Circadian system of mice integrates brief light stimuli

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Van den Pol, Anthony N., Vinh Cao, and H. Craig Heller. Circadian system of mice integrates brief light stimuli. Am. J. Physiol. 275 (Regulatory Integrative Comp. Physiol. 44): R654–R657, 1998.—Light is the primary sensory stimulus that synchronizes or entrains the internal circadian rhythms of animals to the solar day. In mammals photic entrainment of the circadian pacemaker residing in the suprachiasmatic nuclei is due to the fact that light at certain times of day can phase shift the pacemaker. In this study we show that the circadian system of mice can integrate extremely brief, repeated photic stimuli to produce large phase shifts. A train of 2-ms light pulses delivered as one pulse every 5 or 60 s, with a total light duration of 120 ms, can cause phase shifts of several hours that endure for weeks. Single 2-ms pulses of light were ineffective. Thus these data reveal a property of the mammalian circadian clock: it can integrate and store latent sensory information in such a way that a series of extremely brief photic stimuli, each too small to cause a phase shift individually, together can cause a large and long-lasting change in behavior.

RESULTS

The mice (n = 86) were held under conditions of constant dark for periods of 1–7 mo. Under these circumstances, the free-running circadian rhythms of the animals were disrupted, with periods ranging from 22.5 to 24.5 h. In the presence of light, the circadian rhythms of the animals were entrained to the light-dark cycle, with periods of 24.0 h. The phase of the rhythms was determined by the time of day at which the light was turned on. In the absence of light, the rhythms were free running, with periods ranging from 22.5 to 24.5 h.

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constant environmental conditions, mice demonstrated circadian rhythms with a mean period of 23.5 h ranging from 23.2 to 24.3 h. Most individual mice showed characteristic circadian cycles with periods that varied from one day to the next by only a few minutes per day. Based on wheel size and revolutions per day, most mice ran 2–4 miles/day. Some long-distance runners ran as many as 15 miles during their active periods.

Each of the three multiple photic stimulus regimens described above produced phase shifts of up to several hours in the circadian wheel running rhythms (Fig. 1). These were permanent phase shifts that lasted as long as the rhythms of individual animals were followed or until the animal experienced another phase shifting stimulus. Both male and female mice showed changes in their circadian rhythms in response to these brief light stimuli.

In control experiments we found that single flashes of 2-ms duration were ineffective in causing phase shifts. This is similar to the previous finding in hamsters (12) that single pulses of 3 ms duration were ineffective in causing phase shifts. These data indicate that the circadian system is capable of integrating extremely brief light pulses falling as much as 1 min apart so as to interpret them as being the equivalent of continuous, long-duration photic stimuli.

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The effect of photic stimuli on circadian rhythms is typically investigated by exposing animals to flashes of light usually of durations ranging from minutes to 1 h at different times in the circadian cycle. Plotting the resulting phase shifts as a function of time of light exposure produces a phase response curve. A typical photic phase response curve for an animal held in constant dark would show no phase shifts during the subjective day, phase delays during the early subjective night, and phase advances during the late subjective night.

When the phase responses of all of the mice were plotted as a function of the phases of their rhythms when they received the stimulation, a typical mouse photic phase response curve resulted (Fig. 2A). During the period from circadian time (CT) 0000 to 1200 light flashes had little effect, consistent with the previous demonstration of this light-insensitive period of the circadian cycle (5). Between CT12 and CT18, light flashes caused a robust phase delay. Between CT18 and CT24 a modest phase advance was generated by the light flashes (Fig. 2A). Each species has its own typical phase-response curve (8). The one shown in Fig. 2A is typical for mice, where the phase advance is of smaller magnitude than the phase delay (3, 8). In Fig. 2B a substantial phase delay was generated by one 2-ms flash every 1 min for 1 h; little phase advance is seen. This lack of phase advance could be due to the small sample size during the critical phase advance period or to a decreased phase-advance sensitivity to the brief intermittent light stimulation.

The multiple flashes given between CT14 and CT18 caused a phase shift of 1.63 h (±0.33, SE) based on 11 mice during this period. These results are significantly different from the absence of a significant phase shift in seven mice that received a single flash during the same light-sensitive time period, CT14 to CT18 (t-test, P < 0.002).

Because the data from all trials in Fig. 2A create a typical mouse phase-response curve, it is unlikely that the observed phase shifts of individual animals were random events induced by stress or other perturbations. Occasionally, the same mice were exposed to nonphotic disturbances associated with cage cleaning and to control experiments in which foreign mice were introduced into the cages for 1 h. In no case did these disturbances cause phase shifts of the circadian wheel running rhythms of the experimental mice, suggesting...
we do not know if the 1-s pulses used were capable of producing large-magnitude phase shifts such as the ones shown in the present study. It is possible that a free running rhythm could entrain to a stimulus that would not be of sufficient magnitude to produce a maximal phase shift if it were delivered at appropriate circadian times. The present results differ from all preceding studies in that they demonstrate maximal (for the mouse) phase delays in response to extremely brief exposures to light, amounting to only 120 ms in total duration given 2 ms at time over 5 or 60 min. These results suggest that the total duration of light can be less important than the integration of time over which the light intermittently appears. Furthermore, the results indicate that neurons can store information derived from brief photic stimuli for at least 60 s and probably can accumulate and integrate the brief sensory information over a substantially longer period.

