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Serotonergic Stimulation and Nonphotic Phase-Shifting in Hamsters

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BOBRZYNSKA, K. J., M. H. GODFREY AND N. MROSOVSKY. *Serotonergic stimulation and nonphotic phase-shifting in hamsters.* PHYSIOL BEHAV **59**(2) 221–230, 1996.—Stimuli that make hamsters active, such as dark pulses or triazolam administration, also phase shift their circadian clocks, producing phase advances during the subjective day and phase delays during the subjective night. Activity or its correlate appears to be important in producing the shifts because preventing locomotion blocks the phase shifts associated with these stimuli. The physiological basis of clock resetting induced by activity is not fully understood. The serotonergic (5-HT) projection from the raphe to the suprachiasmatic nucleus (SCN) is a possible route by which nonphotic information could reach the pacemaker. Administration of 8-HYDROXY-2-(DI-*N*-PROPYLAMINO) TETRALIN HYDROBROMIDE (8-OH-DPAT), a 5-HT_{1A} and 5-HT7 receptor agonist, at circadian time (CT) 8 produces phase advances in the circadian rhythms of hamsters. Before concluding that 5-HT mediates the effect of activity on the pacemaker, it must be shown that 5-HT agonists do not produce shifts simply because they make animals more active. Therefore, we investigated the contribution of activity to 8-OH-DPAT-produced shifts. Preventing hamsters from moving around after administering 8-OH-DPAT did not abolish phase shifts. Moreover, higher doses of 8-OH-DPAT diminished activity on the day of injection but did not affect the amplitude of phase shifts. Surprisingly, quipazine (a non specific 5-HT agonist), when injected in the middle of subjective day did not phase shift the activity rhythm of hamsters, as it has been reported to do in rats.

Circadian Activity Serotonin Nonphotic Phase shift Hamster

INTRODUCTION

IN MAMMALS the main circadian pacemaker is the suprachiasmatic nucleus (SCN) of the hypothalamus (19,40). Light at appropriate times resets the pacemaker but nonphotic behavioral stimuli such as social interactions and cage changes can also affect the phase of the biological clock (24). The most powerful behavioral phase-shifting manipulation known is confining a hamster to a novel wheel for 2–3 h (26,28,36). The magnitude of these phase shifts is in the same range as those induced by light. Novelty-induced wheel running produces phase shifts primarily during the subjective day, when light is ineffective in this respect. Recent studies suggest that the neuropeptide Y (NPY) input to the SCN from the IGL (intergeniculate leaflet) is an important neural mechanism underlying nonphotic phase shifting (3,29). However anatomical, behavioral and electrophysiological studies suggest that it is possible that 5-HT is also involved in the nonphotic system.

The SCN receives serotonergic projections from the raphe nucleus (2,4,20). This serotonergic pathway might perhaps be involved in nonphotic phase shifts, since there is a correlation between spontaneous activity in the early part of the night and 5-HT metabolism in the SCN (7–9,16,38). It is possible that activity induced at other times could result in release of 5-HT.

The effects of 5-HT on the SCN in vitro are consistent with a role for 5-HT in nonphotic phase shifting. Quipazine (a non specific 5-HT agonist), and 8-OH-DPAT, have phase-shifting effects on the rhythm of firing rate in the SCN cells of rats (18,32,34,37). The phase-shifting effects of 8-OH-DPAT may be due to its action on the 5-HT7 and not the 5-HT_{1A} receptor subtype in the area of the SCN (14). By using tetrodotoxin , it was found that quipazine acts on clock cells directly (33). Moreover, the shape of the phase response curve (PRC) for quipazine is similar to that for nonphotic stimuli in vitro i.e., in both cases phase advances are produced during the subjective day, and phase delays during the subjective night. This similarity in the PRCs could be an indication of a similar mechanism of action (32).

Similar effects are found when 8-OH-DPAT is injected systemically in vivo in hamsters, which produces maximum phase shifts of about 1 h at CT 8 (42). However, the latter result has to

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be considered carefully before being accepted as evidence for a mediating role of 5-HT in nonphotic clock resetting. Increased activity and arousal can result in phase shifts (22,23), so the phase-shifting effect of a drug could be due to elevated activity, arousal, or some other correlate, rather than to a direct action of the drug on the pacemaker. This is known to apply to triazolam, a benzodiazepine whose phase-shifting effects are blocked when an animal's movement is restricted for a few hours after injecting the drug (25,43). Following 8-OH-DPAT administration there are signs of increased activity in some of the activity records published by Tominaga et al. (42). Therefore we investigated whether 8-OH-DPAT induced phase shifts are mediated by increased activity levels.

In the present paper we describe experiments in which phase shifting after 8-OH-DPAT treatment was measured in hamsters prevented from moving around by confinement to the nest box after they received the drug (Experiments 1 and 2) and by injecting doses of 8-OH-DPAT high enough to reduce activity (15) (Experiment 4). We verified that our method of restriction, confinement to a nest box, was capable of blocking phase shifting at CT 8 to another activity-inducing drug, triazolam (Experiment 3). Finally, we investigated whether systemic administration of quipazine would produce phase shifts in hamsters (Experiment 5) as it has been reported to do in rats (6).

GENERAL METHODS

ANIMALS AND HOUSING

For all of the experiments, male Syrian hamsters (*Mesocricetus auratus*) were purchased from Harlan Sprague–Dawley (Indianapolis, IN). Upon arrival in the laboratory the animals were 40 days old except where noted. In Experiments 1–3 and 5, hamsters were housed individually in transparent plastic-walled cages equipped with a running wheel (17.5 cm in diameter), living area, and a small nest box which could be closed by inserting a plastic door (Fig. 1). The dimensions were the same as shown in the diagram of Mrosovsky and Salmon (25) except that the entrance to the wheel was 9 cm above the foor of the cage rather than level with it. In Experiment 4, animals were housed in individual plastic cages ($44 \times 23 \times 20$ cm) and had continuous access to a running wheel 17.5 cm in diameter. Food (Purina 5001 Rodent Chow) and water were continuously available. The range of temperatures was 20–23°C. Illumination was measured with a Gossen Lunasix 3 light meter. The illumination in the living cage during the light phase was about 50 lx in all of the experiments.

