

Short-term exposure to constant light promotes strong circadian phase-resetting responses to nonphotic stimuli in Syrian hamsters

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Abstract

Behavioral (nonphotic) stimuli can shift circadian rhythms by serotonin (5-HT) and/or neuropeptide Y (NPY) inputs to the suprachiasmatic nucleus (SCN) circadian clock. Based on the idea that behavioral phase resetting is modulated by endogenous changes in postsynaptic sensitivity to such transmitters, hamsters were exposed to constant light (LL; ~250 lx) for 1–3 days, which suppresses locomotor activity and eliminates the daily rhythm of SCN 5-HT release measured by microdialysis. Groups subjected to brief LL or maintained under a light/dark cycle (LD) received phase-resetting treatments with the 5-HT_{1A,7} agonist (±)-2-dipropyl-amino-8-hydroxyl-1,2,3,4-tetrahydronaphthalene (8-OH-DPAT) or sleep deprivation (SD). Animals were released to constant darkness at the start of the treatments. Phase advances to 8-OH-DPAT and SD during the day were 11 and 3 h for LL vs. 2 and 1 h for LD, respectively. Phase delays during the night were –12 and –5 h for LL vs. no responses for LD, respectively. Phase-transition curves for both LL treatments had slopes approximating 0, indicative of Type 0 phase resetting. For all treatments, the degree of locomotor suppression by LL was not correlated with the phase shift magnitude. Re-establishing locomotor activity by overnight food deprivation did not prevent potentiated shifting to SD. However, re-establishing peak extracellular 5-HT levels by intra-SCN 5-HT reverse microdialysis perfusion in LL did significantly reduce potentiated 8-OH-DPAT phase advances. Constant light also enhanced intra-SCN NPY-induced phase advances during the day (6 vs. 2 h for LD). These results suggest that LL promotes Type 0 phase resetting by supersensitizing 5-HT and/or NPY postsynaptic responses and possibly by attenuating the amplitude of the circadian pacemaker, thus enhancing circadian clock resetting nonspecifically.

Introduction

Mammalian circadian rhythms are generated and maintained by a neural clock located in the suprachiasmatic nucleus (SCN; Rusak & Zucker, 1979; Moore, 1983; Klein *et al.*, 1991). Clock timing is regulated by direct photic signaling from the retina to the SCN via the retinohypothalamic tract (Hendrickson *et al.*, 1972; Moore & Lenn, 1972; Pickard, 1982; Youngstrom & Nunez, 1986; Johnson *et al.*, 1988) and an indirect projection from the intergeniculate leaflet, the geniculohypothalamic tract (Card & Moore, 1982; Johnson *et al.*, 1989). Timing is also regulated by nonphotic (behavioral) information provided to the SCN via neuropeptide Y (NPY) release from geniculohypothalamic tract terminals and serotonergic input from the mid-brain raphe (Albers & Ferris, 1984; Biello *et al.*, 1994; Meyer-Bernstein & Morin, 1996; Marchant *et al.*, 1997; Glass *et al.*, 2003). While photic stimulation is considered to be the predominant synchronizer of clock phase, photic and nonphotic stimuli each have phase-resetting actions, albeit with different phase response curves (PRCs). Nonphotic PRCs generally have phase-advance portions in the

subjective day and phase-delay portions towards the end of the subjective night (Bobrzynska & Mrosovsky, 1998; Mrosovsky *et al.*, 1989). Photic PRCs have phase delays during the early subjective night and phase advances in the late subjective night (Johnson, 1990).

Although the circadian phase-resetting actions of nonphotic stimuli have been well studied, the mechanisms mediating their effects are not well understood. The role of serotonin (5-HT) in nonphotic responses remains particularly speculative (Morin, 1999). Although PRCs for 5-HT agonists resemble behavioral PRCs, there are inconsistencies between studies on the putative site(s) of the phase-resetting action of 5-HT as well as differences between *in vitro* and *in vivo* phase-resetting effects of 5-HT agonists applied directly to the SCN (Mistlberger *et al.*, 2000). Also, reports that raphe lesions (Meyer-Bernstein & Morin, 1998), 5-HT antagonist treatments (Antle *et al.*, 1998) and SCN 5-HT depletion (Bobrzynska *et al.*, 1996b) do not block behavioral phase shifts weaken the case for a role of 5-HT in many types of nonphotic phase regulation.

