant dim light (~20 lux). They received two consecutive presentations of either a 2-h auditory or control stimulus from 0100 to 0300 on the second and third nights (presentation order of the stimulus and control was counterbalanced). Core body temperature (CBT) was collected and stored in 2-min bins throughout the study and salivary melatonin was obtained every 30 min from 1900 to 2330 on the baseline and poststimulus/postcontrol nights. Circadian phase of dim light melatonin onset (DLMO) and of CBT minimum, before and after auditory or control presentation was assessed. The auditory stimulus produced significantly larger phase delays of the circadian melatonin (mean ± SD, −0.89 ± 0.40 h vs. −0.27 ± 0.16 h) and CBT (−1.16 ± 0.69 h vs. −0.44 ± 0.27 h) rhythms than the control. Phase changes for the two circadian rhythms also positively correlated, indicating direct effects on the biological clock. In addition, the auditory stimulus significantly decreased fatigue compared with the control. This study is the first demonstration of an auditory stimulus phase-shifting circadian rhythms in humans, with shifts similar in size and direction to those of other nonphotic stimuli presented during the early subjective night. This novel stimulus may be a useful countermeasure to facilitate circadian adaptation after transmeridian travel or shift work.

nonphotic; melatonin; core body temperature; phase shifts; sensory stimuli

Light is considered the primary entrainer of circadian rhythms in mammals, including humans. Nonphotic stimuli, however, including various kinds of locomotor activity, dark pulses, and triazolam also phase shift and entrain circadian rhythms in rodents (reviewed in Refs. 55 and 59). Furthermore, in nonhuman mammals and birds, social communication signals, including olfactory and auditory sensory information, phase shift circadian rhythms (reviewed in Refs. 20 and 55).

Specifically, several studies in birds and primates indicate the biological clock is sensitive to auditory stimuli. For example, daily playback of conspecific vocalizations at fixed times of day in siskins, serins, and house sparrows (32, 52, 61), crowing from conspecific species in domestic fowl (64) and 12-h cycles of playback of conspecific vocalizations in chick-

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CBT minimum before and after auditory or control presentation was assessed. On the basis of the aforementioned nonphotic data, it was hypothesized that the human circadian system, like that of birds and nonhuman mammals, would respond to a nonphotic auditory stimulus. Specifically, it was predicted that an auditory stimulus would elicit phase delays in circadian melatonin and temperature rhythms, based on the human exercise PRC and on evidence that sensory stimuli induce phase delays when presented during the inactive phase in other diurnal species.

MATERIALS AND METHODS

Subjects

Ten subjects, 5 men and 5 women, ages 18–72 (mean age ± SD, 44.7 ± 21.4 yr) participated in this study. Subjects initially were screened by telephone and in-person interviews. Subjects with a history of psychiatric, endocrine, metabolic, or neurologic disorders, extreme morningness or eveningness (assessed by the Morningness-Eveningness Questionnaire; 35) or hearing impairments were excluded. Other exclusionary criteria included the following: use of central nervous system medications, history of smoking, irregular sleep/wake cycles, or shift work or travel across time zones for 2 nights. Subjects were screened by telephone and in-person interviews. Subjects with a morningness or eveningness (assessed by the Morningness-Eveningness Questionnaire; 35) or hearing impairments were excluded. Other exclusionary criteria included the following: use of central nervous system medications, history of smoking, irregular sleep/wake cycles, or shift work or travel across time zones for 2 months before study entry. In addition, women using oral contraceptives or with irregular menstrual cycles were excluded.

Subjects maintained a habitual, individualized wake-up time and bedtime of ~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: 0010 ± 01.34 h; control: 0026 ± 01.21 h) and wake times (stimulus: 0741 ± 01.12 h; control: 0747 ± 1.01 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, maintained throughout all waking periods following study entry, including during exposure to the auditory and control stimulus. Ordinary fluorescent lights in the laboratory did not exceed 16 lux measured at eye level and in the direction of gaze at any position as determined by a digital light meter. For both conditions, including during stimulus presentation, lighting exposure factors such as orientation toward the light source and intensity were identical. For both conditions, lighting exposure factors such as orientation toward the light source and intensity were identical. Furthermore, subjects could not control the lighting conditions at any point during the study.