Data Recording

Wheel revolutions were detected by a magnetic micro-switch, and recorded in 10 min bins by a computer using the Dataquest III (Mini-Mitter Co., Inc., Sunriver, OR) hardware and software. Actograms for all the animals were generated using standard upper and lower limits on the y axis. Activity for each 10 min bin was quantified in one out of 15 levels: the first level was for 1–55 revolutions, the second level for 56–110 revolutions, etc. Data were not clipped (27).

Data Analysis: Type I Experimental Design

Animals were tested in (DD) Aschoff (1). Activity onset was defined as the first 10 min bin with at least 56 wheel revolutions which was followed by another bin with 56 or more revolutions within 40 min. A regression line was calculated for the 7 daily activity onsets (1–7) immediately preceding the injections. A second regression line was calculated for 7 consecutive onsets (11–17) following the injection. The three onsets (8–10) immediately following the injection were not included in the analysis, to minimize the effects of possible transients. The phase shift was defined as the difference between onset 8 (the first posttreatment day) predicted by extrapolation of the prepulse regression line and onset 8 predicted by backward extrapolation of the postpulse regression line.

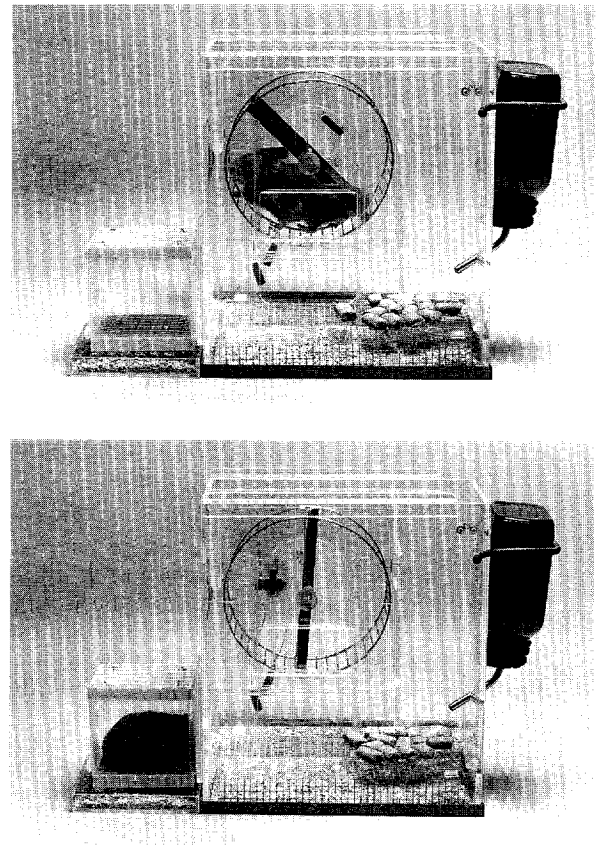

FIG. 1. Cage type used in Experiments 1–3 and 5. (a) hamster free to move in its cage; (b) it is confined to its nest box.

Data Analysis: Type II Experimental Design

Animals were kept in a 14:10 light:dark (LD) cycle until entrainment had been achieved (1). On the day of the experimental manipulation, the lights were turned off at the time of, or shortly after, treatment administration. This procedure is an efficient way to administer drugs to a large number of animals at both the same clock time and circadian time, because the phase angle of entrainment (activity onset relative to dark onset) is generally similar in animals kept under the same LD cycle. Phase shifts were calculated by comparing the onset of activity on the day before experimental treatment to the onset of activity on the first day following treatment.

Statistical Analysis

Significance was tested using two-way ANOVA (Experiments 1–3) or one-way ANOVA (Experiments 4–5), and unpaired student t-tests (two-tailed), with Bonferroni corrections when appropriate.

EXPERIMENT 1

EFFECTS OF ACTIVITY RESTRICTION ON THE PHASE-SHIFTING EFFECTS OF 8-OH-DPAT IN AN ASCHOFF TYPE II EXPERIMENTAL DESIGN

The purpose of this experiment was to look for a possible mediating role of activity, or a correlate, in the phase shifts produced by 8-OH-DPAT. The method used was to confine hamsters to their nest boxes after they received the drug.

Method

Experimental procedure. Animals were kept on a 14:10 LD cycle from 40-60 days of age. At 60 days of age animals received an intraperitoneal (IP) injection at approximately CT 8. Each animal was weighed two days prior to being injected. To specify CT 8 on the day of injection, the time of each animal's activity onset was determined from the activity record of the preinjection day; this time was taken as CT 12 on the day of injection. Animals were injected in the order of their activity onsets. The animal with the earliest activity onset was injected first; this injection was made 4 h prior to the midpoint in the range of the animals' activity onsets.

The animals were randomly divided into 4 groups ($n = 6$ per group). Groups 1 and 2 were injected with 8-OH-DPAT (Research Biochemicals International), dosage 5 mg/kg in 0.9% saline (0.85 ml/kg), and returned to their cages. Animals in Group 1 were free to move in their cages; animals in Group 2 were confined to their nest boxes until 4 h after the last injection. Groups 3 and 4 were injected with 0.9% saline (0.85 ml/kg). The animals were then returned to their cages. Hamsters in Group 3 could move freely in all parts of their cages; Group 4 animals were confined to their nest boxes until 4 h after the last injection. Following the last injection, the lights were turned off, and the animals remained in DD for 5 days. After that, the pretreatment LD 14:10 cycle was reinstated. The animals were reentrained to this cycle over the next 10 days. At the end of this period a cross-over experiment was conducted. Drug treatments were reversed for the previously saline and 8-OH-DPAT groups, however the confined and unconfined conditions remained the same. All other procedures were the same as those in the first part of the experiment.

