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Failure of Triazolam to Alter Circadian Reentrainment Rates in Squirrel Monkeys

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BOULOS, Z. AND T. A. HOUPT. *Failure of triazolam to alter circadian reentrainment rates in squirrel monkeys.* PHARMACOL BIOCHEM BEHAV 47(3) 471-476, 1994.—The short-acting benzodiazepine triazolam has been shown to cause phase-dependent phase shifts of the circadian activity rhythms of squirrel monkeys maintained in constant light. The present study sought to determine whether properly timed triazolam administration can also accelerate the reentrainment of circadian rhythms following phase shifts of the daily light-dark (LD) cycle. Circadian rhythms of telemetered body temperature and locomotor activity were recorded from squirrel monkeys exposed to an 8-h phase advance of the LD cycle, followed 16 days later by an 8-h phase delay. On the day of the phase advance, each animal received a single injection of triazolam (0.3 mg) or of vehicle alone in midsubjective day (circadian time 6 [CT6], where CT0 represents the time of light onset and the beginning of subjective day, and CT12 the time of dark onset and the beginning of subjective night]), 2 h after the new time of dark onset, while on the day of the phase delay the animals received triazolam or vehicle in late subjective night (CT20), just before dark onset. This procedure was then repeated, giving triazolam to animals that had previously received vehicle alone, and vehicle to animals that had received triazolam. The daily acrophases of the temperature and activity rhythms were calculated by cosinor analysis, and exponential functions were fitted to the acrophases that followed each of the phase shifts. The rates of reentrainment of the temperature and activity rhythms, defined as the time required for the exponential functions to reach 90% of the new steady-state phase of entrainment, were slower after the phase advance than after the phase delay, but did not differ significantly between drug and vehicle conditions. Since triazolam was administered either in darkness or just before dark onset, these results suggest that the phase shifting effects of triazolam in squirrel monkeys may be entirely light-dependent.

Triazolam Benzodiazepines Circadian rhythms Body temperature Squirrel monkeys

TRIAZOLAM is one of several benzodiazepines found to be effective in resetting the circadian rhythm of locomotor activity in hamsters [alprazolam (22), diazepam (2), midazolam (21), chlordiazepoxide (5), triazolam (14)]. Single injections of triazolam cause phase advances when administered during the middle of the hamster's subjective day, and phase delays when administered during subjective night and early subjective day (14). These effects are dose-dependent (15) and are obtained in constant light (LL), constant darkness (DD), and in blinded animals (14,17). Furthermore, administration of a single dose of triazolam in midsubjective day on the day of an 8-h phase advance of a daily light-dark (LD) cycle reduces the time required for hamsters to reentrain to the new cycle by about 50% (18). These properties of triazolam and other benzodiazepines, combined with their low toxicity, make them potential candidates for use in the treatment of human circadian disorders, including those associated with jet travel and with changes in shiftwork schedules.

Unlike the case in humans, where triazolam has sedative-hypnotic effects, in hamsters this short-acting benzodiazepine induces hyperactivity, and recent evidence indicates that at least some of the phase-shifting effects of triazolam are mediated by its effects on behavioral activity. Thus, immobilizing hamsters immediately after triazolam administration abolishes the phase-advancing and phase-delaying effects of the drug (19), whereas inducing hamsters to run in activity wheels at different circadian phases causes phase shifts similar to those caused by triazolam (7,10). Inducing locomotor activity in

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midsubjective day has also been shown to greatly reduce reentrainment time following an 8-h advance of the daily LD cycle (8,9).

In a recent study in squirrel monkeys, triazolam administration in LL caused phase-dependent phase shifts similar to those obtained in hamsters (6). Phase advances were obtained after injections in mid- to late subjective day, and phase delays after injections during subjective night and early subjective day. The phase shifts cannot be attributed to an increase in behavioral activity, as triazolam causes sedation in these diurnal primates. The aim of the present study was to determine whether properly timed triazolam injections are also effective in accelerating the reentrainment of circadian rhythms in squirrel monkeys following 8-h phase advances and phase delays of the LD cycle.

METHOD

Animals and Maintenance

Six adult male squirrel monkeys (*Saimiri sciureus*) weighing 725–1040 g at the time of surgery were studied. During experimental sessions, the animals were individually maintained in stainless steel cages (45 × 45 × 60 cm), each enclosed in a light-tight, sound-attenuating, ventilated wooden chamber. Lighting in the chambers was provided by a 22-W circular fluorescent lamp partially covered with electrical tape to reduce illumination intensity to 60 lx (as measured with a Gossen Luna-Pro light meter through a diffusing cap). Temperature in the experimental room was maintained as 22 ± 1°C, but the LD cycle induced a daily oscillation in chamber temperature, from 24–25°C during the dark to 28–29°C during the light. Continuous white noise in the room helped mask extraneous sounds.