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). The sessions 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 0300 to 1200 on nights 2 and 3. They remained in bed in 0 lux if they awakened before 1200.

On nights 2 and 3, subjects received two consecutive presentations of either the stimulus or control, with session order counterbalanced.

During the stimulus session, subjects received a 2-h auditory stimulus (described below) from 0100 to 0300. This time was chosen on the basis of studies in humans that found exercise produced phase delays when presented during the early subjective night (3, 10, 12, 74, 80). During presentation, subjects remained awake, with their eyes open, and remained seated in a chair. No other competing stimuli or activities were allowed during exposure. Infrared closed-circuit cameras and technicians monitored compliance. The control session was identical to the stimulus session except that subjects were informed they were receiving an auditory stimulus above the human hearing range (e.g., ultrasonic, frequency above 15 kHz); however, a blank compact disc was played instead. This procedure ensured subjects were equally attentive to the stimuli in both conditions and controlled for the possibility that phase shifts in the auditory condition could be caused by various protocol factors. To assess transient effects of the auditory stimulus and control, subjects completed the Profile of Mood States (described below) at 0050, 0150, and 0250 on nights 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 protocol throughout the year.

Auditory Stimulus

The auditory stimulus was a compact disc of bird song melodies, enhanced with a classical music background (Unison Music, Nashville, TN). Using a standard player, the compact disc was played continuously for 2 h at 60 dB, intensity equivalent to normal conversation (33). 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, under dim light conditions (described above) 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 (47), 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, Yellow Springs, OH) attached to Mini-loggers (Mini-Mitter, 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 always occurred during each subject’s sleep cycle and thus was not obscured by waking activity (3, 4).

**Mood measures**

**Profile of Mood States Questionnaire.** The Profile of Mood States Questionnaire (POMS; 51) is a 65-item self-report scale that assesses transient affective states in response to various stimuli, including auditory cues (69, 71). The POMS has been validated in repeated-measures designs (see review, Ref. 66). Each item is rated on a scale from 0 (not at all) to 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 (TMD), 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 (2) assessed DLMO and CBT minimum phase shifts at baseline (night 1) and after exposure (night 4) between the auditory stimulus and control sessions and determined baseline differences in phase markers between sessions. Repeated-measures ANOVA examined differences in POMS scores for nights 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; 16). Data are presented as means ± SD; $P \leq 0.05$ was considered significant for all statistical analyses.

**RESULTS**

**Circadian Rhythm Phase Shifts**

**Prestimulus.** On night 1, the stimulus and control session DLMOS (2119 ± 0.80 h vs. 2132 ± 0.86 h; $Z = -0.97$, $P = 0.33$; $r = 0.78$, $P < 0.009$, $n = 10$) and CBT minimums (0427 ± 1.26 h vs. 0444 ± 1.08 h; $Z = -0.87$, $P = 0.39$; $r = 0.71$, $P < 0.05$, $n = 10$) did not differ significantly and were significantly related. The timing of the auditory and control stimuli did not differ significantly in relation to hours after baseline DLMO (4.68 ± 0.80 h vs. 4.46 h ± 0.86 h; $Z = -0.97$, $P = 0.33$) or hours before baseline CBT minimum (2.45 ± 1.26 h vs. 2.73 ± 1.08 h; $Z = -0.87$, $P = 0.39$). Moreover, in all cases, stimulus presentation occurred after DLMO and before CBT minimum.