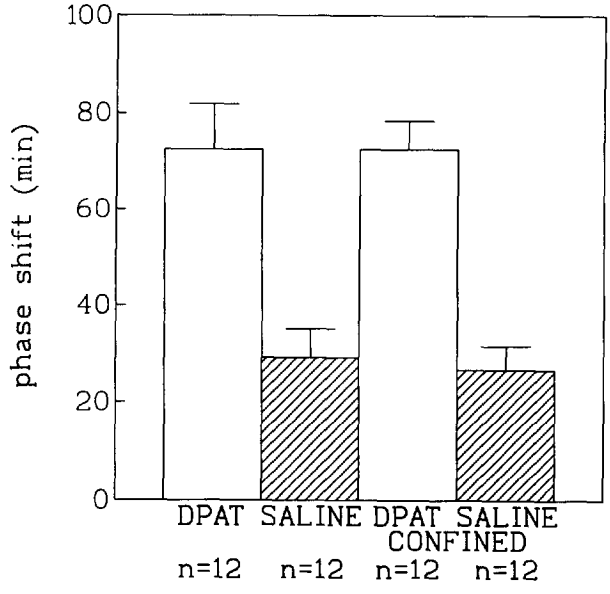

FIG. 2. Mean phase shifts ± SEMs for Experiment 1 (Aschoff type II design).

Activity quantification. Least Squares Linear Regression was used to determine whether a correlation existed between the total wheel revolutions within the 2-h posttreatment period and phase shifts in the activity rhythms. This 2-h interval was selected to exclude any activity which might possibly be a part of an immediately phase-advanced activity onset produced by the experimental manipulation. This assumes phase advances do not exceed 2 h [see Tominaga et al. (42), Fig. 2].

RESULTS

The effect of condition (non confinement vs. confinement to the nest box) on the magnitude of phase shifts was not significant ($F = 0.04$, $p = 0.85$, two-way ANOVA; Fig. 2). However, the effect of treatment (saline or 8-OH-DPAT injections) was significant across the four groups, ($F = 45.22$, $p = 0.0001$). 8-OH-DPAT produced phase shifts which were significantly different from the saline group ($t = 3.43$, $p < 0.008$ after Bonferroni correction for six comparisons).There was no correlation between total wheel revolutions in the 2 h postinjection period and the magnitude of phase shifts for the 8-OH-DPAT injected group ($r = -0.46$, $p = 0.13$, least squares linear regression, two-tailed). Also, there was no such correlation in the saline injected group ($r = -0.11$, $p = 0.74$). Activity records for animals in each treatment group are shown in Fig. 3.

EXPERIMENT 2

EFFECTS OF ACTIVITY RESTRICTION ON THE PHASE-SHIFTING EFFECTS OF 8-OH-DPAT IN THE ASCHOFF TYPE I EXPERIMENTAL DESIGN

Experiment 1 demonstrated that the phase-shifting effects of 8-OH-DPAT are not mediated through activity, using the Aschoff Type II experimental design in which animals were transferred from LD to DD following the drug injection. The saline injected control group shifted about 30 min in that experiment, which could either be due to the phase-shifting effects of saline (11,17,41) or the change from LD to DD. If this phase shift is due to the change in lighting conditions in the Aschoff Type II design, then approximately 30 min of the phase shift produced by 8-OH-DPAT could also possibly be attributed the LD to DD transition. However, it is conceivable the effects of the drug and the transition from LD to DD do not interact in an additive way. Maybe this transition in some way amplified the effect of the drug. The purpose of the next experiment was to determine the effects of activity restriction on the phase shifts produced by 8-OH-DPAT, in absence of changes in lighting, in the Aschoff Type I design.

Method

Subjects. Upon arrival in the laboratory the animals were 20 days old. These hamsters were previously used in an experiment in which they received a 3 h exposure to a novel wheel. No drugs were administered to these animals previously.Experimental design. Animals were in LD 14:10 from 64–74 days of age. They were then put into DD for 14 days prior to receiving IP injections starting at CT 8. Each animal was weighed on the last day of the LD cycle. CTs were calculated with reference to onset (CT 12) on day 8, the day of injection, as predicted by extrapolation of the prepulse regression line. For each animal CT 8 was 4

circadian h prior to the extrapolated onset on day 8. Each circadian hour was calculated by dividing the free-running period (τ), which indicates the slope of the prepulse regression line, by 24.

The animals were randomly divided into 4 groups. The treatment groups, and injection procedures were the same as in Experiments 1 and 2. Three groups had $n = 6$. However the group free to move in its cage following an injection of 8-OH-DPAT had $n = 5$, since one animal died of unknown causes. The animals remained in DD for 10 days after the injection. At the end of this period a cross-over experiment was conducted. Both the confined vs. unconfined conditions and the injected substances were reversed for each group.

Activity quantification. Activity was quantified in the 2 h postinjection interval as described for Experiment 1.

RESULTS

The effect of condition (non confinement vs. confinement) on the magnitude of phase shifts was not significant ($F = 0.41$, $p = 0.53$, two-way ANOVA; Fig. 4). However, the effect of treatment (saline or 8-OH-DPAT injections) was significant across the four groups, ($F = 36.4$, $p = 0.0001$, two-way ANOVA). 8-OH-DPAT produced phase shifts which were significantly different from the saline group ($t = 4.08$, $p < 0.008$ after Bonferroni correction for six comparisons). There was no significant correlation between the number of wheel revolutions in the 2 h postinjection interval and the magnitude of phase shift in the 8-OH-DPAT injected group ($r = 0.31$, $p = 0.35$),least squares linear regression, two-tailed. Also no significant correlation was found for the saline injected group ($r = 0.39$, $p = 0.20$). Activity records for animals in 8-OH-DPAT and 8-OH-DPAT/confined groups are shown in Fig. 5.

EXPERIMENT 3

EFFECTS OF ACTIVITY RESTRICTION ON PHASE-SHIFTING EFFECTS OF TRIAZOLAM AT CT 8 IN THE ASCHOFF TYPE II DESIGN

Nest box confinement was the method used in Experiments 1 and 2 to prevent activity following saline and 8-OH-DPAT administration. This method has been found to be effective in blocking phase shifts produced by triazolam given at CT 6 (25).

