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Neonatal clomipramine treatment of Syrian hamsters: effect on the circadian system

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Abstract

The circadian behavior of male Syrian hamsters injected with the serotonin/norepinephrine reuptake inhibitor clomipramine (15 mg/kg from postnatal days 8 to 21) was examined. Clomipramine treatment significantly augmented mean activity values of wheel running rhythm, as well as delayed its acrophase. After a 6-h phase advance of the light–dark cycle, reentrainment of clomipramine-treated hamsters took significantly longer than controls. Clomipramine-treated hamsters exhibited a shorter circadian period than controls in constant light conditions, but no differences were found in constant darkness. Light pulses applied at late subjective night to clomipramine-treated hamsters caused significantly reduced phase advances as compared to controls, while no differences were found in phase delay magnitudes when light pulses were applied during early subjective night. Administration of the 5-HT_{1A} receptor agonist 8-hydroxy-2-(di-*n*-propylamino) tetralin (8-OH-DPAT) at circadian time 8 significantly advanced the onset of activity to a greater extent in clomipramine-treated hamsters than in controls. The results indicate that neonatal clomipramine treatment of hamsters causes long-lasting changes in the circadian system, by increasing activity levels and by partially inhibiting light-evoked responses. An enhancement of a non-photic, serotonergic-induced response was also unveiled.

Keywords: Circadian rhythms; Clomipramine; Hamsters; 5-HT (5-Hydroxytryptomine, serotonin); Depression

1. Introduction

The suprachiasmatic nuclei of the hypothalamus serve as the pacemaker responsible for the generation and entrainment of circadian rhythms in mammals (Klein et al., 1991). Photic information is conveyed to the suprachiasmatic nuclei via the retinohypothalamic tract, and subsidiary via the geniculohypothalamic tract (Morin, 1994). These inputs terminate in the ventrolateral part of the suprachiasmatic nuclei, which is also innervated by serotonergic nerve terminals originated in dorsal and median raphe nuclei (Morin et al., 1992). In rodents, raphe projections to the suprachiasmatic nuclei represent one of the most dense concentrations of serotonergic terminals in the brain (Moore et al., 1978). Exogenous serotonin (5-hydroxytryptamine, 5-HT) and selective 5-HT_{1A} receptor agonists, as well as endogenous 5-HT, induced changes in the circadian system, as well as inhibited a variety of light-evoked responses on circadian behavior (Glass et al., 1995; Rea et al., 1994; Selim et al., 1993), the data supporting serotonergic regulation of circadian photic responses.

A compelling amount of evidence now indicates that disorders of peripheral and central 5-HT activity are implicated in the pathophysiology of major depression (Meltzer and Lowy, 1987). On the other hand, several hypotheses of circadian rhythm dysfunction in depression have been entertained, holding in common an impairment of efficiency, loss of strength or decreased coupling potency of central pacing functions (Healy, 1987; Wehr and Wirz-Justice, 1982). In addition, several investigations demonstrated the effects of antidepressant drugs, most of them 5-HT reuptake inhibitors, on the circadian system. In the majority, but not all studies, drugs that augmented the availability of 5-HT at the synaptic cleft altered the activity profile by increasing overall activity level, duration, and circadian rhythm amplitude, as well as lengthened the period of the circadian pacemaker and/or delayed its phase position (Duncan et al., 1988; Wollnik, 1992; Rosenwasser and Hayes, 1994; Wehr and Wirz-Justice, 1982). However, period shortening (Possidente et al., 1996; Wollnik, 1992) or no consistent changes in period (Klem-

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fuss and Kripke, 1994; Refinetti and Menaker, 1993) have also been reported during chronic treatment with antidepressant drugs. On the other hand, it has been reported that photic responses (i.e., photic entrainment, phase-shifting response to light) could be affected by antidepressant treatment. For example, clorgyline (a type A monoamine oxidase inhibitor that augments 5-HT availability at the synaptic cleft), chronically administered to hamsters, diminished the phase advance and potentiated the phase delay response to brief light stimulation (Duncan et al., 1988). Moreover, it delayed the phase position of the wheel-running rhythm under entrained conditions (Klemfuss and Kripke, 1994).

