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Effects of Imipramine on Circadian Rhythms in the Golden Hamster

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REFINETTI, R. AND M. MENAKER. *Effects of imipramine on circadian rhythms in the golden hamster.* PHARMACOL BIOCHEM BEHAV 45(1) 27-33, 1993.—The effects of the antidepressant imipramine on circadian organization were studied in wild-type and tau-mutant golden hamsters. Chronic imipramine treatment in doses ranging from 0–50 mg $kg^{-1} \cdot day^{-1}$ depressed general activity and body temperature and caused a reduction in body weight but had no significant effect on circadian organization. Imipramine treatment did not affect the rate of reentrainment after a 6-h advance in the light–dark cycle, did not alter the advanced-phase angle of entrainment of tau-mutant hamsters, did not affect the free-running period of wild type hamsters, and did not alter the phase-response curve to light pulses. Because imipramine, a clinically effective antidepressant, did not have any measurable effect on the circadian system in these experiments, our results do not provide support for the hypothesis that the antidepressant action of imipramine is mediated by alterations in the circadian system.

Circadian rhythms Imipramine Animal models of depression

ALTHOUGH patients afflicted by depression have been successfully treated with a number of antidepressant drugs for many years, there is still no consensus about the physiological mechanisms responsible for depression and the physiological action of antidepressant drugs (4,27). Observations that depressed patients have circadian rhythms with abnormal phases of entrainment (21,38) as well as reduced amplitude (32,34), and that at least some forms of depression can be effectively treated with appropriately timed pulses of bright light (3,20), led several authors to suggest that the physiological mechanism responsible for depression is a disruption in the circadian organization of physiology and behavior (9,18,24). Accordingly, the therapeutic action of antidepressants would be attained by a restoration of circadian organization. However, little research has been done to examine the effects of clinically effective antidepressants on basic mechanisms of circadian organization (13,37). The present article describes experiments that investigated the effects of the antidepressant imipramine on circadian organization in golden hamsters.

Imipramine is the oldest member of the family of tricyclic antidepressants, the most widely used drugs in the treatment of depression (4,16,27). In animal studies, imipramine has been shown to reverse learned helplessness, an animal model of depression (10). Our experimental approach is based upon the contention that if a) circadian disruption is the process responsible for depression and b) imipramine is an effective antidepressant, then c) imipramine should have measurable effects on circadian organization. Two previous laboratory studies suggested that imipramine may affect circadian organization (11,12), whereas two others failed to detect such an effect (2,5).

Circadian disruption may result from an internal desynchronization of multiple biologic rhythms or from an abnormal relationship between the subject's rhythms and the external zeitgeber. Our experiments concentrated on the relationship between circadian rhythms and the environmental zeitgeber (light–dark cycle).

GENERAL METHOD

Male golden hamsters (2–4 months old) were used in all experiments. They were maintained at 22°C with free access to Purina Lab Chow and water. Except for the tau-mutant hamsters used in Experiment 2, all animals were purchased from Charles River Laboratories (Wilmington, MA).

Two types of recording equipment were used. In Experiment 1, body temperature and locomotor activity were recorded by telemetry. Animals were housed in individual plastic cages (21 × 30 × 20 cm) lined with wood shavings. Radio transmitters (Model VM-FH, Mini-Mitter Co., Sunriver, OR) were implanted intraperitoneally under sodium pentobarbital anesthesia (80 mg/kg). Locomotor activity and body temperature were monitored continuously by radio receivers placed under the animal cages (Model RA-1010, Mini-Mitter) and recorded every 6 min by a computerized data acquisition system (Dataquest III, Data Sciences, Inc., St. Paul, MN). In

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Experiments 2–4, running-wheel activity was recorded. Animals were housed in individual plastic cages (25 × 46 × 20 cm) lined with wood shavings and fitted with 17 cm diameter running wheels. Microswitches attached to the wheels allowed continuous recording of running-wheel activity by a computerized data acquisition system (Dataquest III).

For drug treatment, imipramine pellets (Innovative Research of America, Toledo, OH) were implanted subcutaneously in the abdomen (Experiment 1) or interscapular region (Experiments 2–4). Based upon the mean body weight of 145 g and the duration of drug release from the pellets (3 weeks), pellets containing 0, 15, 35, 75, and 150 mg imipramine were used to produce approximate doses of 0, 5, 12, 25, and 50 mg $kg^{-1} \cdot day^{-1}$. The intermediate dose (12 mg $kg^{-1} \cdot day^{-1}$) corresponds approximately to the dose usually prescribed to human patients (200 mg per day) after correction for the difference in metabolic body mass ($kg^{0.75}$).