The chambers were opened daily during the light portion of the LD cycle to allow visual inspection of the animals and to replenish food (Teklad diet TD76357, supplemented with fresh fruit) and water supplies. Litter was changed twice a week.

Data Collection

Body temperature and locomotor activity were recorded at 10-min intervals by telemetry, using battery-operated transmitters (Mini-Mitter model VM-FH disc with extended life batteries) implanted in the peritoneal cavity. The transmitters radiate a signal at a frequency proportional to temperature and an amplitude that varies with the animal's position in the cage (changes in signal amplitude above a preset threshold provide activity counts). The signals were picked up by receivers mounted on the rear wall of the cage and relayed through a switching matrix to a computer equipped with Dataquest III hardware and software. Additional analysis was performed on a Macintosh SE/30 computer.

Procedure

The monkeys were anesthetized with Halothane and the transmitters were surgically implanted in the peritoneal cavity under sterile conditions. The animals were allowed a minimum of two weeks for recovery, during which time they were kept in the colony room under LD 12 : 12 (L : 0800–2000, EST). They were then transferred to the isolation chambers and kept under the same LD cycle for 10 days. On day 11, the LD cycle was advanced by 8 h by shortening the duration of the daily light segment, and each animal received an IP injection of 0.3 mg triazolam in 0.5 ml dimethyl sulfoxide (Me_2SO) vehicle (n = 3) or of vehicle alone (n = 3). The injections were administered at 1400 (±0.5 h), 2 h after the new time of dark onset, with the aid of an infrared light source and viewer (FJW Optical Systems). On day 27, the LD cycle was delayed by 8 h by lengthening the duration of the light segment, and the animals received a second injection of triazolam or of vehicle alone between 1930 and 2000, just before dark onset. The two injection times therefore corresponded to circadian time 6 (CT6, where CT0 represents the time of light onset and the beginning of subjective day, and CT12 the time of dark onset and the beginning of subjective night) for the phase advance and CT20 for the phase delay. The animals were allowed 15 days to reentrain to the shifted LD cycle and were then returned to the colony room.

Three weeks later, the monkeys were put back in isolation chambers with the aim of repeating the procedure of the first experimental session, giving triazolam to animals that had previously received vehicle alone and vice-versa. Two days after the first phase shift/injection, however, one of the monkeys was found lying on the floor of the cage. It was treated with antibiotics and dexamethasone but died overnight (an autopsy report concluded that the animal died from acute peritonitis caused by leakage of intestinal contents in the abdomen, due either to accidental perforation of the ileum during the injection or to local intestinal necrosis caused by the large numbers of microfilaria found in the abdomen). The following day, a malfunction of the light-scheduling equipment caused a disruption of the LD cycle. The remaining five animals were therefore returned to the colony room, and the entire experimental session was repeated three weeks later. The animal that died was not replaced.

Data Analysis

The daily phases of the circadian rhythms of body temperature and locomotor activity were obtained by cosinor analysis, which is included in the Dataquest III software. A 24-h cosine function was fitted to consecutive 24-h data segments by least-squares, and the time of the peak of the fitted curve was used to index the daily acrophase of the rhythm. Reentrainment rates were estimated by fitting exponential functions to the acrophase for the day before the phase shift and to the eight acrophases that followed the phase shift (the acrophase for the day of the phase shift was omitted, as it would have been influenced by the direct effects of triazolam on temperature and activity) and calculating the time required for the fitted function to reach 90% of the new entrained steady-state, defined as the mean acrophase for the last five days of each condition. In the case of body temperature, exponential functions were fitted to the data from individual animals as well as to the group mean data. Daily locomotor activity patterns, however, were much more variable both between and within animals, and exponential functions were fitted only to the group mean data. Analysis of variance (two-factor repeated-measures design) was performed on the acrophase data, with each animal serving as its own control.

RESULTS

All monkeys showed stable entrained temperature rhythms with a daily range of 2–3°C. Body temperature started to increase 1–2 h before lights-on, reaching maximum levels shortly before the end of the light segment. The daily acrophases obtained by cosinor analysis preceded dark onset by about 5 h.