Among the various animal models of depression that have become available, Vogel et al. (1990) reported that rats subjected to a neonatal chronic treatment of clomipramine, a 5-HT/norepinephrine reuptake inhibitor, exhibited a pattern of behavioral, physiological and neurochemical changes in adulthood that may model clinical signs of depression, in particular regarding to active sleep, aggressive, sexual and reward-seeking behaviors (Mirmiran et al., 1981; Vogel et al., 1990). Other reports showed an increased electrodermal response to sound stimulation (Guinjoan et al., 1996), decreased spontaneous firing-rate of 5-HT neurons in raphe nuclei (Yavari et al., 1993), hyposensitivity of 5-HT neurons to citalopram, a 5-HT reuptake inhibitor (Maudhuit et al., 1995), and decreased hypothalamic levels of 5-HT in rats neonatally treated with clomipramine (Feenstra et al., 1996), as well as with other antidepressants monoamine reuptake inhibitors, including desipramine (a norepinephrine-preferring agent) (Hilakivi et al., 1987). Together, these data suggest that neonatal antidepressant treatment produces an impairment in monoaminergic neurotransmission, supporting the current view of a link between the monoamine systems and the pathophysiology of depressive disorders.

Among the little information available on the circadian organization in rodents neonatally treated with monoamine reuptake inhibitors, Rosenwasser and Hayes (1994) described period lengthening, increased circadian amplitude and altered effects of both photic and pharmacological treatments on free-running period after neonatal desipramine treatment in rats. We considered it worthwhile to extend this subject by examining the circadian system of Syrian hamsters neonatally treated with clomipramine. The hamster was selected because of its regular and well-defined circadian onset of running-wheel activity, which provides a clear phase marker of the circadian pacemaker (Pittendrigh and Daan, 1976). The effect of neonatal clomipramine treatment on hamsters photic, i.e., entrainment and photic phase shifts, and non-photic, i.e., 8-hydroxy-2-(di-*n*-propylamino) tetralin (8-OH-DPAT)-induced phase shift responses, was examined. While these studies were being completed, circadian rhythm alterations in rats neonatally treated with clomipramine were reported in abstract form by Rosenwasser et al. (1996).

2. Materials and methods

2.1. Animals

A breeding colony of Syrian hamsters (*Mesocricetus auratus*), derived from a Charles River original strain, was maintained in a temperature-controlled room (21°C) under a light–dark of 14:10 cycle (14 h of light per day), with food and water ad libitum. Neonatal clomipramine treatment was given as described by Vogel et al. (1990) for rats, except for some modifications to obtain similar survival rates in hamsters. In rats, the survival rate was approximately 80–95%. However, with the original protocol (15 mg/kg twice per day), we found a deleterious effect in hamsters. First, neonatal manipulation twice daily resulted in a major increase of maternal cannibalism, so we tried to repeat the experiment with a single administration. We sampled 30 to 10 mg/kg injections, and found a near 100% mortality with 30 or 20 mg/kg doses. As the survival rate was similar for 15 and 10 mg/kg (approximately 70%), we decided to use a single injection of 15 mg/kg. With this dose, no significant changes of body weight were found, and a marker of developmental stage such as eye opening was unaffected. Moreover, epidermic manifestations of clomipramine administration were apparent, in a similar fashion to what others and we have observed in rats. Briefly, hamsters received 0.1 ml clomipramine hydrochloride (15 mg/kg) dissolved in sterile saline solution s.c. once daily at 1600–1800 h, from day 8 through day 21 of life. Control hamsters received 0.1 ml saline s.c. After weaning, hamsters were left undisturbed (4–5 animals per cage) until they reached the age of 4 to 7 months. Although a cross-fostered procedure was not possible in this species because of a strong stress factor, genetic influences were balanced as a total of 14 litters were used in these experiments. Male hamsters were employed in all cases. Entire litters were assigned to treatments, and females were allowed to remain in the litters. It should be noted that each experimental group included animals from several litters. For each experiment (i.e., free-running and entrainment, circadian response to light pulses at circadian time 14 or circadian time 19, and 8-OH-DPAT response) a new group of inexperienced clomipramine and saline-treated animals was used.