Nevertheless, potent phase-resetting actions of 5-HT have been observed under conditions where the serotonergic postsynaptic response is enhanced. For example, inhibiting 5-HT synthesis potentiates *in vivo* phase advances induced by intra-SCN perfusion of (±)-2-dipropyl-amino-8-hydroxyl-1,2,3,4-tetrahydronaphthalene (8-OH-DPAT), the 5-HT_{1A,7} agonist (Lovenberg *et al.*, 1993), indicating

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that diminished 5-HT output can up-regulate serotonergic phase resetting (Ehlen *et al.*, 2001). Also, the large phase-advancing effects of 5-HT agonists in the deafferented SCN slice have been attributed to a similar depletion-induced hypersensitivity (Mistlberger *et al.*, 2000). From this we speculate that nonphotic phase resetting is influenced not only by the circadian phase of stimulus application but also by the degree of serotonergic activity preceding the stimulus. To test this, experiments were undertaken by exposing hamsters to brief duration (1–3 days) constant light (LL) exposure which eliminates the daily rhythm of SCN 5-HT release and potentiates serotonergic and behavioral phase resetting (Knoch *et al.*, 2002). Pharmacological and behavioral trials were undertaken in which the effects of LL on 8-OH-DPAT and behavioral [sleep deprivation (SD)] PRCs were assessed. The actions of LL on NPY and wheel-running phase-shifting responses were also examined to determine if LL has wide-ranging effects in the circadian nonphotic clock-resetting mechanism.

Materials and methods

Animals

Adult male Syrian hamsters (*Mesocricetus auratus*) reared from breeder pairs obtained from Harlan Sprague Dawley (Madison, IL, USA) were housed in a 22°C climate-controlled vivarium. The animals were kept individually in polycarbonate cages under a 14 h light : 10 h dark cycle (LD) with average light intensity of ~250 lx. For animals used in the behavioral (SD) trials, cages were equipped with a 35-cm diameter running wheel. Rodent chow (Prolab 3000; PMI Feeds, St Louis, MO, USA) and water were provided *ad libitum*. The experiments were conducted using the National Institutes of Health Guidelines for the Care and Use of Laboratory Animals.

Circadian activity measurements

In all experiments, locomotor activity was measured for a minimum of 7 days under LD prior to experimentation. Locomotor activity was recorded using infrared sensors or magnetic switches attached to running wheel assemblies for the 8-OH-DPAT and SD experiments. These measurements provided data on the circadian rhythms of general locomotor activity and voluntary wheel-running activity, respectively. For both measurement systems, output was interfaced with a computerized data acquisition system (Dataquest III; Mini-Mitter Co., Sunriver, OR, USA). Circadian phase resetting was assessed using a modified Aschoff type II procedure (Aschoff, 1965), where animals were released into constant darkness (DD) at the time of drug or behavioral treatment. Experiments were performed after a minimum of 7 days of stable entrainment under LD. Phase shifts were calculated as follows: a line based on the five preceding days was extrapolated to the day of treatment under LL and then a regression line based on days 3–10 post-treatment was back-extrapolated to the same day of treatment. The difference between these two extrapolated lines on the day of treatment is considered the phase shift. Activity onset was defined as the first bout of activity sustained for at least 30 min as recorded from infrared sensors or the first 15-min period in which the total number of wheel revolutions exceeded 50% of the maximum number of revolutions/10 min measured that day and that was followed by at least 1 h of sustained activity.

By definition, the 24 h time designation for animals held under LD is referred to as zeitgeber time (ZT; with ZT 12 designated as the time of lights-off). For clarity of data presentation, however, ZT rather than the circadian time convention for free-running conditions with no ZT was used to designate the circadian phase of treatments delivered during the short-term LL exposures. There were no perceptible changes in

phase or period of the free-running circadian activity rhythm during these brief exposures, so ZT is equivalent to circadian time as a phase marker.

Microdialysis-HPLC

Concentrically designed microdialysis probes were constructed from a 26-gauge stainless steel outer cannula (Small Parts, Miami, FL, USA) into which was inserted a beveled 32-gauge fused silica tube (Poly-micro Technologies, Phoenix, AZ, USA). Hemicellulose dialysis tubing (12 kDa MW cutoff; 230 µm OD; Spectra-por; Fisher Scientific, Pittsburgh, PA, USA) was inserted approximately 1.0 mm into the outer cannula and secured with epoxy glue. The distal end of the tubing was cut to a length of 1.5 mm and the tip sealed with epoxy, providing an active dialysing length of 1.0 mm.

Procedures for microdialysis of the SCN have been described previously (Dudley *et al.*, 1998, 1999). Briefly, the probes were implanted in the SCN under nembutal (50 mg/kg) anaesthesia.

Microdialysis was performed 1 day after probe implantation, beginning at ZT0 by continuously perfusing probes with filtered artificial cerebrospinal fluid (NaCl, 147 mM; KCl, 4.0 mM, CaCl₂, 1.8 mM; pH 7.2) at a flow rate of 1.2 µL/min using a calibrated syringe pump (CMA/100; Bioanalytical Systems, Inc., West Lafayette, IN, USA) attached to an overhead liquid swivel (Instech, Plymouth Meeting, PA, USA). The assembly allowed the animals freedom of movement within the cage throughout the duration of the experiment. For all experiments, measurement of extracellular 5-HT in the SCN was aided by adding 4.0 µM citalopram to the perfusate. The sampling interval was 1 h. Probe tip location was determined from cresyl violet-stained frozen sections at the end of the experiment.