The temperature records of a representative animal during

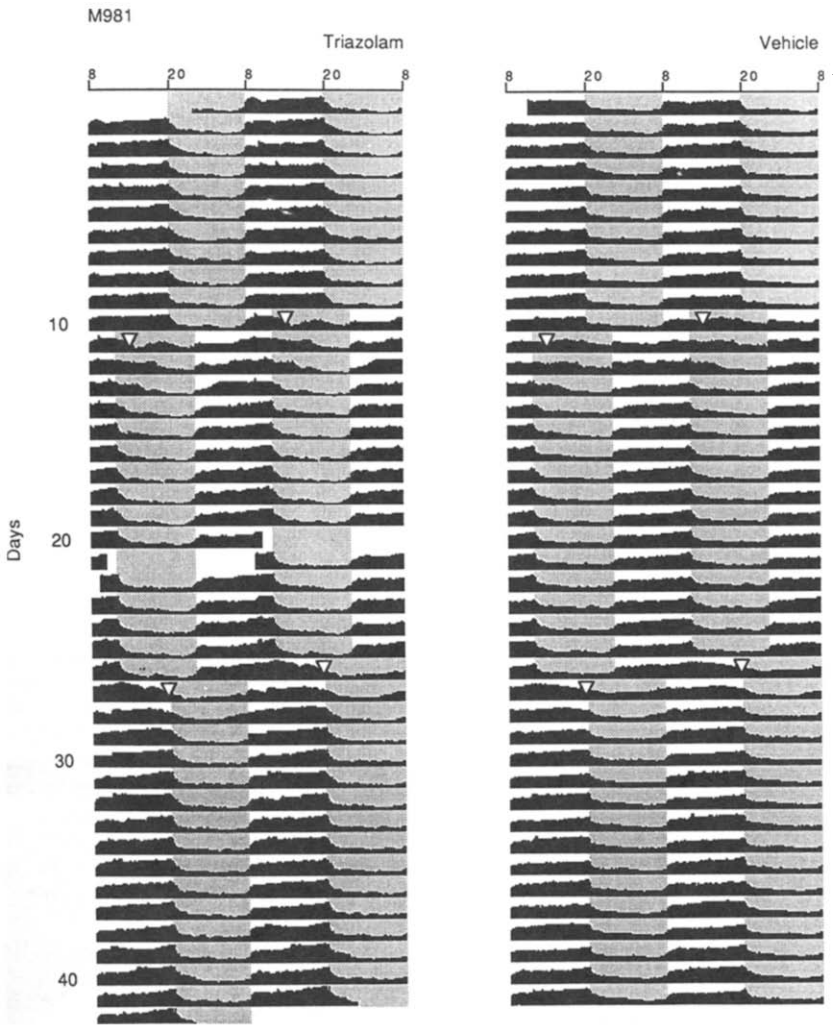

FIG. 1. Double-plotted records of body temperature for monkey M981 during triazolam and vehicle conditions. The daily light-dark (LD) cycle (hours of darkness indicated by shading) was advanced by 8 h on day 11 and delayed by 8 h on day 27. Injection times are indicated by inverted triangles. The gap in one of the records was due to recording equipment failure.

drug and vehicle conditions are shown in Fig. 1. Triazolam administration had a marked sedative effect which lasted 2–3 h. During that time, the monkeys were usually found lying on the cage floor, rather than in their typical sleep position (crouching with head tucked between the legs), and they showed little or no response to external stimulation (noise or touch). Sedation was accompanied by a lowering of body temperature, especially during the phase advance portion of the study when the injections were administered in the middle of the high temperature phase of the rhythm.

Light exerted a direct, masking effect on body temperature in all animals. Thus, on the first few days after the LD cycle was advanced light onset was immediately followed by a moderate increase in temperature, but temperature increased again several hours later, reaching its normal daytime level a little earlier each day. A similar, gradual advance was seen in the timing of the daily fall in temperature. Following the phase delay, temperature started to increase a few hours later on each of the next 3–4 days, but remained at a high level until dark onset.

The daily locomotor activity patterns were much more variable than those of body temperature and were subject to stronger masking effects by light and darkness. In general, however, reentrainment patterns of the activity and temperature rhythms were similar, and there was no indication of any dissociation between the two.

Figure 2 shows the mean daily acrophases of the temperature and activity rhythms during drug and vehicle conditions for the five monkeys that completed the study. In the case of temperature, the two sets of data are virtually identical. Analysis of variance on the first eight acrophases following the phase shifts showed a highly significant time effect ($p = .0001$ for both advances and delays), but no significant drug effect ($p > .05$) or Drug × Time interaction ($p > .05$) for either advances or delays. Similar results were obtained with the activity acrophases.