**Poststimulus.** The auditory stimulus produced significantly larger phase delays than the control in DLMO ($Z = -2.56$, $P = 0.009$; $d = 2.04$; Fig. 2A) and CBT minimum ($Z = -2.36$, $P = 0.02$; $d = 1.37$; Fig. 2B). Individual CBT and DLMO phase changes in response to the auditory stimulus and control are plotted in relation to CBT minimum and DLMO in Figs. 3 and 4, respectively. The timing of CBT minimum or DLMO and the magnitude of phase shifts were not significantly related in either session (control: CBT; $r = -0.10$, $P = 0.79$, $n = 10$; DLMO: $r = -0.07$, $P = 0.85$, $n = 10$; stimulus: CBT: $r = -0.44$, $P = 0.21$, $n = 10$; DLMO: $r = -0.02$, $P = 0.96$, $n = 10$; Figs. 3 and 4). Overall, the auditory stimulus produced phase delays in CBT minimum and DLMO in nine subjects. Similarly, the control stimulus produced phase delays in CBT minimum and DLMO in 9 and 10 subjects, respectively (Fig. 5).

**Pre- and Poststimulus relationships.** Within each session, night 1 and 4 DLMOs (control: $r = 0.98$, $P < 0.001$, $n = 10$; stimulus: $r = 0.90$, $P < 0.001$, $n = 10$) and CBT minimums (control: $r = 0.97$, $P < 0.001$, $n = 10$; stimulus: $r = 0.84$, $P < 0.003$, $n = 10$) were significantly related.

**Relationships between circadian phase markers.** The stimulus and control sessions showed similar mean timing differences between DLMO and CBT minimum on night 1, ranging on average from 7.13 to 7.19 h. Moreover, phase shifts of these
markers correlated significantly (control: $r = 0.76, P < 0.05, n = 10$; stimulus: $r = 0.74, P < 0.05, n = 10$). These measures also related positively and significantly on nights 1 and 4 for both the control (night 1: $r = 0.89, P < 0.002, n = 10$; night 4: $r = 0.87, P < 0.002, n = 10$) and auditory stimulus sessions (night 1: $r = 0.71, P < 0.05, n = 10$; night 4: $r = 0.69, P < 0.05, n = 10$).

Mood Measures

Fatigue scores showed a significant session effect on night 3, whereby the auditory stimulus decreased fatigue compared with the control ($7.03 \pm 5.39$ vs. $10.60 \pm 8.07$; $F(1,10) = 4.70, P = 0.05, d = 0.52$). There were no other significant differences in POMS measures.
DISCUSSION

An auditory stimulus produced significantly larger phase delays than the control in both DLMO and CBT minimum, representing the first demonstration of such phase shifts in humans. Furthermore, these results indicate that humans, like other species, show circadian sensitivity to sensory stimuli, and as such, they add to the growing database of nonphotic stimuli that phase-shift human circadian rhythms. Auditory stimuli may be beneficial in situations requiring circadian adaptation, which result from altered timing and changes in the light-dark cycle, such as transmeridian travel or shift work.

This study’s results extend findings obtained in birds and nonhuman mammals that demonstrate phase-shifting effects of various conspecific and nonconspecific auditory stimuli on circadian rhythms (14, 24, 26, 32, 50, 52, 61, 64). Furthermore, the observed phase delays in response to auditory presentation in the early subjective night are comparable in direction and/or relative size to those produced by other nonphotic stimuli in humans, including exercise (3, 6, 10, 12, 74) and triazolam administration (9).