2.2. Drugs

Clomipramine hydrochloride (Anafranil) was kindly provided by Ciba-Geigy, Argentina. The 5-HT_{1A} receptor agonist 8-OH-DPAT was purchased form Research Biochemicals, Natick, MA.

2.3. Experimental protocol

Hamsters were transferred to individual cages equipped with running wheels (14-cm diameter) fixed to the roof of

the cage, and their locomotor activity was recorded with Dataquest III (Minimitter, Sunriver, OR). Animals were initially exposed to a 14:10 light–dark cycle (LD) for at least 10 days (light intensity 750 lux) and their activity onset relative to the time of lights off (ψ) was determined. The onset of activity (defined as circadian time 12) in each circadian cycle was used as a phase marker to calculate phase shifts to light pulses and drug injections in constant darkness. Phase shifts were calculated by determining the least squares linear regression to consecutive onsets of activity. Actograms whose pre- or post-pulse onsets did not fit a linear regression model, were discarded for the analysis. Lines through the onsets of 7 days prior to the day of a pulse and/or injection, and through the onsets of days 4–10 post-pulse, were extrapolated to circadian time 12 on day 1 (the day following the manipulation). The phase shift was defined as the difference between the timing for circadian time 12 on day 1 as projected by the pre-pulse line, and the post-pulse lines. Free-running periods (τ) were determined from the slope of eye-fitted lines drawn through onsets on days 4–12 in any given condition (day 1 being the day of start of condition), and were confirmed with periodogram analysis. The length of daily activity bouts (α) was also determined in light–dark and constant darkness conditions from the corresponding actograms. Activity onsets were determined as the time of the first of 3 consecutive 5-min activity bouts with over 20 wheel-turns (by constructing actograms with a 'high-pass' filter over 20 turns). Activity offset was determined as the first of five consecutive 5-min activity bouts showing no activity. In the cases in which activity was fragmented, three observers subjectively estimated the offset. Alpha was determined as the difference between these two time points.

In order to test the reentrainment to a 6-h phase advance of the light–dark cycle and free-running periods, clomipramine- and saline-treated hamsters ($n = 10$/group) were used. Wheel-running activity rhythm parameters were analyzed during the first 12 days. After a baseline recording of the wheel-running rhythm, a 6-h phase advance of the light–dark cycle was performed (new lights off time at 1400 h). After stable entrainment for at least 10 days, animals were placed under constant darkness conditions, and their circadian period and activity characteristics were recorded.

In order to determine the circadian response to a phase-modifying light pulse, independent groups of clomipramine- and saline-treated hamsters were employed. After baseline recording in light–dark conditions, animals were kept in constant darkness for at least 12 days until a 10-min long, 100-lux light pulse (white light from a fluorescent tube) was delivered at circadian time 14 ($n = 6$/group) or circadian time 19 ($n = 10–12$/group). The recording continued until the new stable phase was achieved. After stable baseline recordings (for at least 2 weeks) another set of light pulses (at circadian time 14 or 19) was administered. After a 10-day reentrainment period in light–dark conditions, animals were maintained in constant light (60 lux at cage-levels) for another 3-week period.

To examine 8-OH-DPAT-induced phase advances, clomipramine-treated and saline-treated hamsters ($n = 10$/group) were kept in light–dark for baseline recording, and were then maintained in constant darkness conditions for at least 12 more days. 8-OH-DPAT (2.5 mg/kg, i.p., dissolved in 0.1 ml saline solution) was administered at circadian time 8, and the animals were left undisturbed for at least 2 week. This dose was chosen as the minimal effective dose that produces significant phase advances of the wheel-running rhythm, at the time of maximal effectiveness for the drug (Cutrera et al., 1996).

2.4. Statistical analysis

Differences between means were tested using the Student's *t*-test. Two circadian parameters (i.e., amplitude and acrophase of the rhythm) were calculated using the Dataquest cosinor analysis (an iterative least-squares nonlinear regression procedure to fit sinusoidal functions to the data). Mean activity value was taken using the TAU software (Minimitter) analysis of waveform (representation of the real data). As amplitude of the cosinor function is commonly correlated with its mesor (mean level value of the adjusted data), amplitude/mesor ratio values are also provided.