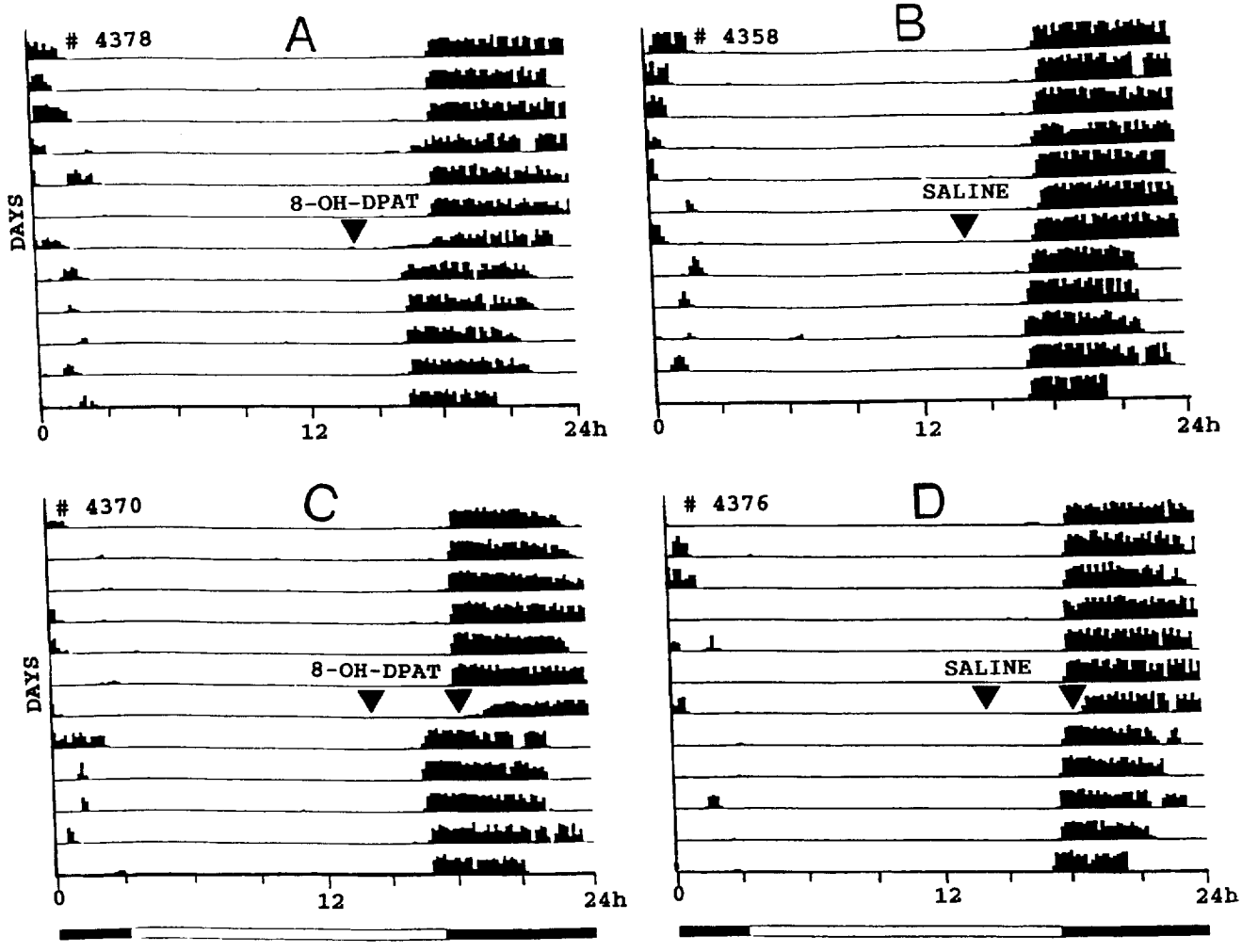

FIG. 3. Actograms illustrating phase shifts after 8-OH-DPAT or saline administration at CT 8. Times of injection are indicated by an arrow on day 7. DD started immediately after all the animals were injected. Animals in actograms (A) and (B) were free to move in their cages, those in (C) and (D) were confined to a nest box for 4 h after the injection. For actograms (C) and (D) the first arrow indicates the time of injection and start of confinement. The second arrow indicates the time at which the animal's nest box was opened.

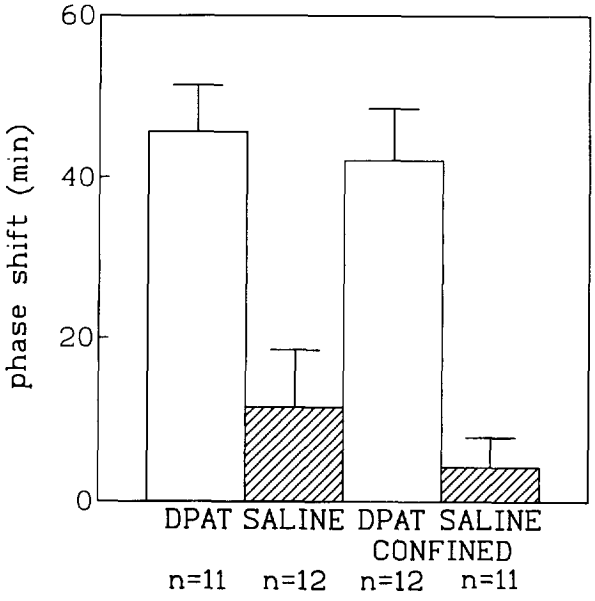

FIG. 4. Mean phase shifts ± SEMs for Experiment 2 (Aschoff type I design).

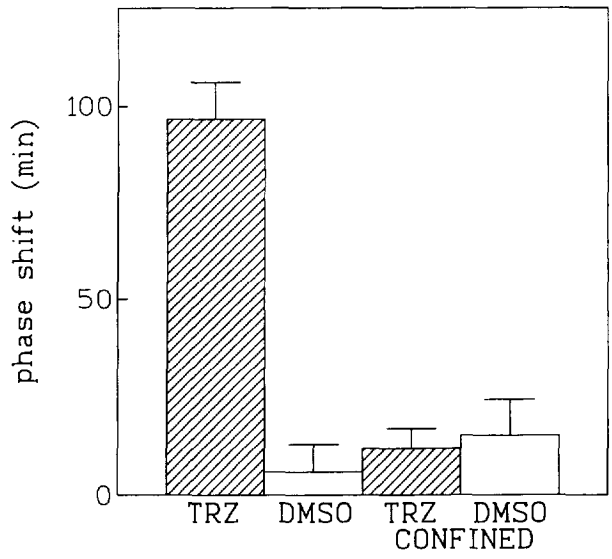

FIG. 6. Mean phase shifts ± SEMs for Experiment 3.