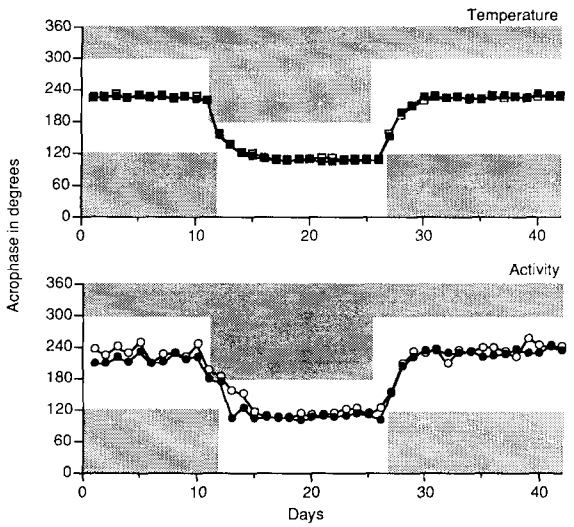

FIG. 2. Mean daily acrophases in degrees (0 degrees represents midnight) of the body temperature (top) and locomotor activity rhythms (bottom) for the five squirrel monkeys during triazolam (filled symbols) and vehicle conditions (open symbols). Shading indicates the dark portion of the light-dark (LD) cycle.

Reentrainment rates for the body temperature rhythms varied widely between animals, but within animals the values for drug and vehicle conditions were remarkably similar. For example, during the phase advance portion of the study monkey M982 was the fastest to reentrain after both triazolam and vehicle injections (1.98 days in both conditions), while monkey M990 was the slowest (7.90 and 8.10 days, respectively). The parameters of the exponential functions fitted to the mean daily acrophases of the temperature and activity rhythms under each condition are given in Table 1. For the group as a whole, advances of the temperature rhythms required 4.64 days after triazolam and 4.60 days after vehicle administration, while delays required 3.32 days (triazolam) and 3.61 days (vehicle). Corresponding values for the activity rhythms were 3.82 (triazolam) and 4.20 days (vehicle) for advances, and 3.71 (triazolam) and 2.89 days (vehicle) for delays.

Although the direct effect of light did not entirely mask the endogenous oscillation in body temperature, it could have affected the daily acrophase determinations sufficiently so as to obscure any effect of triazolam on reentrainment rate. To examine this possibility, we compared body temperature at the time of light onset following drug and vehicle administration. Since temperature in the entrained steady-state starts to rise before light onset, this measure provides an index of circadian entrainment that is not affected by masking. Figure 3 shows that temperature at lights-on decreased following the phase advance and increased following the phase delay, returning to its preshift level approximately eight and four days later, respectively. Even by this criterion, however, reentrainment rates after triazolam and vehicle administration did not differ significantly. Analysis of variance showed a strong time effect ($p = .0001$), but no significant drug effect or Drug × Time interaction ($p > .05$) for either advances or delays.

DISCUSSION

The failure of triazolam to accelerate circadian reentrainment was unexpected, as the injection phases (CT6 and CT20) corresponded to those at which injections in LL caused phase shifts of up to 3 h in magnitude (6). Based on the temperature data, vehicle-injected animals reentrained at an average rate of 1.7 h/day during the phase advance and 2.2 h/day during the phase delay and a 3-h triazolam-induced phase shift would therefore have reduced reentrainment time by one to two days in both conditions. The triazolam dose used in the present study (0.3 mg) was higher than that used in LL (0.2 mg), but it was chosen only after a pilot experiment failed to show any effect at the lower dose.

There is, however, another difference between the two studies: Our injections were administered either in total darkness or just before dark onset, whereas injections in the earlier study were administered in the light. There is evidence that the circadian effects of some benzodiazepines are strongly

TABLE 1

PARAMETERS OF EXPONENTIAL FUNCTION FITTED TO THE MEAN DAILY ACROPHASES OF THE BODY TEMPERATURE AND LOCOMOTOR ACTIVITY RHYTHMS

		A	k	C	t(90%)
Temperature					
Advance	Triazolam	117.08	−0.48	105.53	4.64
	Vehicle	118.08	−0.49	107.86	4.60
Delay	Triazolam	−120.40	−0.71	226.70	3.32
	Vehicle	−117.09	−0.65	225.46	3.61
Activity					
Advance	Triazolam	119.88	−0.53	105.20	3.82
	Vehicle	136.29	−0.43	114.10	4.20
Delay	Triazolam	−128.61	−0.75	229.80	3.71
	Vehicle	−108.16	−1.24	231.54	2.89

The exponential function $Ae^{kt} + C$ was fitted to the mean daily acrophases that followed an 8-h phase advance and an 8-h phase delay of the light-dark (LD) cycle for triazolam and vehicle conditions. In the equation, k is the time constant, t the time in days, and C the asymptotic value of the fitted function in degrees. Also shown is t (90%), the time in days required for the fitted function to reach 90% of the final steady-state acrophase.