EXPERIMENT 1

To investigate whether imipramine can facilitate the synchronization of biologic rhythms with the external world, it is necessary to have subjects in a desynchronized state. Transient desynchronization may be obtained by abrupt shifts in the phase of the light–dark (LD) cycle, as it takes a number of days for animals to reentrain to the new LD cycle. We treated hamsters with different doses of imipramine, subjected them to a 6-h phase shift in the LD cycle, and observed the rate of reentrainment of their rhythms of locomotor activity and body temperature.

Thirty hamsters were used. Six animals were randomly assigned to each of the five doses of imipramine. Animals were maintained under a 14 L : 10 D cycle that was synchronized with the LD cycle in the animal colony. After 10 days of baseline recording, the LD cycle was advanced by 6 h and animals were monitored for another 12 days. The transition between the two LD cycles was obtained by shortening the L phase on the transition day.

Individual results were evaluated by nonparametric analysis of phase markers (29) and group results by one-way and factorial analysis of variance (ANOVA) (15). Phase markers of both activity and body temperature were determined by the method of onsets and the method of acrophases (29). Onsets were determined by visual inspection of actograms produced by filling the space for each 6-min bin if the activity counts for that bin exceeded the mean activity counts of that day by more than 80% or, for temperature actograms, if the temperature reading exceeded the daily mean temperature by more than 1%. The number of days required for reentrainment was calculated as the number of days between the LD shift and the first occurrence of onsets within ±1 SD of the new phase of entrainment (for most animals, 1 SD corresponded to approximately 29 min). For the determination of acrophases, the raw data were filtered by an 8-h moving average procedure and the time of the highest value for each day in the smoothed curves was considered the acrophase for that day.

Control Animals

Six of the 30 subjects received pellets containing no imipramine and provided control data for the effects of the LD shift. Actograms of locomotor activity and body temperature of a control hamster are shown in Fig. 1, which also shows the acrophases for the same animal. After the 6-h advance in the LD cycle, both activity and temperature advanced gradually over several days until a new stable phase of entrainment was established. As expected, the acrophases occurred consistently later than the onsets, but the two types of phase markers seemed to correlate well. It should be noted that the onsets of activity do not correspond exactly to the activity onsets in actograms based upon running-wheel activity. The activity onsets obtained by telemetry records with cutoff at 80% above the daily mean occur approximately 1 h earlier than the onsets obtained by running-wheel records.

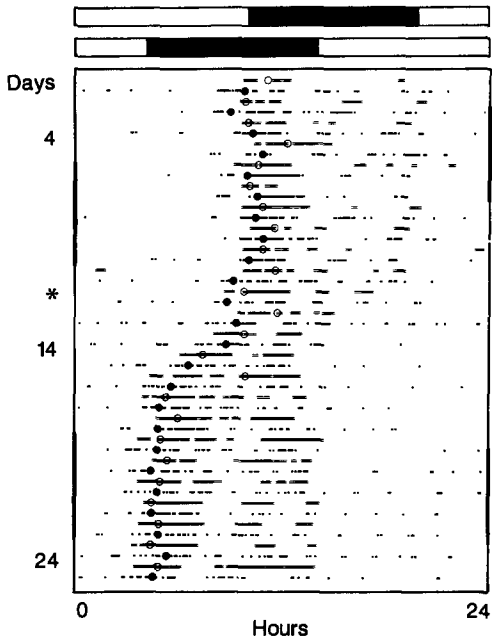

FIG. 1. Body temperature and activity rhythms of a control hamster subjected to a 6-h phase advance of the light–dark (LD) cycle on the day indicated by the asterisk. The data are plotted in the form of an actogram, from which the onsets can be determined by visual inspection. The daily acrophases (calculated from the raw data) are indicated by circles superimposed on the actogram. Each pair of lines (open symbols, body temperature; closed symbols, activity) corresponds to 1 day. Successive days are plotted on successive pairs of lines. The bars at the top of the actogram represent the LD cycle (top bar, days 1–10; bottom bar, days 12–24).

