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The benzodiazepine triazolam phase-shifts circadian activity rhythms in a diurnal primate, the squirrel monkey (*Saimiri sciureus*)

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Triazolam can shift the phase of circadian rhythms in hamsters recorded in constant light or dark. This effect is apparently mediated by physical activity stimulated by the drug. We examined whether triazolam can shift the phase of circadian rhythms in a diurnal primate, the squirrel monkey, that is sedated by triazolam. Single injections of triazolam at 0.15–0.20 mg/kg induced phase advances or delays of monkey activity rhythms recorded in constant light. The phase–response curve is similar to that obtained for hamsters. Behavioral activation is evidently not necessary for a phase-shifting action of triazolam in this primate. A companion study in which triazolam was administered to squirrel monkeys in the dark in a light–dark cycle reentrainment paradigm failed to find evidence for a phase shifting action of triazolam. Shifts induced by triazolam in monkeys recorded in constant light may thus reflect changes in light exposure as a consequence of sedation or altered retinal processing of light.

Single injections of the benzodiazepines (BZDs) diazepam [6], midazolam [15] and triazolam [11] can shift the phase or lengthen the period (τ) [8] of circadian wheel-running rhythms of hamsters maintained in constant light (LL) or dark (DD). The direction and magnitude of BZD-induced phase shifts depend on the circadian phase at which the drug is administered; injections in the mid-to-late inactive phase of the wheel-running rhythm produce phase advances, whereas injections early in the inactive phase and throughout the active phase generally produce phase delays. The phase–response curve (PRC) thus described is similar to those for injections of neuropeptide Y [1], glutamate [9] and muscimol [10] and for 2–6 h dark pulses applied to hamsters in LL [3]. It differs from the PRC for light pulses [4] and the cholinergic agonist carbachol [5].

BZDs are notable in being the first pharmacological agent with a significant phase-shifting action that has the reasonable potential for human application. BZDs are already widely prescribed for their anxiolytic, antidepressant and hypnogenic properties but whether they can shift human rhythms is unknown. The present study was designed to assess whether triazolam can shift circadian rhythms of another diurnal primate, the squirrel monkey, using the rigorous methods of environmental control that are routinely applied in rodent circadian studies. After the study was initiated, it was discovered that phase shifts induced by triazolam in hamsters are dependent on a feedback effect of physical activity on the circadian clock [14]. Paradoxically, triazolam at the doses used triggers behavioral activity in hamsters; if wheel-running is prevented for several hours following the injection, no phase shift is ultimately observed. The present study thus has the added significance of using a species that is sedated, not activated, by moderate doses of triazolam.

Twenty four adult, male, wild caught Peruvian and Bolivian monkeys were used. Body weights ranged from 850 to 1100 g. The animals were housed individually in stainless-steel cages enclosed within ventilated wood isolation chambers. Each cage was equipped with one or two 8″–12″ perches. Movements on or off the perch were detected by microswitches that were monitored continuously by Apple11 computer. Data were summed and stored in 10 min intervals and analysed offline using an Atari or MacintoshII computer. Constant light was provided by cool white fluorescent tubes (30–60 lux). A white noise generator helped mask auditory communication among the 8–12 monkeys recorded in the room. Food and water were checked each day at variable times between 08.00 and 24.00 h.

Individual monkeys were recorded for up to 3 months. They received triazolam (dissolved in dimethyl sulfoxide,

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DMSO) or vehicle injections about every 3 weeks, given a suitably stable activity rhythm. To administer drug or vehicle, the animals were captured by hand or hook pole, weighed and immobilized manually. Injections were delivered intraperitoneally via a 1 ml syringe with a 25 gauge needle. The monkeys were returned to their cages and viewed periodically until recovered. After 3 months, the monkeys were transferred to vivarium cages in a group colony for at least one month. Some monkeys with good rhythms were reused. Animals receiving multiple triazolam injections were tested at different phases or doses.