However, those data were not extensive. The purpose of the present experiment is to verify that also at CT 8 nest box confinement is a good method of blocking phase shifts associated with activity inducing stimuli.

Method

Procedure. The animals were in LD 14:10 from 40–54 days of age. When the animals were 54 days old, they received an IP injection at about CT 8. Injection and weighing procedures were the same as in Experiment 1. The animals were randomly divided into 4 groups ($n = 6$ per group). Groups 1 and 2 were injected with triazolam (Upjohn, MI), dosage 5 mg/kg in dimethyl sulfoxide (DMSO), 0.85 ml/kg. Groups 3 and 4 were injected with DMSO (0.85 ml/kg). The animals were then returned to their cages. Hamsters in Groups 1 and 3 could move freely in all parts of their cage, whereas those in Groups 2 and 4 were confined to their nest boxes for 4 h from the time of the last injection. A cross over was conducted at the end as described in Experiment 2.

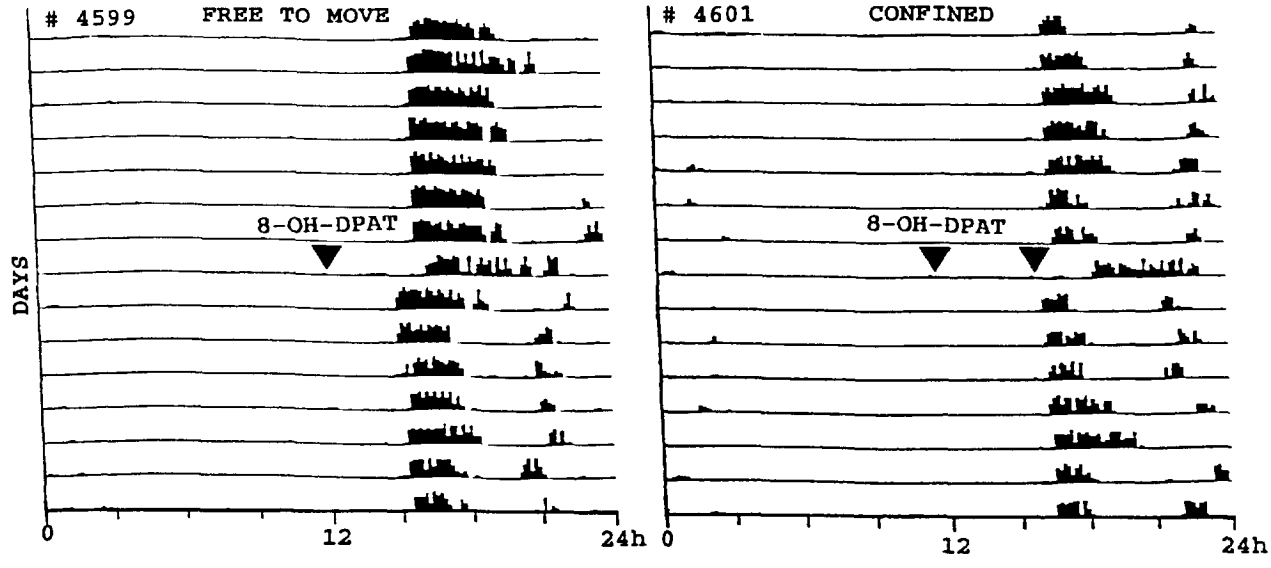

FIG. 5. Actogram on the left illustrates phase shifts of activity rhythms in hamsters injected with 5 mg/kg 8-OH-DPAT. Time of injection is indicated by the first arrow on day 8. The actogram on the right illustrates the activity rhythm of a hamster injected with 5 mg\kg of 8-OH-DPAT and confined to its nest box for 4 h after the injection. Time of injection and start of confinement is indicated by the first arrow on day 8. The second arrow indicates the time at which the animal's nest box was opened. The animals were maintained in darkness.

RESULTS

The effect of condition (non confinement vs. confinement to the nest box) on the magnitude of phase shifts was significant ($F = 34.8$, $p = 0.0001$, two-way ANOVA; Fig. 6). The effect of treatment (DMSO or triazolam injections) was also significant across the four groups, ($F = 34.82$, $p = 0.0001$). The Bonferroni multiple comparisons test revealed that nest box confinement significantly attenuated phase-shifting effects of triazolam ($t = 8.79$, $p < 0.001$, two-tailed t-test). Also, in the unconfined animals the phase shifts after triazolam, were significantly greater than those after DMSO ($t = 8.87$, $p < 0.001$).

EXPERIMENT 4

DOSE-RESPONSE CURVE FOR PHASE SHIFTING BY 8-OH-DPAT IN THE ASCHOFF TYPE I EXPERIMENTAL DESIGN

Another way to investigate the possibility that phase shifting by 8-OH-DPAT is mediated by increased activity is to use doses of 8-OH-DPAT sufficiently high to produce the serotonin syndrome and the associated reduction in locomotion (15). The purpose of the next experiment was to establish the dose–response curve for 8-OH-DPAT, and to establish a relationship between the phase-shifting effects of this drug and serotonin syndrome.

Method

Subjects. All of the animals had previously received a 3 h activity pulse in another experiment, but were pharmacologically naive.