On average, the "onsets" of the activity rhythm occurred 53 min before lights off prior to the LD shift and 45 min before lights off on days 7–12 postshift, and this difference was not statistically significant, $t(5) = 0.52$, $p > 0.05$. The activity onsets preceded the temperature onsets by 13 min before the LD shift and by 20 min after reentrainment to the new LD cycle. This difference was not statistically significant, $t(5) = 0.39$, $p > 0.05$, which indicates that entrainment was fully reestablished. Reentrainment of the activity rhythm took 6.7 days (or 5.2 days by the method of the acrophase), consistent with previous observations that reentrainment requires approximately 1 day for each hour of shift in the advance direction (14,36,39). Reentrainment of the temperature rhythm took 5.8 days (or 6.3 days by the method of the acrophase), not significantly different from the 6.7 days required for reentrainment of the activity rhythm, $t(5) = 0.82$, $p > 0.05$.

General Effects of Imipramine

Some animals, mostly in the group that received the highest dose, developed mild skin irritations at the site of the pellet

implant. The highest dose of imipramine also caused a significant reduction in body weight (Fig. 1A). Three of the original six subjects in this group died before the end of the experiment and were replaced. Proportional dose-dependent effects were observed in the amount of daily activity and the mean level of body temperature. As shown in Fig. 2B, activity levels during the first day after drug implant were reduced in a dose-dependent manner. Although the activity levels of all groups increased 1 week later, animals receiving higher doses were still less active. A similar phenomenon was observed for body temperature (Fig. 2C).

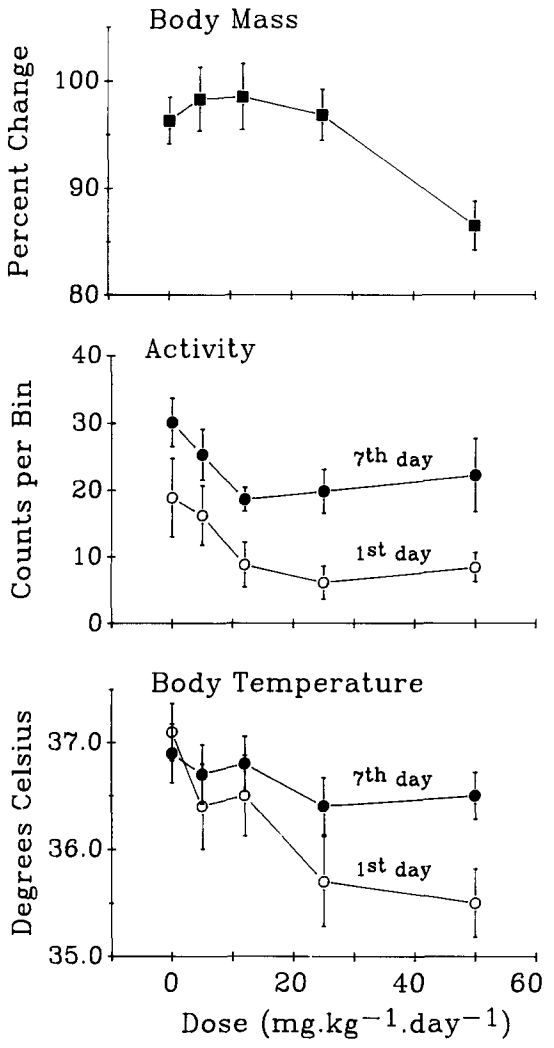

FIG. 2. Body mass change, locomotor activity, and body temperature of hamsters treated with different doses of imipramine. Each point corresponds to the mean (±SE) of six animals. Mean body mass prior to drug administration was 145 g. One-way analysis of variance revealed a significant effect of imipramine dose on body mass as a percentage of baseline, $F(4, 25) = 3.65$, $p < 0.02$. Factorial analyses of variance revealed significant effects of time (1st day vs. 7th day) and dose on locomotor activity, $F(1, 10) = 21.36$, $p < 0.01$, and $F(4, 40) = 3.35$, $p < 0.02$, respectively, as well as a significant effect of dose on body temperature, $F(4, 40) = 6.69$, $p < 0.01$. No significant interaction effects were observed ($p > 0.10$).