Changes in phase and period of activity rhythms were detected using a program that fits lines with least square linear regression to activity onsets for 7–14 cycles before and after each injection. The first cycle after an injection was sometimes omitted if a shift was apparently not yet complete. Activity onset was defined as the first data point above an activity threshold after 4 h below threshold. The threshold was set according to the average amount of activity displayed by each monkey; usually it was 15 or 20 counts in a 10 min time bin. Phase shifts were calculated by extrapolating fitted lines for pre- and post-injection days to the injection day and measuring their displacement. Period changes were found from the slope of these lines.

Several initial injections served to establish a dose at which reliable behavioral effects could be observed. During the injection procedure the monkeys resisted handling and usually vocalized. A dose of 0.01 mg/kg had no observable sedating effect. At 0.05 mg impaired motor coordination and light sedation were usually apparent within 10 min. At 0.15 and 0.2 mg sedation was rapid and lasted 1–3 h. Motor coordination was poor and orienting responses to auditory or tactile stimuli were sluggish. One injection of 0.4 mg and one of 0.3 mg produced heavy sedation for up to 6 h.

A PRC for 38 injections of 0.15–0.20 mg and for 15 DMSO injections is presented in Fig. 1. Circadian time 0 (CT0) is defined as the onset of the animals' daily active period, which typically lasts 10–14 circadian hours (1/24th of τ). Injections in the mid-active phase (CT6–10) produced phase advances of the free-running activity rhythm. Injections in the monkeys' mid-inactive phase (CT17–22) produced phase delays. DMSO injections were associated with small advances or delays with no consistent relation to circadian time of administration. Examples of phase delays and advances are provided in Fig. 2. Phase shifts induced by DMSO were not readily detectable by eye in the actograms and presumably reflect the standard error about the regression lines for any given set of days. The mean phase shift ($\pm$ S.E.M.) to triazolam injections between CT6–10 was 2.08 $\pm$ 0.52 h, versus -0.22 ± 0.07 h for DMSO ($P<0.001$, t-test, 2-tailed). The mean shift to triazolam between CT17–22 was -1.65 ± 0.27 h, versus -0.18 ± 0.22 h for DMSO ($P<0.001$). Injections of 0.01 mg, 0.05 mg and 0.10 mg ($n=5$–7 at each dose) did not produce a significant mean phase shift at CT6–10, although the largest individual shift (+4.16 h) was observed after a 0.05 mg injection.

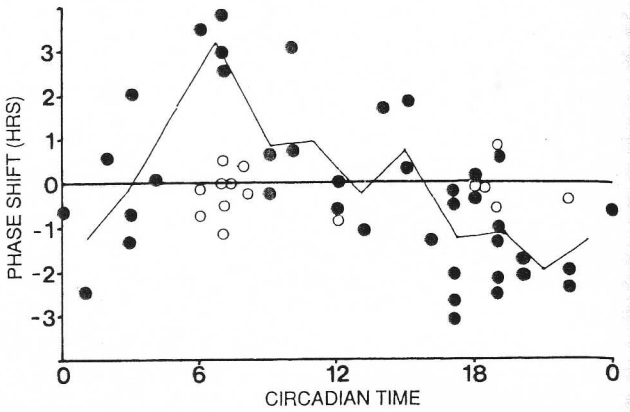

Fig. 1. Phase–response curve for triazolam (0.15–20 mg; ●) and DMSO (○). Phase shifts are plotted against circadian time of injection. Positive values represent advance shifts, negative values delay shifts. Circadian time 0 is the onset of the daily active period. Center weighted sliding average responses at CT0–1–2, 2–3–4 etc. for triazolam are connected by the solid line.