3. Results

3.1. Effect of neonatal clomipramine treatment on free-running period in constant darkness and entrainment

Free-running period (τ) and activity length (α) after two weeks in constant darkness (DD), as well as α and the phase angle (ψ) values of clomipramine- and saline-treated hamsters kept in light–dark (LD), are summarized in Table 1. α_{LD} (assessed from an average of 12 days) was slightly

Table 1
Effect of neonatal clomipramine treatment on free-running period and entrainment of the wheel-running rhythm

	Clomipramine-treated ($n = 10$)	Saline-treated ($n = 10$)
τ_{DD}	23.98 ± 0.01	23.9 ± 0.04
α_{DD}	9.58 ± 0.72	9.50 ± 0.67
α_{LD}	12.6 ± 0.76	10.72 ± 1.09
ψ_{LD}	19.59 ± 0.36	19.7 ± 0.39

Shown are the means ± S.E.M.
τ (the period of the circadian rhythm), ψ (phase-angle) and α (length of nocturnal activity bouts) are expressed in hours, and were taken from at least 10 consecutive days in light–dark (ψ_{LD}, α_{LD}) or constant darkness conditions (τ_{DD}, α_{DD}).
Time of lights off in light–dark was 2000 h.

different among groups, without reaching statistical significance (clomipramine-treated hamsters showing a longer length of activity than controls). No significant differences in period values (τ_{DD}) nor in the length of nocturnal activity under constant darkness (α_{DD}) were detectable among clomipramine- and saline-treated hamsters. Likewise, ψ in clomipramine- or saline-treated hamsters maintained in light–dark for at least 12 days did not differ significantly.

In order to check whether these τ_{DD} values reflect any after-effects of the reentrainment paradigm, we measured τ_{DD} after a short initial light–dark entrainment in another group of animals in Experiment 2. These τ_{DD} values were very similar to those reported in Table 1 (clomipramine-treated: 24.02 ± 0.02; saline-treated: 23.96 ± 0.04).

Table 2 summarizes the effect of neonatal clomipramine treatment on amplitude, acrophase and mean activity values of the wheel-running rhythm in clomipramine- and saline-treated hamsters maintained in light–dark for at least 12 days. As revealed by Cosinor analysis, neonatal clomipramine injection significantly increased amplitude ($t = 2.70$, $P < 0.05$) of the wheel running rhythm, when compared to the saline treatment. However, when expressed as amplitude/mesor for each animal, these differences were no longer apparent (clomipramine-treated: 1.5 ± 0.16, saline-treated: 1.57 ± 0.19). Acrophase was significantly delayed in clomipramine-treated hamsters ($t = 2.82$, $P < 0.01$). The adjustment to the cosine functions was highly significant in all cases ($P < 0.0001$), and P.R. values (goodness-of-fit measure) ranged from 30% to 60% in both clomipramine- and saline-treated group. In addition, analysis of a 12 day-long period in light–dark demonstrated a significant increase in mean activity values in the clomipramine-treated group ($t = 2.28$, $P < 0.05$).

Representative actograms of clomipramine- and saline-treated groups that were reentrained after a 6-h phase advance of the light–dark cycle are shown in Fig. 1A,B. There was a statistically significant difference among groups in the resynchronization time (clomipramine-treated: 11.44 ± 0.43 vs. control: 9.88 ± 0.39, $t = 2.5$, $P < 0.05$).