Experimental design. At 91 days of age animals were put into LD 14:10 for 9 days. They were then put into DD for 10 days prior to receiving IP injections starting at CT 8. The animals were randomly divided into 5 groups with 8 hamsters in each group. Each animal was weighed on the last day of the LD cycle. Group 1 received injections of 0.9% saline solution (0.85 ml/kg). Groups 2–5 received the following doses of 8-OH-DPAT (RBI) respectively: 0.01, 0.1, 1 and 10 mg/kg dissolved in 0.9% saline (0.85 ml/kg). Animals were injected under a dim-red safe light, and their behavior was observed with the aid of an infrared viewer. Each animal's behavior was observed within 10–20 min after the injection. The following behaviors were noted: hind limb abduction, straub tail, lethargic, immobile, and normal. Hind limb abduction is a feature of the serotonin syndrome (15); in this condition the hind limbs are often extended backwards, making it difficult for the animal to move. Straub tail is also a feature of the serotonin syndrome; the tail and hind area are extended upward. Lethargic refers to animals that were moving but only very slowly, with apparent difficulty. Immobile was used to describe animals that exhibited one or more of the symptoms of the serotonin syndrome and also were not moving; immobile does not imply that the animal was sleeping, since animals in this condition, although motionless, had unusual body postures and open eyes. Animals could exhibit one or more of these abnormal behaviors. When no abnormal behaviors were observed the hamsters were classified as normal.

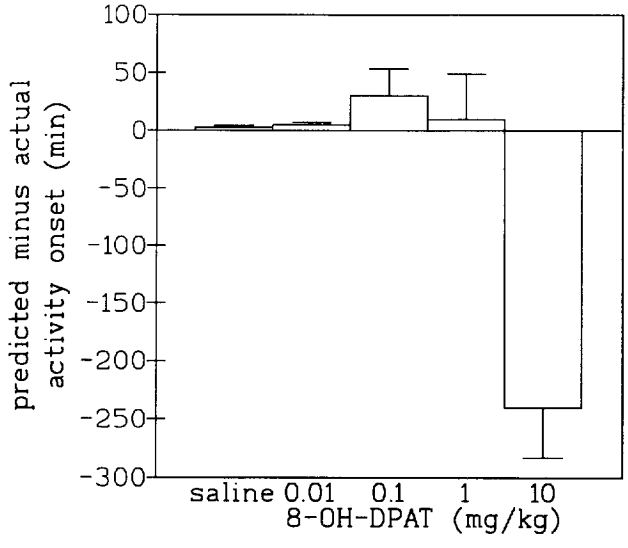

FIG. 7. Time of activity onset (mean ± SEMs) on the day of injection in Experiment 5 as a function of dose of 8-OH-DPAT. Negative values mean that animals became active later than predicted.

TABLE 1
BEHAVIOR OF HAMSTERS AFTER RECEIVING 8-OH-DPAT OR SALINE IN EXPERIMENT 4

Dose of 8-OH-DPAT (mg/kg)	Normal Behavior	Hind Limb Abduction	Straub Tail	Lethargic	Immobile
Saline	100	0	0	0	0
0.01	100	0	0	0	0
0.1	50	0	0	50.0	0
1	0	37.5	62.5	62.5	0
10	0	66.7	83.3	16.7	83.3

The numbers are the percent of animals within a treatment group which exhibited a particular behavior.

Data analysis. To determine the immediate effects of the various doses of 8-OH-DPAT on levels of activity, the difference between the predicted and the actual activity onset on the day of injection was determined. Activity onset on the day before injection was used as the predicted activity onset on the day of injections. A negative number implies that the actual activity onset occurred after the predicted onset. A positive number implies the opposite.

RESULTS

Figure 7 illustrates how varying doses of 8-OH-DPAT affected activity onset on the day of injection. ANOVA showed that the variation among the group means was extremely significant ($F = 16.01$, $p < 0.0001$). The results of behavioral observations are presented in Table 1. Most of the animals given 10 mg/kg of 8-OH-DPAT behaved abnormally, and had signs of the serotonin syndrome. However, even at the 1 mg/kg dose the syndrome was generally evident, although immobile behavior was not seen. The onsets of activity on the day of the injection in the 10 mg/kg group were 4 h later than normal (Fig. 7). This group was the only one for which onsets were significantly different from those of saline-injected animals ($p < 0.001$). Despite differences in behavior in the 10 mg/kg and 1 mg/kg groups in the postinjection period (Table 1), both these groups showed similar phase shifts (Fig. 8). ANOVA revealed that the differences among the group means were very significant ($F = 11.1$, $p = 0.0001$). The groups with phase shifts significantly different from the saline control group were the ones which received 0.1 mg/kg ($p < 0.05$), 1 mg/kg ($p < 0.001$), and 10 mg/kg ($p < 0.001$) of 8-OH-DPAT.

Two animals from the group injected with 0.01 mg/kg of 8-OH-DPAT and two from the 10 mg/kg group had to be excluded from all data analyses because they were not active enough on a sufficient number of postinjection days to meet the

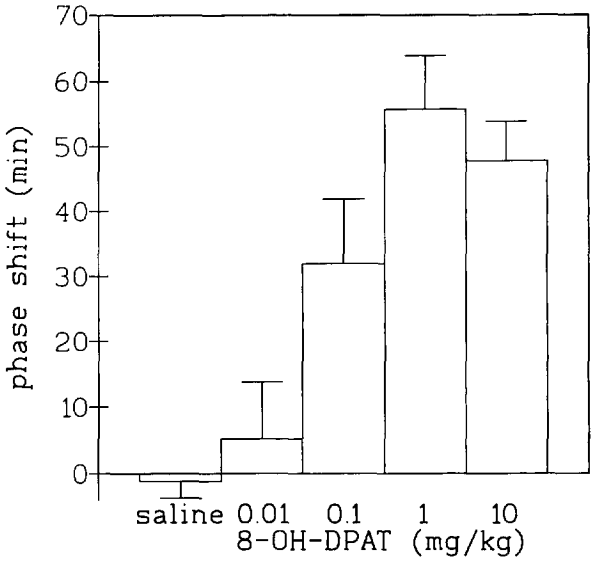

FIG. 8. Relationship between the dose of 8-OH-DPAT and phase shift (means ± SEMs) in Experiment 4.

criteria for activity onset. This might have resulted from problems with data recording apparatus (i.e., faulty microswitch, or from long-lasting effects of the drug on activity).

EXPERIMENT 5

PHASE-SHIFTING EFFECTS OF QUIPAZINE IN THE ASCHOFF TYPE II EXPERIMENTAL DESIGN

Edgar et al. (6) have shown that in rats systemic administration of quipazine produces a nonphotic type PRC. Since the role of 5-HT in the circadian system of both rats and hamsters is unclear, and since few data are available for hamsters, this study was designed to investigate whether the general 5-HT agonist quipazine would have phase-shifting effects in hamsters similar to those found in rats.