Small changes in τ following drug or vehicle injections were common. The mean τ change at CT6–10 was -0.18 h $\pm$ 0.19 h (lengthening of τ) for triazolam and -0.06 ± 0.11 h for DMSO ($P>0.1$), and at CT17–22 was -0.006 ± 0.13 h for triazolam and 0.06 $\pm$ 0.05 h for DMSO ($P>0.1$). The average lengthening of τ following triazolam at CT6–10 was significant ($P<0.05$) but this was skewed by a single large τ change of 0.84 h. One DMSO injection was also followed by a large lengthening of τ (0.34 h). There was no significant relation between the direction of τ and phase changes following injections.

This study demonstrates that acute administration of triazolam can shift the phase of free-running circadian rhythms of squirrel monkeys in LL. The shape of the PRC is consistent with that obtained for various BZDs in hamsters in LL or DD [6, 11, 15]. However, unlike hamsters, the squirrel monkey is sedated by the doses of triazolam used. Thus, it appears that the phase-shifting actions of triazolam in this species are not dependent on behavioral activity, as has been demonstrated for hamsters [14]. Nor can these actions be attributed to the acute stress of the injection procedure; vehicle injected animals were similarly stressed but did not exhibit significant mean phase shifts at CT6–10 or 17–22.

One animal did exhibit a relatively large τ change after a vehicle injection. This may reflect an effect of stress on the clock or it may reflect coincidence. We have observed