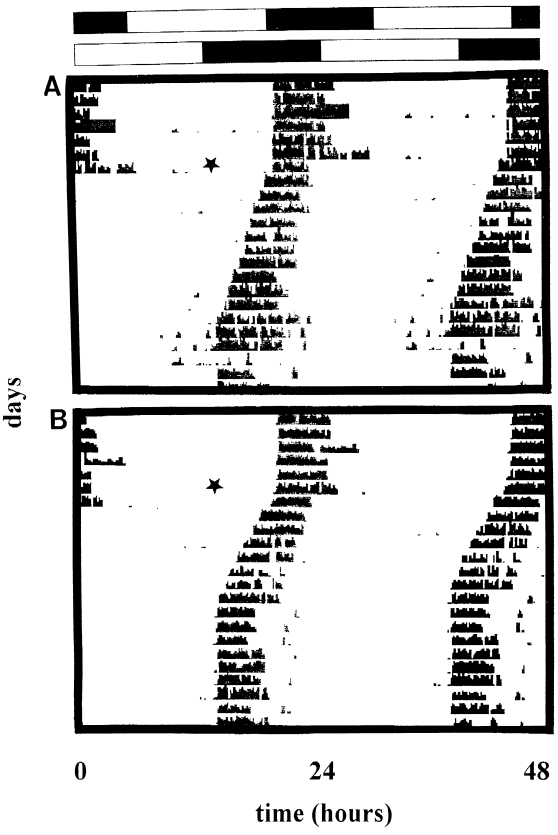

Fig. 1. Reentrainment of locomotor activity rhythms after a 6-h advance of the light–dark cycle. Initial and final photoperiods are shown at the top of the figure. Representative actograms depict the reentrainment rate for (A) clomipramine-treated; (B) saline-treated hamsters. The star indicates the day when the dark portion of the light–dark cycle was phase-advanced. Actograms were double-plotted so that 48 consecutive hours are shown on each line. The apparent increase in activity shown for actograms A and B on the third day of the record is an unexplained artifact created by the Dataquest III system.

Table 2
Effect of neonatal clomipramine treatment on acrophase, mean activity and amplitude of the wheel-running rhythm under light–dark conditions

	Mean activity (counts/5 min)	Amplitude (counts/5 min)	Acrophase (deg)
Clomipramine-treated ($n = 10$)	33.1 ± 4.06[a]	50.6 ± 7.2[b]	357.1 ± 4.1[c]
Saline-treated ($n = 10$)	21.5 ± 2.10	33.8 ± 3.7	342.8 ± 1.8

Shown are the means $\pm$ S.E.M. obtained from an average of 10 days. Acrophases are shown in degrees ($360° = 24$ h).
[a,b,c] designate significant differences as compared to saline-treated controls in a Student's t-test.
[a]Mean activity: $t = 2.28$, $P < 0.05$.
[b]Amplitude: $t = 2.70$, $P < 0.05$.
[c]Acrophase: $t = 2.82$, $P < 0.05$.

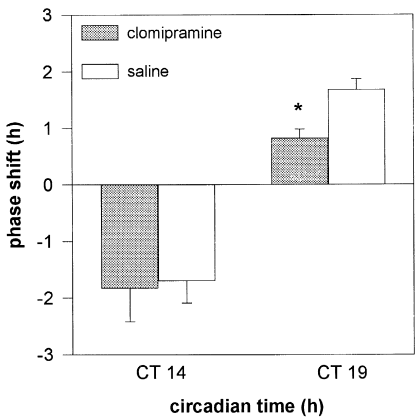

Fig. 2. Average phase shift responses to light in control and clomipramine-treated animals. Clomipramine significantly increased the response at circadian time 19. ($* P < 0.05$, Student's t-test).