Method

Subjects. On arrival in the laboratory, two groups of hamsters were housed in two separate rooms. The animals were studied previously in unrelated behavioral experiments, in which they were confined to a novel running wheel on various occasion for 3 h at different circadian times.

Procedure.

Test 1. Animals were kept in the 14:10 LD cycle for 12 days before injections. Since hamsters were entrained to the LD cycle by the day of injection, Zeitgeber time (ZT) was close to circadian time (CT). When the animals were 90 days old, they received a subcutaneous (s.c.) injection of quipazine at ZT 5 (i.e., 7 h before dark onset). Each animal was weighed just before the injection. Weighing, injecting, and returning all the animals to their home cages took about 20 min, after which the room lights were turned off for 5 days. The hamsters received either quipazine dimaleate (RBI), $n = 6$, dosage 10 mg/kg in of 0.9% saline (1.33 ml/kg), or vehicle ($n = 5$), (1.33 ml/kg). Following the 5 days in DD, the hamsters were allowed to reentrain to the LD cycle for 24 days. Ten extra animals of the same age were used in the second part of this test; they had previously been injected once with saline ($n = 5$) or with quipazine ($n = 5$). Animals were then randomly divided into 5 groups. Each group received a different dose of quipazine, dissolved in approximately 0.9 % saline (1.17 ml/kg): 5 mg/kg ($n = 4$), 10 mg/kg ($n = 4$), 20 mg/kg ($n = 4$), 50 mg/kg ($n = 4$) or 100 mg/kg ($n = 5$). All animals were weighed and then injected s.c. at ZT 6.5. After the last injection, the room lights were turned off and the animals remained in DD for 5 days.

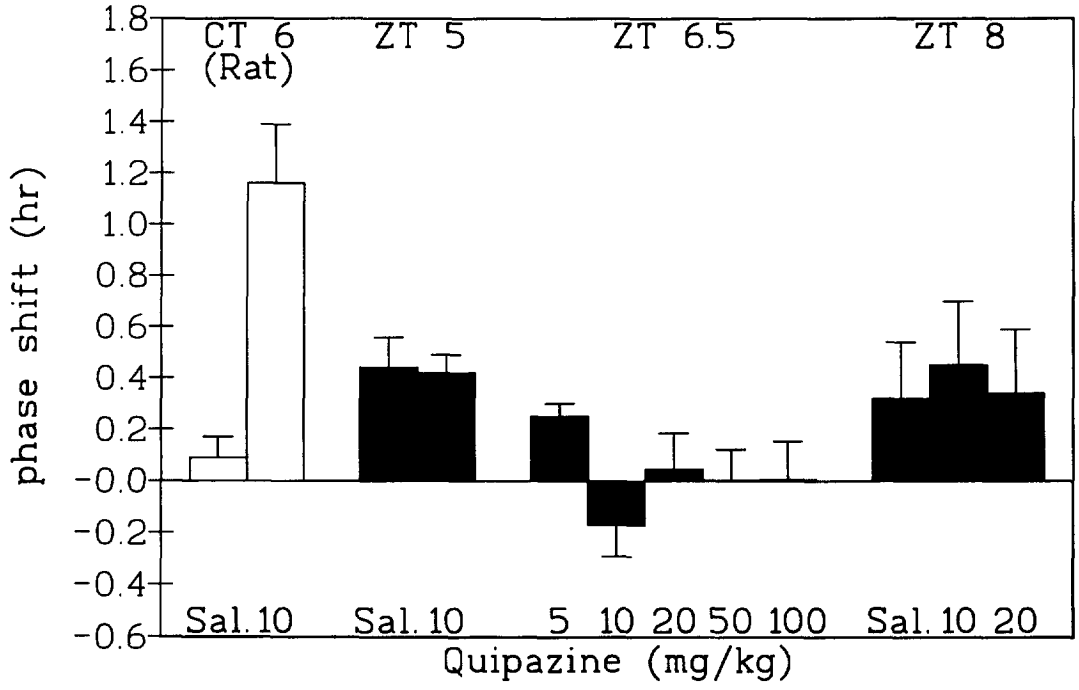

FIG. 9. Mean phase shift ± SEMs of all tests with quipazine or saline (Sal.) at different CTs in Experiment 5. Dosages shown at the bottom of each bar are in mg/kg. Data for CT 6 are for rats injected s.c. with quipazine or saline [replotted from Edgar et al., (6)].

Test 2. The second group of animals was maintained in LD 14:10 for 11 days before injections. At 102 days of age they were weighed. Three days later they were injected s.c. at CT 8 with either quipazine 10 mg/kg ($n = 8$), dissolved in approximately 0.2 ml of 0.9 % saline, or saline ($n = 8$) at. CT 8 was calculated as in Experiment 1. Animals were returned to their home cages, and the lights were turned off for 5 days.

RESULTS

No evidence was obtained in any of the tests, that quipazine produces phase shifts in hamsters ($p > 0.3$, one-way ANOVA, two-tailed, Fig. 9). Although there were some phase advances observed after quipazine administration, they were not significantly different from those of saline controls. The injections of quipazine, especially at higher doses, often decreased the total amount of wheel running on the day of the injection.

DISCUSSION

Preventing hamsters from moving around by confining them to their nest boxes failed to reduce 8-OH-DPAT-induced shifts, both under Aschoff type I and with Aschoff type II designs (Experiments 1 and 2). The phase advances attributable to 8-OH-DPAT were similar with these two procedures. With the Aschoff type II design, the total shift averaged 70 min, but since saline-injected animals advanced by 30 min on average, as is usual with this procedure (13,28), only 40 of the 70 min is attributable to the drug. This is close to the mean shift of 43 min obtained with Aschoff type I design in which there was no change in lighting conditions; saline treated animals in this test shifted only 9.3 min.