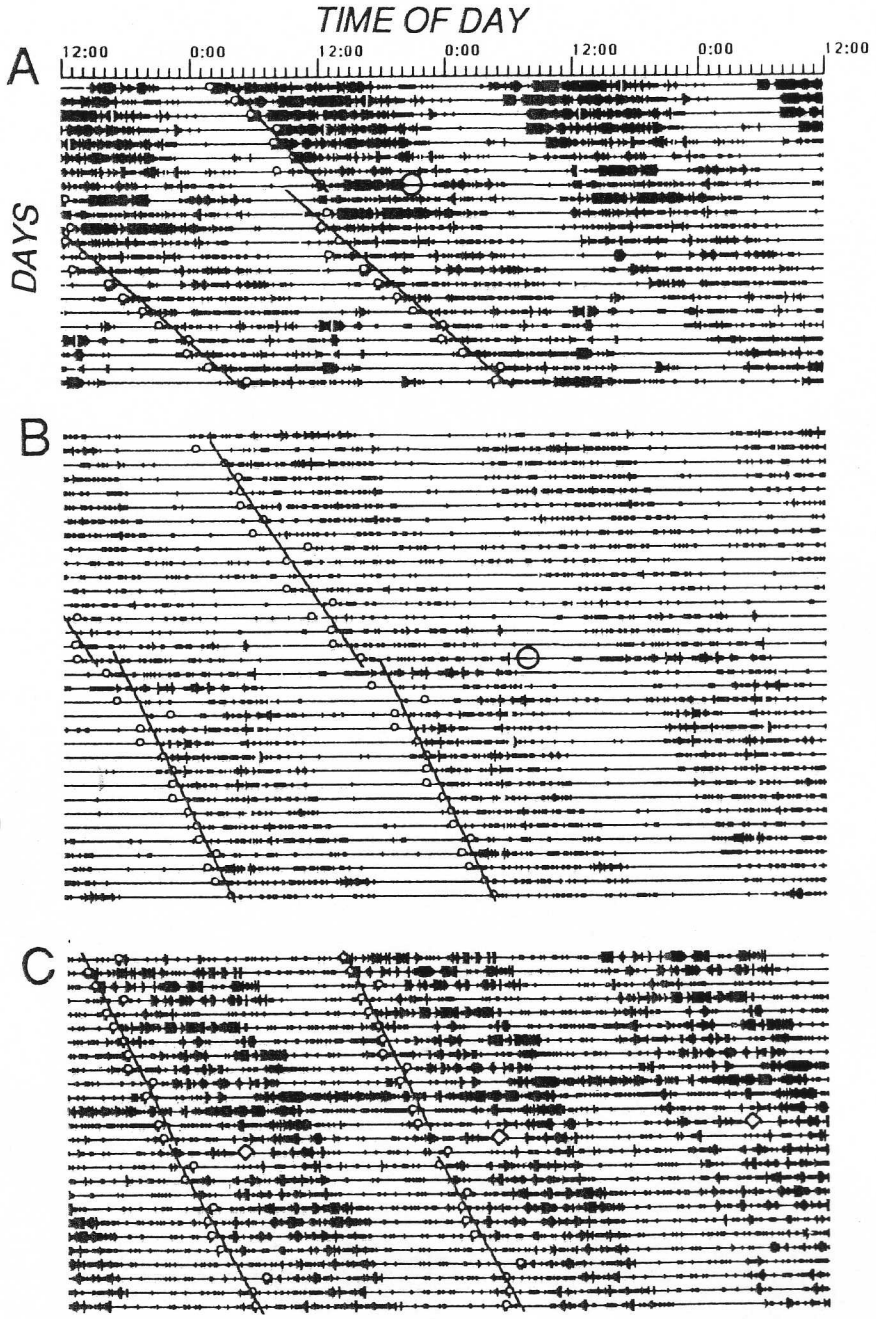

Fig. 2. Actograms of perch activity of three squirrel monkeys in LL. The charts are triple plotted; each line represents 3 consecutive days, and consecutive days are also aligned top-to-bottom. Time within a day is plotted left-to-right in 10 min bins. Vertical deflections from the lines represent time bins during which activity counts were registered. Higher deflections indicate more activity. Open circles represent the onset of the daily active phase as selected by computer algorithm. Solid regression lines were also fitted by computer. Time of injections are indicated by large open circles. A: a large phase advance shift (+4.16 h) to a 0.05 mg injection at CT8. B: a large phase delay shift (−2.1 h) to 0.20 mg at CT17. C: a small phase advance (+0.50 h) to DMSO at CT7.

several instances of large, abrupt, apparently spontaneous τ changes in squirrel monkey activity rhythms in LL.

Overall, changes in τ after triazolam injections were inconsistent. There was a trend for a lengthening of τ after injections at CT6–10 but no significant trend at other circadian phases. The lack of relation between direction of τ change and direction of phase shift is consistent with results from a similar study of hamsters [8]. This contrasts with the τ and phase changes that follow exposure of mice and hamsters in DD to 1 h light pulses; phase advances tended to be associated with τ shortening (D. Nelson, cited in ref. 8), phase delays with τ lengthening [4, 8]. The mechanisms for independent shifting of τ and phase of the circadian clock remain to be elaborated.

The ability of BZDs to shift the phase of circadian

rhythms raises the possibility that the drug could be used to accelerate the rate at which circadian rhythms adapt to a shift of the light-dark (LD) cycle. Accelerated reentrainment has been demonstrated in hamsters [13]; however, this is presumably mediated by the bout of wheel-running triggered by triazolam [14]. Studies of primates have failed to reveal a similar clear acceleration of reentrainment by triazolam. One study of humans reported that timed injections of triazolam provided only a transitory improvement in the reentrainment rate of cortisol and melatonin rhythms following an 8 h delay of the LD cycle [12]. A similar study of squirrel monkeys found no effect of triazolam injections on the rate of reentrainment to 8 h delays or advances of the LD cycle [2]. Injection times were selected based on the PRC presented here. The results would thus appear to be at odds with the present study, since no change in reentrainment rate implies that triazolam had no effect on the phase of the monkeys' clock. However, injections in that study were made at or after dark onset of the LD cycle, whereas all of the injections in the present study were made in LL. This suggests that the phase shifts observed here in response to triazolam may have resulted from changes in light exposure as a consequence of sedation (unlikely, since monkeys usually nap at CT6, but do not phase shift) or altered retinal processing of light [2, see also 7]. Possible tests of this hypothesis include repeating the triazolam PRC study using blind monkeys or monkeys maintained in very dim LL, or assessing the phase responses to 2–3 h of darkness in monkeys maintained in bright LL.

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