In addition to exercise, the phase delays are consistent in direction with those produced by light. In humans, phase shifts to light occur according to a well-described PRC. Light presented before core body temperature minimum induces phase delays, while light presented after core body temperature minimum induces phase advances (36, 54, 73). However, despite an average phase shift of 1.16 h for temperature and 0.89 h for melatonin—and maximum shifts of 1.3–2.0 h—the magnitude of phase delays produced by the auditory stimulus was, on average, smaller than those produced by a single light pulse (31, 36, 54, 73). Direct comparisons between these two stimuli, however, must be qualified since the aforementioned studies used constant routines or free-running protocols, as well as longer light pulse durations and/or more frequent presentations. Such factors, as well as lighting intensity and wavelength, affect the magnitude of photic phase shifts (see Ref. 49). Indeed, when auditory phase shifts are compared with photic phase shifts obtained using protocols under similarly entrained conditions, circadian phase delays are of similar magnitude (e.g., 44, 62, 63, 78). Furthermore, auditory stimulus presentation at other circadian times may produce larger phase delays than those observed in this study. Despite significant phase delays, there was no relationship between the timing of CBT minimum or DLMO and the size of phase shifts. Such a lack of a relationship presumably is due to the small range of time points (3.5–4.0 h) during which the stimulus was presented (i.e., limited to early subjective night) and has been found in other studies using photic and nonphotic stimuli (3, 31). At present, it remains unknown whether an auditory PRC will include an advance region and/or dead zone when the stimulus is presented in the morning after CBT minimum or in the afternoon and early evening, before DLMO. Thus, despite its similarity to both light and exercise in the early subjective night, whether a complete auditory PRC will resemble light or exercise or neither stimulus awaits further investigation.

The mean phase delays of the circadian temperature and melatonin rhythms produced by the auditory stimulus showed large effect sizes compared with the control. Smaller mean phase changes were observed for DLMO compared with CBT minimum, as has been noted in numerous other studies using nonphotic and photic stimuli (19, 31, 42, 68, 80). DLMO is less susceptible than CBT minimum to masking effects, and it is therefore considered a more sensitive marker (38, 47, 48). Such decreased susceptibility is evidenced in this study by the smaller variation in DLMO phase changes, particularly in the auditory condition; other studies have also shown smaller variations in DLMO (3, 31, 38).

Both CBT minimum and DLMO were closely related at baseline in the control and stimulus sessions and also were closely related to each other, demonstrating internal stability for each phase marker and a consistent temporal relationship between markers within individuals (3, 10, 40, 67, 73, 80). Furthermore, the direction and magnitude of DLMO and CBT minimum phase shifts were positively related to each other, indicating a direct effect on the intrinsic circadian timing system, in concordance with other studies (e.g., 7, 18, 19, 23, 31, 40, 67). At baseline and postexposure, DLMO occurred between 7.13 h and 7.19 h before CBT minimum, with the two markers showing a positive relationship similar to previous reports from other laboratories (e.g., 8, 13, 23).

The dim light control protocol induced small phase delays (0.27–0.44 h) in the circadian melatonin and temperature rhythms, respectively. Various other laboratories using dim light conditions have reported phase delays of approximately similar size (a 0.2 h delay per day; 3, 6, 7, 10, 12, 19, 31, 67, 73). These delays are likely due to the slightly longer than 24 h endogenous human pacemaker period length (e.g., 18, 36, 79) and to the fact that subjects were required to remain in darkness.
until 12 noon, thereby missing the normal phase advance to morning light.

The auditory stimulus used in this study has several potential advantages over other nonphotic stimuli such as exercise. Exercise increases body temperature (10) and affects melatonin concentration (3, 10, 12, 15, 58); although unknown, it is unlikely that the auditory stimulus directly affects either of these physiological variables. Moreover, some subjects may find exercise strenuous or inconvenient and thus not feasible; by contrast, the auditory stimulus is easily administered. The auditory stimulus also may be beneficial for a heterogeneous population, since both young and older subjects showed phase delays; similarly, older individuals have shown phase-shifting responses to exercise (3). Further research using larger sample sizes is needed to determine whether the circadian phase-shifting effects of auditory stimuli differ by age; if no such differences exist, this auditory stimulus may be a viable, easily administered nonpharmacologic alternative to exercise or bright light in the elderly.