That activity restriction did not alter the phase-shifting effect of 8-OH-DPAT might be unsurprising to pharmacologists. Yet it contrasts with what is found with triazolam (Experiment 3). Preventing hamsters from moving around after triazolam injections virtually abolishes phase shifts (25,43). We verified that blocking of triazolam-induced shifts by activity restriction occurred also at CT 8 (Experiment 3), the time that 8-OH-DPAT was given in our tests. That activity restriction does not affect 8-OH-DPAT-induced shifts suggests that this drug does not affect the clock simply because it makes the animal more active. This conclusion is supported by the results with high doses of 8-OH-DPAT (Experiment 4). When 10 mg/kg of this substance was given to hamsters they became lethargic or immobile; this influence of the drug lasted a number of hours, and the animals did not start to run in their wheels until about 4 h later than usual. Despite this inactivity, phase advances in hamsters receiving 10 mg/kg were similar to those of hamsters receiving a tenth as much 8-OH-DPAT; most of the latter were not made inactive, and they started to run in their wheels close to the expected time (Fig. 7).

Thus, observations of behavior, taken together with the results from the activity restriction experiments, make it unlikely that phase shifting by 8-OH-DPAT is mediated by increased activity. If this is accepted, then the relationship between activity and serotonin can be approached in a different way. The question becomes not whether shifts induced by 8-OH-DPAT or other serotonin agonists are mediated by activity, but whether activity-induced shifts are mediated by serotonin.

The possibility that the serotonergic projections from the raphe nucleus to the SCN are important in behavioral nonphotic shifting was raised by Mrosovsky (23). This suggestion was given life by demonstrations that serotonin agonists applied to SCN slices in vitro resulted in phase shifts with PRCs similar to those produced by novelty-induced running and by triazolam, that is, with phase advances in the subjective day (32,33). Unfortunately the data are not conclusive from studies in which serotonin has been depleted. Smale et al. (39) found that 5-HT depletion by intraventricular 5,7-DHT did not prevent triazolam-induced shifting, but they pointed out that this could conceivably have depended on a few residual serotonergic fibers. In other studies, phase-shifting of hamsters by triazolam was abolished by depletion of 5-HT in the SCN (5), and by a general brain depletion (30), but since the chemical manipulations used to deplete 5-HT made the animals less active, the lack of shifting after triazolam may well have been due to inadequate nonphotic input from activity or its correlates. In hamsters the amount or duration of activity is often related to the size of the subsequent shifts (13,25).

In a further study, Penev et al. (31) quantified the activity levels in 5-HT depleted with p-chloroamphetamine and non depleted animals, and argued that the depleted animals no longer shifted to triazolam even though there was no significant difference in the levels of wheel-running activity in these two groups in any of the six 1 h activity pairs after the injection. A closer inspection of the data reveals that the mean wheel-running levels in the depleted animals in the 6 h taken as a whole, were in fact only about 60 % of those found in the control animals. However, the variability in the data was quite large which might have obscured a detection of any significant differences between these groups. These results are also surprising considering that only 25 % of 5-HT was depleted in the hypothalamus. The implication of previous studies is that only high levels of depletion may be capable of attenuating phase shifts to triazolam (5,39).

Overall, therefore, the evidence for involvement of 5-HT in nonphotic behavioral shifting remains circumstantial. It rests largely on the fact that both induced activity and serotonin agonists produce phase advances in the subjective day.

Apart from the work of Tominaga (42), no studies on the phase-shifting effects of serotonergics have been done in hamsters. Therefore we decided to try quipazine in an Aschoff type II design, which in rats is reported to phase shift when injected in vivo at CT 6 (6) in an Aschoff type I design, and in vitro even earlier (32,34). No phase shifts after quipazine injections were detected. The higher doses of quipazine (20–100 mg/kg) did reduce the wheel running on the day of injection, just as was observed in the animals injected with the 10 mg/kg of 8-OH-DPAT. It is conceivable that this reduced activity could have produced phase delays in wheel-running rhythm, similar to those found when the animals are restrained in small plastic tubes (44). It is possible that any such delays might have cancelled out any phase advances produced by quipazine. However, this explanation is unlikely since the animals injected with the highest dose of 8-OH-DPAT were immobile when observed and started running several hours later than usual, yet shifted in a manner similar to the animals given 1 mg/kg which did not produce a pronounced reduction in activity (Fig. 8).

Since quipazine is a nonspecific agonist, it is possible that when injected systemically some other actions of the drug may have cancelled out its phase-shifting effects. This would not in itself explain why in rats quipazine is effective. Possibly there is a different receptor profile; there are precedents for species differences in 5-HT receptor profiles (12). In the light of failure to obtain shifts with quipazine in hamsters, one may look more closely at the data for rats by Edgar et al. (6). The actogram given by those authors appears to represent the largest effect obtained with the drug; the phase shift was more than 2 standard deviations away from the mean. Moreover, this animal was quite active after the injection, which might have contributed to the

phase shift. Taking this one animal out of the analysis reduces the differences between the groups. Further work on the effects of quipazine in rats in vivo is desirable.

In summary, the role of 5-HT in nonphotic phase shifting remains unclear. Given the accumulating evidence that, at least in hamsters, for novelty-induced running, the NPY projections from IGL to SCN are critical for shifts (3,29), one may wonder if the 5-HT input to the SCN has some other function. If both photic and nonphotic inputs are capable of clock resetting, then there must be physiological mechanisms for mediating not only the phase shifts to the zeitgebers in isolation, but also the interactions that occur when the two different zeitgebers arrive silmultaneously. Perhaps the main role of 5-HT in the SCN is in such interactions. Morin and Blanchard (21) found that in the 5-HT depleted hamsters, the phase angle of entrainment to a LD cycle become more positive, and the light PRC shifted to the right by 30 min. As well, serotonin agonists attenuate photic phase shifting and Fos protein induction by light (10,35); and the electrophysiological responses to light of many SCN neurons are attenuated by application of serotonin agonists (45). Also serotonin and 8-OH-DPAT inhibit field potentials in the SCN in response to optic nerve stimulation in vitro (35). When these data are considered together, it suggests that rather than the mediation of nonphotic shifts in themselves, it might be that the main function of the serotonergic input to the SCN is in the interactions between nonphotic and photic events on circadian clock resetting.

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