The observation that a series of 2-ms pulses of light causes a permanent alteration in the timing of circadian rhythms is an interesting case of temporal summation or integration of environmental information by the circadian system. The effect of this temporal summation is the induction of large and long-lasting phase shifts of the endogenous activity cycle. It is interesting to note that a phase shift means that the circadian system will respond differently to the same stimulus applied 24 h later than it would have responded if no phase shift had occurred. Thus the phase shift is a response modification that resulted from experience, which may be considered a simple form of learning (18). There are other instances in which long-term behavior modification has been associated with phase shifting of circadian rhythms. Circadian “aftereffects” were found in a paradigm in which animals were entrained to a short (22 h) light-dark cycle. After being released into constant conditions, they continued to show relatively short free running periods for several weeks (15). In another study, Pavlovian conditioning of the circadian system was demonstrated. When nonphotic sensory stimuli were paired with light cues, they became capable of inducing phase shifts when tested alone (1).

Fig. 2. A: combined data for several experiments, including both the stimuli at 1 flash and 5 flashes/5 s. Endogenous circadian time (CT) for each animal was defined on the basis of the start of running wheel activity being CT12. Light flashes caused either phase delays or modest advances depending on the mouse’s CT. Each point represents the phase shift in the circadian rhythm, comparing a control period 7 days before the light flash with the 7 days after the light stimulation. Little effect of light was found between CT0 and CT12, the subjective day for these nocturnal rodents, a time when they are inactive. Light flashes consistently caused a phase delay between 1200 and 1800. B: a single 2-ms pulse of light has little effect on the phase of the circadian rhythm. In striking contrast, a series of 2-ms photic pulses at 60 1-min intervals produced a substantial phase delay, but little apparent phase advance.

that the responses found here are selective to stimulation of the visual pathway.

DISCUSSION

What is remarkable about the results of this study is that maximal phase delays were produced with extremely brief total exposures to light. Previous experiments have commonly used 15-min periods of light, which were considered short, to generate maximal phase shifts in circadian rhythms (16). Experiments with hamsters demonstrated that pulses of strong light of 3-s duration produced relatively small phase shifts (12). In a study of nocturnal flying squirrels, 1-s light pulses at 24-h intervals were sufficient to entrain the animals’ activity rhythms (4). A phase-response curve was not reported in that study, however, and therefore we do not know if the 1-s pulses used were capable of producing large-magnitude phase shifts such as the ones shown in the present study.
activation, then a phase shift should be generated by a single flash, but in our experiments a single flash was ineffective in causing phase shifts. Thus the photoreceptors in the retina do not appear to be the site of integration of the trains of photic stimuli delivered in our experiments.

It is most likely that a central nervous system site, either the SCN or the intergeniculate leaflet of the thalamus, is responsible for the temporal summation of photic information observed in these experiments. Candidate mechanisms have been described that may act as a substrate for neuronal changes in response to photic stimulation. Neurmodulators that may be involved in photic signaling can exert long-term actions on both intracellular calcium levels and on electrical activity of SCN neurons, probably through G protein-coupled second messengers (13, 19). The mechanism for this neurmodulatory action appears to be a long-term depression of synaptic activity. Perhaps such a mechanism enables the SCN to integrate over time extremely brief stimuli that individually would be ineffective in inducing phase shifts.

This study points out what is probably a major adaptive difference between the visual system and the circadian system. It is adaptive for the visual system to register brief photic stimuli as discrete events to be maximally responsive to rapid changes in visual information. For the circadian system of a nocturnal animal, however, it is more important to be able to register the presence of low levels of light even when the animal is active in an environment (e.g., under tree or brush cover) that precludes continuous exposure to the stimulus.

There may be a practical application for the fact that the circadian system temporally integrates photic stimuli. Bright light therapy is being used to entrain the circadian system temporally integrates photic stimulation. Neuromodulators that may be involved in photic signaling can exert long-term actions on both intracellular calcium levels and on electrical activity of SCN neurons, probably through G protein-coupled second messengers (13, 19). The mechanism for this neurmodulatory action appears to be a long-term depression of synaptic activity. Perhaps such a mechanism enables the SCN to integrate over time extremely brief stimuli that individually would be ineffective in inducing phase shifts.

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REFERENCES