Effects of Imipramine on Circadian Organization

As the analysis based upon the acrophases gave similar results to that based upon the onsets, only the onset data will be discussed. As shown in Fig. 3, the small differences in the mean latencies for reentrainment among the various doses of imipramine were all within 1 SEM, and this was true for both the activity rhythm and the temperature rhythm. Factorial ANOVA revealed no effect of variable type (activity or temperature) or of drug dosage, $F(1, 25) = 0.01$, $p > 0.10$, and $F(4, 25) = 0.30$, $p > 0.10$, respectively.

EXPERIMENT 2

In this experiment, we investigated whether imipramine can influence a synchronized but abnormal relationship with the zeitgeber. A number of clinical observations suggest that depressed patients are phase advanced as compared to healthy individuals (38). As an animal model for this condition, we used the tau-mutant hamster. As the result of a single-gene mutation, tau-mutant hamsters have circadian periods much shorter than 24 h (28). They entrain to a 24-h LD cycle with a greatly advanced phase, so that much of their activity takes place during the L phase of the cycle rather than during the D phase as in normal hamsters (22). We treated mutant hamsters with different doses of imipramine and examined the phase angle of their entrainment before and after the treatment.

Twenty-five homozygous tau mutant hamsters were used. Because of their short free-running circadian period (mean = 20.4 h), tau-mutant hamsters do not easily entrain to a 24-h LD cycle. To increase the number of animals that would entrain to the LD cycle, we used a 23-h cycle (L 13 : D 10, 100 lux). Animals entrain to this LD cycle with a strongly advanced phase. Animals were left undisturbed, except for weekly checks of food and water, for approximately 4 weeks until stable entrainment was observed. Animals that had not entrained (16% of the total) were discarded and replaced at a later time. Animals with stable entrainment received imipramine implants. Five animals were randomly assigned to each dose and their running-wheel activity was monitored for another 3 weeks.

For data analysis, individual records were plotted as actograms with 6-min bins, and the phase angle of entrainment

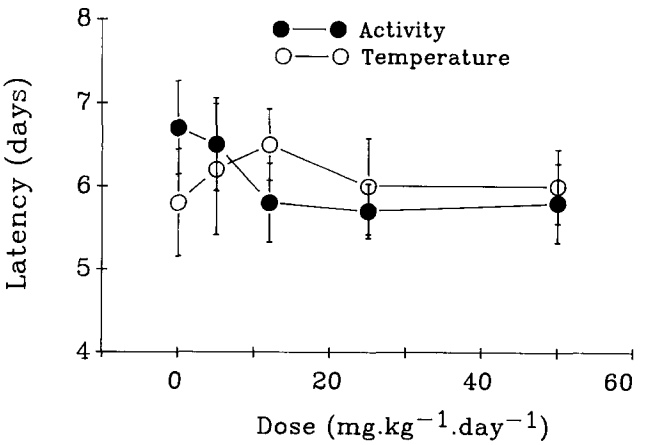

FIG. 3. Mean latency to reentrain after a 6-h phase advance of the light-dark (LD) cycle, as determined by the method of the onsets. Each point corresponds to the mean (±SE) of six hamsters.

was determined by visual inspection for the last 6 days before the implant and for days 9–14 after the implant. The "daily" onsets of activity were used as phase markers.

The records of locomotor activity of an animal kept under L 13 : D 10 for 42 "days" are shown in Fig. 4. The animal seemed to entrain to the LD cycle after about 10 days. After stable entrainment was attained (days 23–42), the onsets of running activity were approximately 3 h before lights off. The record of this animal, which did not receive imipramine, is representative of the records of most experimental subjects prior to the implants. The mean onset (± SE) for the 25 experimental subjects prior to the pellet implants was 4.2 ± 0.5 h before lights off. In contrast, the running-wheel onsets of wild-type hamsters under a normal (24 h) LD cycle occur at or slightly after lights off.

The mean changes in the phase angle of entrainment for the five dose groups are shown in Fig. 5. Although there was a trend for higher doses to produce larger phase delays, the treatment effect did not reach statistical significance, $F(4, 20) = 1.05, p = 0.41$.

EXPERIMENT 3

The pattern of entrainment of circadian rhythms to an LD cycle is dependent upon two parameters of the circadian pacemaker: its free-running period and its time-dependent sensitivity to light (25). In the present experiment, we examined the effects of imipramine on the free-running period. Effects on the time-dependent sensitivity [the phase-response curve (PRC)] were examined in Experiment 4.