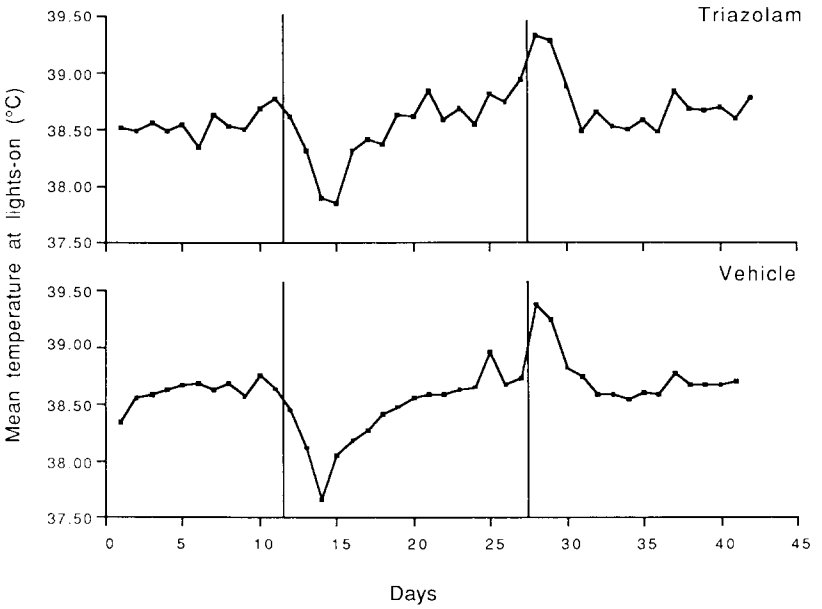

FIG. 3. Mean body temperature at lights-on for the five squirrel monkeys during triazolam and vehicle conditions. The days of the phase shifts are indicated by vertical lines (day 11 = phase advance, day 27 = phase delay).

light-dependent. The clearest example is diazepam, which causes phase-dependent phase shifts in hamsters maintained in LL (2) but has little or no effect in blinded hamsters (3). In the case of triazolam, the similarity between the phase shifts obtained in LL, DD, and in blinded hamsters would suggest that these effects are independent of lighting condition. However, this does not preclude the possibility that the circadian effects of triazolam in squirrel monkeys are light-dependent, since triazolam-induced phase shifts in hamsters are largely attributable to the stimulatory effects of this benzodiazepine on behavioral activity (19).

Triazolam has also been given to human subjects in an attempt to facilitate their adjustment to a shift in sleep–wake schedule. In one study (20), rotating shift workers were administered triazolam at bedtime on the first two days of their night shift tour. Triazolam increased sleep efficiency and total sleep time on these two days, but daytime sleep was not facilitated on the next two drug-free days. In another study (13), subjects received triazolam at bedtime on the first three days following a 12-h shift in sleep–wake schedule. Triazolam resulted in a significant improvement in sleep, as well as in alertness and performance during the shifted wake period, but the latter effect could have been due to the increase in prior sleep efficiency rather than to faster reentrainment rates.

Circadian reentrainment rates were studied in subjects given triazolam on the five days following an 8-h delay of their sleep–wake and light–dark schedule (16). On the first day, triazolam was given 3 h before the new bed- and dark onset time, and resulted in a delay in the end of the quiescent period for plasma cortisol and in the daily rise of plasma melatonin. On the next four days, triazolam was given at bedtime, but the differences between triazolam and placebo conditions on the third day were no longer significant, despite the fact that neither hormonal rhythm had fully reentrained by that time. Thus, the delay achieved on day 1 does not appear to have been a permanent or sustained phase shift.

Benzodiazepines have also been administered to transmeridian travelers in an attempt to alleviate symptoms of jet lag. Under these conditions, triazolam (11,12), midazolam (4), and temazepam (1) were all found to improve sleep, particularly after eastward flights. However, only one of these studies included circadian phase assessments, and that study provided no evidence that temazepam altered the rates of reentrainment of urinary 6-sulphatoxymelatonin and cortisol excretion rhythms (1).

In summary, the available data indicate that triazolam and other benzodiazepines may facilitate the adaptation to abrupt shifts in sleep–wake or light–dark schedules in humans by increasing sleep efficiency, but there is no convincing evidence in either human or nonhuman primates that triazolam accelerates the reentrainment of circadian rhythms.

ACKNOWLEDGEMENTS

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