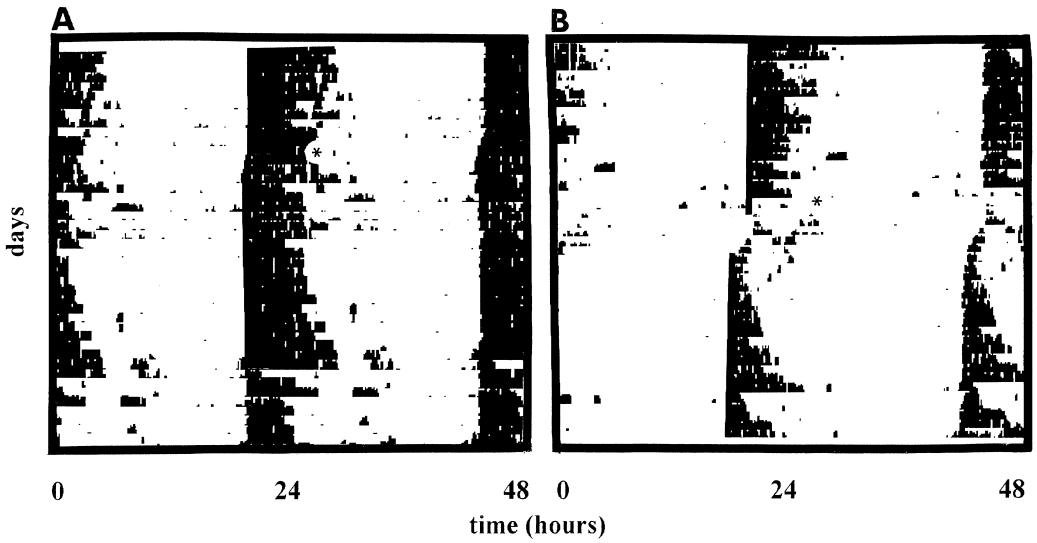

Fig. 3. Representative actograms showing the phase-advance response to a 10-min light pulse at circadian time 19 in (A) clomipramine-treated and (B) control hamsters. The day and time of the photic stimulation are indicated by (*). On the first portion of the double-plotted actogram, the eye-fitted lines corresponding to activity onsets were drawn for the pre- and the post-pulse periods.

3.2. Effect of neonatal clomipramine treatment on circadian photic responses

Fig. 2 depicts the effect of 10-min long, 100-lux light pulses administered either at late (circadian time 19) or early (circadian time 14) subjective night to clomipramine- and saline-treated hamsters kept in constant darkness. Neonatal clomipramine treatment significantly reduced the magnitude of phase advances when a light pulse was applied at circadian time 19 (clomipramine: 0.82 ± 0.16 h; control 1.68 ± 0.19; $t = 3.36$ $P < 0.05$) (Fig. 3), whereas light-induced phase delays (at circadian time 14) were not affected (clomipramine: 1.82 ± 0.6; control 1.69 ± 0.4; $t = 0.03$).

Since results from light-induced phase shifts and resynchronization time showed significant effects of neonatal clomipramine treatment on photic responses, we examined whether clomipramine- and saline-treated hamsters exhibited changes in period under constant light conditions (LL). Significant differences were found in constant light period (τ_{LL}) between groups, τ_{LL} being shorter in clomipramine-treated hamsters (24.47 ± 0.07 h) than in saline-treated controls (24.86 ± 0.13 h) ($t = 2.82$ $P < 0.01$). τ_{LL} was significantly larger than τ_{DD} in both groups of animals (clomipramine-treated: mean values 24.47 ± 0.07 h vs. 24.02 ± 0.02 h, $t = 7.88$, $P < 0.0001$, paired t-test; saline-treated: mean values 24.86 ± 0.13 h vs. 23.96 ± 0.04 h, $t = 6.61$, $P < 0.001$, paired t-test).

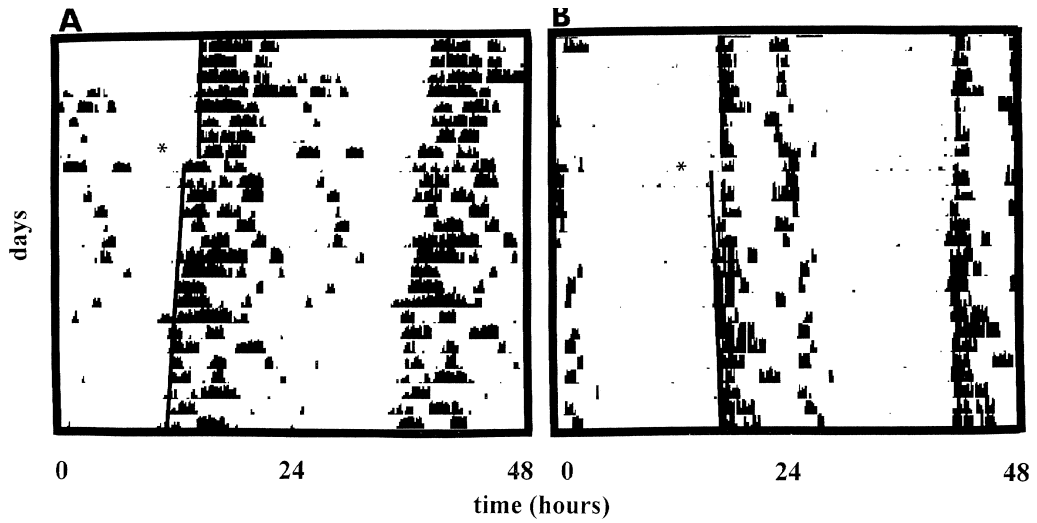

Fig. 4. Phase advances in response to 8-OH-DPAT (2.5 mg/kg) at circadian time 8 in (A) clomipramine-treated and (B) control hamsters. Activity onsets are indicated by an eye-fitted line before and after the stimulus.