Thirty (wild-type) hamsters were used. Prior to the experiment, animals were maintained in 14 L : 10 D for 3 or more weeks. Six animals were randomly assigned to each of five groups and implanted with imipramine pellets, as previously described. They were then placed in constant darkness for 3 weeks and their running-wheel activity was continuously recorded. The free-running period for each animal was determined for days 7–16 after the implant. Two methods were used: linear regression of daily onsets (26) and χ^2 periodogram (31).

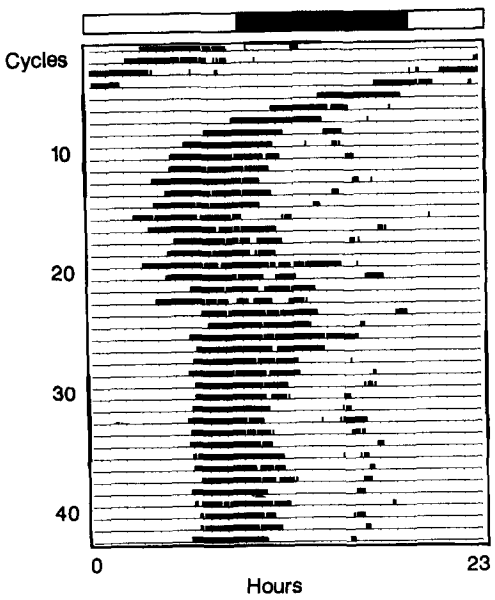

FIG. 4. Records of running-wheel activity of a tau-mutant hamster maintained under a 23-h photoperiod (13 L : 10 D). The bar at the top of the actogram represents the light-dark (LD) cycle.

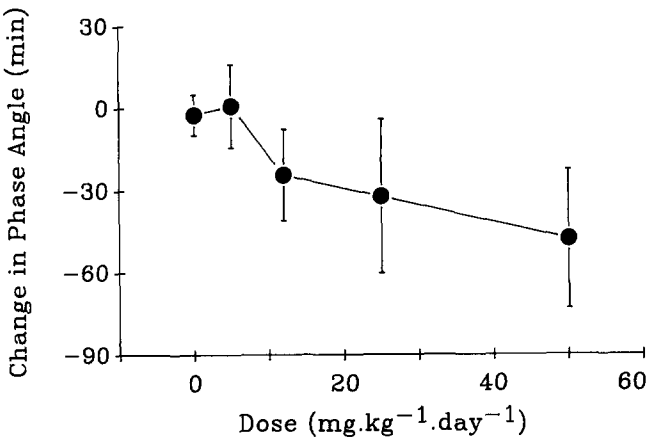

FIG. 5. Mean change in the phase angle of entrainment of tau-mutant hamsters maintained in 13 L : 10 D and treated with different doses of imipramine. Each point corresponds to the mean change (± SE) of five animals. Mean onset prior to the pellet implants was 4.2 h before lights off. +, advances; −, delays.

The two methods of period analysis gave identical results for the accuracy of the periodogram analysis used here (0.1 h). The mean free-running periods of all animals, as determined by the method of linear regression of the onsets, are shown in Fig. 6. There was no significant effect of imipramine on the free-running period, $F(4, 25) = 0.22, p > 0.10$.

EXPERIMENT 4

This experiment examined whether imipramine can affect the time-dependent sensitivity to light (i.e., the PRC).

Thirty-six hamsters were used. Prior to the experiment, animals were maintained in 14 L : 10 D. Half the animals were implanted with a 75-mg pellet of imipramine (approximate dose of 25 mg kg^{-1} · day^{-1}); the other half served as controls. Animals were placed in constant darkness for 3 weeks and their running-wheel activity was continuously recorded. On

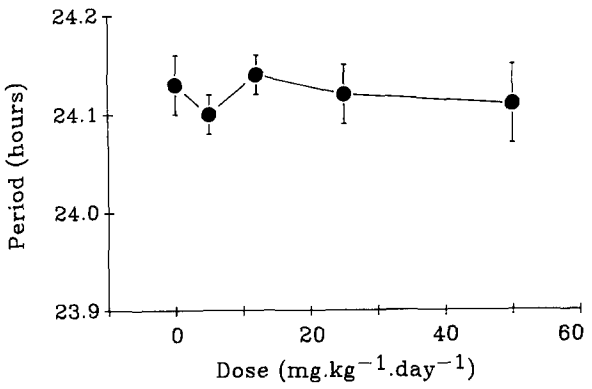

FIG. 6. Mean free-running period of golden hamsters treated with different doses of imipramine. Each point corresponds to the mean (± SE) of six animals.

the 11th experimental day, light pulses (white light, 300 lux, 1 h) were delivered at one of three circadian times: CT 6, CT 14, or CT 18 (the onset of activity being defined as CT 12). Animals in each group of 12 animals (6 experimental, 6 control) received one pulse. In untreated hamsters, pulses at CT 6 cause no shifts whereas pulses at CT 14 cause phase delays and pulses at CT 18 cause phase advances (35).