The results from this study suggest that environmental sounds may phase-shift light-dark and sleep-wake schedules in humans. However, it is unknown which particular component of the stimulus produces circadian phase shifts. In other species, auditory stimuli without ecological content phase shift circadian rhythms (50, 61); therefore, any sound component of the stimulus, and not necessarily the bird song melody, may be the salient cue. Thus sound may have confounded other circadian studies in which the timing of lights on and off, and therefore wake time and bedtime, was announced acoustically (see Ref. 77). Interestingly, auditory deprivation also affects circadian rhythms. For example, hearing-impaired children show a higher incidence of circadian rhythm disorders compared with nonimpaired children (60). In golden hamsters, cochlear lesions decrease locomotor rhythm amplitude and delay phase angles but do not alter the reentrainment rate to an 8-h phase advance or alter tau (17). Finally, the circadian respiratory rhythm amplitude (as measured by carbon dioxide emission) is higher in deaf compared with nondeaf mice (70).

The auditory stimulus decreased fatigue scores compared with the control stimulus on night 3, indicating that this particular stimulus may be arousing. An alerting auditory stimulus could significantly increase cutaneous sympathetic nerve activity and produce vasoconstriction, which could lead to reduced heat loss and phase delays of the circadian body temperature rhythm (see Refs. 5 and 41). Of interest, other nonphotic stimuli produce acute states of elevated activity/ arousal that relate to circadian phase shifts (reviewed in Refs. 55 and 59). It should be noted, however, that in a previous study, this particular auditory stimulus lowered POMS anger scores within 15 min of exposure (25) and was rated as mildly sedative (3.47 ± 0.18 on a 7-point Likert scale ranging from “very sedative” to “very stimulating”); N. Goel and G. Ettarou, unpublished observations). Differences in the two sets of results may be due to the timing of stimulus presentation: in the latter study, the stimulus was presented between 1900 and 2100, while in the present study, the stimulus was presented from 0100 to 0300, when subjects normally do not experience such cues. Thus whether the stimulus is arousing or relaxing remains to be clarified. Other possible putative mechanisms include nonspecific correlates of behavioral state such as hormonal changes (reviewed in Ref. 55).

At present, the neuroanatomical pathways mediating auditory stimuli’s effects on the circadian system are unknown. Auditory stimuli are transduced from the ear to the auditory cortex through a well-characterized multisynaptic pathway, including the cochlear nucleus, superior olivary nucleus, the inferior colliculus (IC), and the medial geniculate nucleus (27). Some of these nuclei have connections to the SCN in rodents. For example, the IC projects directly to the suprachiasmatic nuclei (SCN) in rats (43) and indirectly to the SCN via the lateral geniculate nucleus in common moles (21, 45). The intergeniculate leaflet (IGL) also projects directly to the SCN. The IGL mediates nonphotic stimuli such as wheel access and triazolam in hamsters (59); however, it does not mediate all nonphotic information, and thus its role may vary by species and/or stimulus (28). Notably, both the IGL and SCN in rats contain the orphan nuclear receptor, ROR×, which regulates genes whose products are involved in both sensory input integration and circadian timing (65). Finally, the paraventricular thalamic nucleus and raphe nucleus both project to the SCN directly in rats and have been implicated in nonphotic entrainment (1, 57).

Perspectives

Beyond providing information about the formal properties of the human biological clock, this study’s results have valuable applications for facilitating adaptation of circadian rhythms. The auditory stimulus used in this study may be useful for phase-advanced elderly subjects who cannot tolerate bright light treatment and may be advantageous in situations where light is improperly timed or insufficient, such as following transmeridian travel or shift work or during space travel. Furthermore, the auditory stimulus may benefit the blind and those with circadian rhythm-based sleep disorders, such as advanced sleep phase syndrome. Finally, the auditory stimulus may serve as an easily administered adjuvant to light or exercise, since these latter two stimuli also phase delay circadian rhythms during the early subjective night.

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