3.3. Effect of neonatal clomipramine treatment on 8-OH-DPAT-induced phase advances

Administration of the 5-HT_{1A} receptor agonist 8-OH-DPAT (2.5 mg/kg, i.p.) at circadian time 8 significantly advanced the onset of activity in clomipramine- and saline-treated hamsters maintained under constant darkness for 10 days (Fig. 4A,B). The extent of advance was significantly greater in the clomipramine-treated group (clomipramine-treated: 1.21 ± 0.12 h vs. saline-treated: 0.66 ± 0.16 h, $t = 2.69$, $P < 0.05$).

4. Discussion

The objective of the present study was to determine whether the administration of clomipramine from day 8 to 21 of life, a validated animal model of depression in rats (Vogel et al., 1990), brings about permanent changes of the circadian system in adult Syrian hamsters. As a general effect of neonatal clomipramine treatment, a widespread increase in activity levels of entrained animals and a partial inhibition of light-evoked responses of the circadian system were found. An enhancement of serotonergic-induced responses was also uncovered.

Antidepressants chronically administered to hamsters affected the general pattern of locomotor activity rhythms, decreased the magnitude of the phase advance in response to a light pulse, and augmented the magnitude of the light-induced phase delay (Duncan et al., 1988; Han, 1984). Our foregoing results demonstrate that neonatal clomipramine treatment of hamsters affected light-evoked responses, like photic phase shifting or entrainment. It is noteworthy that clomipramine-treated hamsters took longer to reentrain than control animals after a 6-h advance of the light–dark cycle. Likewise, neonatal clomipramine treatment decreased the capacity of light pulses applied at circadian time 19 to phase advance the onset of wheel running rhythm in hamsters kept in constant darkness, without affecting light-induced phase delays at circadian time 14. Collectively, these results, together with the finding that in constant light conditions clomipramine-treated hamsters showed a shorter circadian period than control hamsters, suggest that some photic responses may be permanently impaired in adult hamsters receiving neonatal injections of clomipramine.

It has been hypothesized that the midbrain serotonergic input plays an important role in modulating photic entrainment. Chemical lesions of the mesencephalic serotonergic system by 5,6-dihydroxytryptamine alter the photic phase response curve, suggesting that endogenous 5-HT regulates the response of the suprachiasmatic nucleus to light (Morin and Blanchard, 1991). These and other observations are consistent with the interpretation that any treatment that increases traffic signal in the serotonergic pathway to the suprachiasmatic nucleus can decrease the response of the circadian clock to photic stimulation (Glass et al., 1995).

It seems possible that neonatal clomipramine treatment alters the capacity of light to entrain the suprachiasmatic nucleus due to an altered 5-HT signaling at the pacemaker level, or its influence on plasticity of specific neural structures during development. A putative mechanism could involve direct effects on the suprachiasmatic nucleus or indirect effects through other structures relevant for photic entrainment. The observed effects of neonatal clomipramine treatment in Syrian hamsters could be explained by a combination of actions on cells within the retina, the raphe complex or lateral geniculate nucleus neurons that project to the suprachiasmatic nucleus.

A possible interpretation in changes under constant light condition is that they might arise by a change in the photic phase response curve (e.g., Golombek and Ralph, 1994). However, parametric effects of light might not only be based upon non-parametric effects such as phase shifts, but rather on a different light-sensitive system. It might be the case that monoamine-related mechanisms (the ones that are most possibly affected by the clomipramine treatment) modulate parametric and non-parametric effects of light by different neurochemical pathways. Moreover, it was reported that neonatal desipramine treatment of rats blunted the effect of constant light on circadian period (Rosenwasser and Hayes, 1994).