The mean results are plotted in Fig. 7 together with the hamster phase-response curve as determined in a previous study with similar experimental methods (35). Advances are denoted by a positive sign; delays are denoted by a negative sign. The data points for our animals are all within ±1 SE from the normal PRC. This is true for both control and imipramine-treated animals. Therefore, imipramine treatment did not affect the phase-response curve as determined by light pulses given at these three time points.

DISCUSSION

Our results indicate that imipramine has a dose-dependent effect on several physiological variables, such as body weight, locomotor activity, and body temperature. However, we found no evidence that imipramine affects the circadian system. Chronic imipramine treatment in five different doses did not affect the rate of reentrainment after a 6-h advance in the light-dark cycle, did not alter the advanced phase angle of entrainment of tau-mutant hamsters, did not affect the free-running period of wild-type hamsters, and did not alter the phase-response curve to light pulses.

In studies of this kind, there is always legitimate concern about the adequacy of animal models for the study of the pharmacological action of drugs in humans. The highest dose of imipramine used in this study on golden hamsters (50 mg $kg^{-1} \cdot day^{-1}$) was higher than the doses used in all previous animal studies and four to five times higher than the therapeutic dose for human patients. However, the absorption of the drug seems to differ considerably for SC administration in rodents and oral administration in humans. According to the manufacturer of the imipramine pellets (Innovative Research of America), a dose of 50 mg $kg^{-1} \cdot day^{-1}$ SC produces a blood concentration of approximately 30 ng $\cdot$ ml^{-1} in golden hamsters. In humans, the usual dose of 200 mg per day orally (i.e., 3 mg $kg^{-1} \cdot day^{-1}$ for a 70-kg person) produces a blood concentration of 200 ng $\cdot$ ml^{-1} (17). This would seem to suggest that the highest dose used in this experiment was equivalent to 15% of the therapeutic dose of imipramine. On the other hand, hamsters treated with 50 mg $kg^{-1} \cdot day^{-1}$ showed clear signs of overdosing: They were motionless for several days, their body temperature fell several degrees below the normothermic level of 37°C, and three of the animals died within 1 week. This indicates that 30 ng $\cdot$ ml^{-1} in hamsters has stronger physiological effects than 200 ng $\cdot$ ml^{-1} in humans.

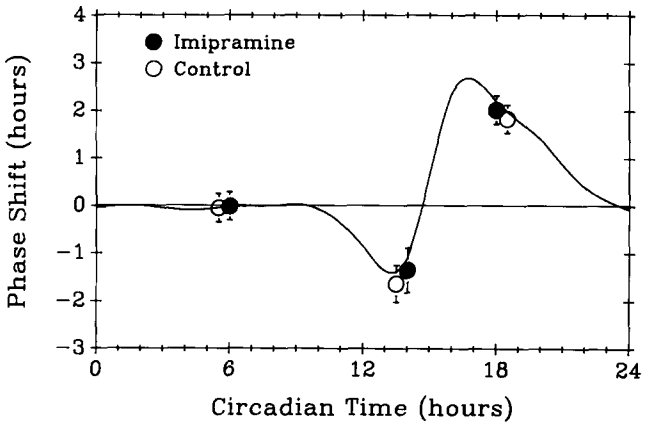

FIG. 7. Mean phase shifts caused by light pulses delivered at different circadian times. Each point corresponds to the mean (±SE) of six hamsters. Some points are plotted 1 h earlier or later than the actual time for graphic clarity. The curve represents the phase-response curve (PRC) obtained from a large sample of normal hamsters (35). +, advances; −, delays.

In Experiment 1, chronic imipramine treatment did not affect the rate of reentrainment after a 6-h advance in the light dark-cycle. These results provide no support for the hypothesis that the antidepressant effect of imipramine results from its ability to alleviate a condition of desynchronization between the internal clock and the external zeitgeber. Our animals were not studied for longer than 3 weeks (the full life of the imipramine pellets), but the therapeutic action of imipramine in humans is usually attained within 2 weeks of treatment (4,16,27).

It has been reported that, in rats, clomipramine (10 mg $kg^{-1} \cdot day^{-1}$) may accelerate reentrainment after a 6-h phase advance of the LD cycle (6). On the other hand, the time required for reentrainment after a 12-h phase shift was not reduced by chronic imipramine administration in rats (5). Aschoff treated golden hamsters with imipramine in the drinking water (28 mg $kg^{-1} \cdot day^{-1}$) and observed no change in the latency to reentrain after a 6-h phase shift (2). In the present experiment in golden hamsters, we used doses of up to 50 mg $kg^{-1} \cdot day^{-1}$ and did not observe a reduction in the time required for reentrainment after a 6-h shift of the LD cycle. The transient state of desynchronization between the circadian system and the LD cycle was not abbreviated by imipramine administration.

In Experiment 2, imipramine treatment had no significant dose-dependent effect on the phase angle of entrainment of tau-mutant hamsters. Therefore, the data provide no support for the hypothesis that the antidepressant action of imipramine is due to its ability to influence the phase angle of entrainment. In a previous study in rats, induction of behavioral depression by inescapable shock (learned helplessness) was not accompanied by changes in the phase angle of entrainment (33) or in the circadian organization of sleep (1). Also in rats, imipramine administration did not affect the acrophase of the rhythm of blood corticosterone concentration (12). In rats and golden hamsters, administration of imipramine through the drinking water (28 mg $kg^{-1} \cdot day^{-1}$) did not affect the phase angle of entrainment of the activity rhythm (2).

In Experiment 3, imipramine treatment did not affect the free-running period of wild-type hamsters. Previous studies of the effects of mood-affecting drugs on the free-running period of rodents have reported a small period lengthening (up to 0.4 h) induced by chronic administration of lithium (19,30) or clorgyline (8,11). In golden hamsters given imipramine (20-30 mg $kg^{-1} \cdot day^{-1}$), the free-running period was either not affected (2) or lengthened by 0.1 h (11). Our results indicated no significant change in period for any of the doses of imipramine. It is possible that longer durations of treatment might produce a significant lengthening, but the results reported by Goodwin and colleagues (11) suggested that the effects of imipramine could be observed within 2 weeks postimplant. Moreover, a period lengthening of only 0.1 h would seem rather small to be of therapeutic value. Thus, we con-

clude that imipramine does not have a significant effect on the free-running period. It might be noted in this connection that, in a previous study in rats, induction of behavioral depression by inescapable shock was not accompanied by consistent alterations in free-running period (33).

In Experiment 4, imipramine treatment did not alter the phase–response curve to light pulses. In a previous study in golden hamsters, chronic administration of clorgyline reduced the phase advance caused by a light pulse at CT 18 from 1.64 h to 0.75 h (7). In the present study, imipramine treatment with several doses did not affect the phase shifts caused by light pulses. The most parsimonious explanation for this discrepancy is that clorgyline and imipramine have different effects on the circadian system. If this is the case, the antidepressant action of clorgyline might indeed be mediated by alterations in the circadian system but the mechanism of action of imipramine remains unaccounted for. More importantly, our data do not support the general idea that the action of antidepressant drugs is mediated by alterations in the circadian system. It is quite possible that the effects of clorgyline on circadian organization are independent of its antidepressant effects.

This series of experiments was designed to evaluate in an animal model the hypothesis that depression is the result of a disruption of the circadian system. Because imipramine, a widely used antidepressant drug, did not have any measurable effect on the circadian system in our experiments, our data do not support the idea that the antidepressant action of imipramine is mediated by alterations in the circadian system. In our view, our results cast doubt on the hypothesis that circadian disruption is the mechanism responsible for depression. Admittedly, our experiments involved golden hamsters and searched for the effects of an antidepressant in subjects that (presumably) were not in a depressed state. However, if one assumes that depression is the result of circadian disorganization, and it is known that antidepressants relieve depression, then one must expect that antidepressants will affect circadian organization. Further studies involving established animal models of depression will certainly contribute to the eventual elucidation of this issue.

ACKNOWLEDGEMENTS

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