Neonatal clomipramine treatment also increased non-photic phase advances induced by the 5-HT_{1A} receptor agonist 8-OH-DPAT when injected at circadian time 8. This drug modified the circadian phase after systemic administration (Cutrera et al., 1996) and also induced phase shifts in hamster locomotor activity after microinjection in the midbrain raphe, but not in the suprachiasmatic nucleus or the intergeniculate leaflet (Mintz et al., 1997). Hypersensitivity to 8-OH-DPAT could be the consequence of clomipramine-induced increases in 5-HT receptor levels in the suprachiasmatic nucleus and/or midbrain raphe targets. Preliminary results from our laboratory indicate that the 5-hydroxyindoleacetic acid/5-HT ratio, an indicator of 5-HT metabolism, increases in the midbrain raphe nucleus of clomipramine-treated hamsters (Yannielli et al., unpublished data). These data, together with the decreased activity in raphe neurons reported in clomipramine-treated rats (Yavari et al., 1993), may reflect an attenuated serotonergic neurotransmission with a concomitant up-regulation of postsynaptic 5-HT receptors. Indeed, Feenstra et al. (1996) have recently reported that neonatal clomipramine treatment resulted in a decrease in hypothalamic serotonin levels in adult animals. Consistent with this interpretation, chronic treatment of adult rats with imipramine, another tricyclic antidepressant, increased the neurophysiological response of neurons to iontophoretic application of 5-HT in the suprachiasmatic nucleus and the lateral geniculate nucleus (Meijer and Groos, 1988). However, it should be noted that there are at least two reports

in which significant changes in 5-HT neurotransmission could not be found in rats neonatally treated with clomipramine (Maudhuit et al., 1995; Dewar et al., 1993)

Circadian rhythm dysfunction has been widely reported in humans (Wehr and Wirz-Justice, 1982) as well in some putative animal models of depression, like chronic mild stress, social isolation or olfactory bulbectomy in rats (Gorka et al., 1996; Greco et al., 1990; Possidente et al., 1996; Rosenwasser and Wirz-Justice, 1997). Circadian rhythm alterations in rats neonatally treated with clomipramine were also reported recently, including an increase in drinking activity rhythm amplitude and changes in free-running period (Rosenwasser et al., 1996), extending previous findings of long lasting effects of neonatal desipramine in rats (Rosenwasser and Hayes, 1994). Our results in hamsters are in agreement with these studies, although we could not detect any change in the free running period in constant darkness.

Hyperactivity is a behavioral feature found in several animal models of depression, like neonatal clomipramine treatment, olfactory bulbectomy, or long-term social isolation in rats (Garzón and Del Río, 1981; Hartley et al., 1990; Possidente et al., 1996). Recent results of our laboratory indicate that neonatally clomipramine-treated hamsters exhibited higher activity levels than control animals as assessed in an activity-meter at diurnal and nocturnal time points (Yannielli et al., submitted). It should be noted that psychomotor disturbance is an important marker of depression. Since alterations in activity levels can phase shift, entrain, or alter the period of free running circadian rhythms in experimental and clinical conditions, depressed-related psychomotor disturbances may be viewed as a cause of, rather than an effect of, altered rhythmicity (Rosenwasser and Wirz-Justice, 1997).

A note of caution regarding the use of hamsters as experimental animals should be exercised. The various studies performed in rats do not always inject clomipramine from postnatal days 8 to 21, but rather use a number of administration protocols. We assumed that days 8 to 21 in the hamster would cover approximately the same developmental stages than those that were used in the rat. Both in rats and hamsters, the serotonergic innervation from the raphe nuclei to the suprachiasmatic nucleus exhibits a similar maturation rate, approximately from day 3 to day 21 (Orpen and Steiner, 1994), so if these neonatal manipulations rely upon effects on these early plasticity events, then we would be on the right developmental time to perform the clomipramine injections.

In summary, the present experiments provide evidence that chronic neonatal treatment with clomipramine alters the hamster circadian system. Although further studies are necessary to establish whether neonatal clomipramine treatment in hamsters can be considered a suitable animal model of endogenous depression, some features described herein could contribute to the understanding of the circadian system role in major depression, as well as to study the relation between the serotonergic and the circadian system in a pharmacological model of depression.

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