Microdialysate was analysed for 5-HT using HPLC (Bioanalytical Systems, Inc.) with amperometric electrochemical detection. Samples were injected onto a 100 × 1 mm 3-µm C-18 reverse-phase microbore column. The mobile phase consisted of 9.45 g monochloroacetic acid (Fisher Scientific), 0.2 g octanesulfonic acid and 0.25 g Na₂ EDTA (Eastman Kodak Co., Rochester, NY, USA) dissolved in 1.0 L HPLC-grade distilled water (pH 3.1). Tetrahydrofuran (6.0 mL) was added after filtration. The flow rate of the mobile phase through the column was 90 µL/min. A 3.0-mm glassy carbon radial-flow electrochemical detector (BAS Bioanalytical Systems Inc., West Lafayette, IN) set at a potential of 590 mV relative to an AgCl reference electrode was used to measure 5-HT. The lower level of sensitivity (signal ≥ 5 × background) was approximately 500 fg. Electrode output was interfaced with an IBM-compatible computer which recorded and analysed the data. Authenticity of the 5-HT peak in SCN microdialysate has been verified by predictable changes in its size following electrical and pharmacological stimulations of the raphe and localized administration of pharmacological agents to the SCN via the dialysis probe (Dudley *et al.*, 1998).

Experimental protocols

Effect of constant light exposure on 8-OH-DPAT-induced phase resetting

The protocol used throughout this study for administering the LL exposures is presented diagrammatically in Fig. 1. The LL (keeping the ~250 lx environmental lighting on) begins at ZT 12 and proceeds from 1 to 3 days depending on the experiment. As an example, treatments administered at ZT 6 and 18 in animals of a 2-day LL group would fall on 42 and 54 h of LL exposure, respectively.

As short-term LL exposure eliminates the nocturnal peak of SCN 5-HT release, it is possible that this could cause a subsequent up-regulation of 5-HT postsynaptic response. It is hypothesized that such

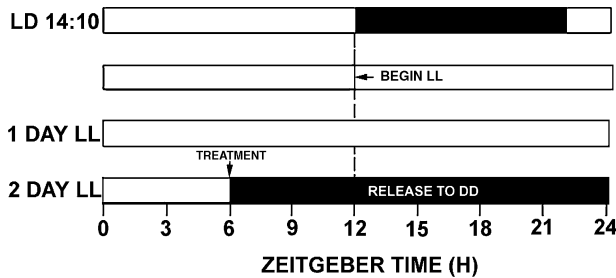


FIG. 1. Protocol for assessing the effects of brief constant light (LL) exposure on behavioral and serotonergic [(±)-2-dipropyl-amino-8-hydroxyl-1,2,3,4-tetrahydronaphthalene-induced] phase-resetting responses. Depicted is a treatment delivered at zeitgeber time 6 of the second day of LL exposure (referred to as 2-day LL exposure). Animals are released from LL to constant darkness (DD) at the outset of a behavioral or drug treatment. The top black bar represents the dark phase of the initial 14 h light: 10 h dark cycle (LD).

up-regulation could potentiate serotonergic circadian phase-resetting effects. This idea was tested by measuring phase-resetting responses induced by systemic application of the 5-HT_{1A,7} receptor agonist 8-OH-DPAT with and without LL exposure. Animals were released into LL on the morning of the seventh day of activity recording under LD and the phase-resetting effects of 8-OH-DPAT were assessed after 1, 2 or 3 days of LL. An i.p. injection of 8-OH-DPAT (5 mg/kg; 5 mg/mL dimethylsulfoxide) or injection vehicle (dimethylsulfoxide) was administered at ZT 6 or 18 and the animals immediately released to DD according to the Aschoff type II protocol. Controls consisted of animals receiving 1, 2 or 3 days of LL treated at ZT 6 or 18 with vehicle injection or no injection and groups maintained under LD that received 8-OH-DPAT injection, vehicle injection or no injection. An additional LL group received an i.p. injection of the 5-HT_{1,2,7} receptor antagonist metergoline at ZT 6 at a dose that blocks behavior-induced 5-HT release in the SCN (5 mg/kg; Glass *et al.*, 2003). Phase resetting in all control groups was assessed after release into DD using the Aschoff type II procedure.

Phase response curves for 8-OH-DPAT

To determine if LL exposure can affect the shape of the 8-OH-DPAT PRC, phase-resetting responses to i.p. 8-OH-DPAT (5 mg/kg) were determined over eight equally spaced time-points spanning the 24-h day. The PRCs for 8-OH-DPAT were generated for groups that received 2 days of LL or were maintained under LD. Control PRCs were generated for groups receiving 2 days of LL and treated with vehicle injection or no injection and for animals maintained under LD that received only vehicle injection.

Suprachiasmatic nucleus serotonin perfusion and constant light-potentiated 8-OH-DPAT phase resetting

The elimination of the nocturnal peak in SCN 5-HT release under LL suggests that depletion-related hypersensitivity could underlie the potentiating effect of LL on serotonergic phase resetting. To test this, 3-h reverse microdialysis perfusions of the SCN with 5-HT (50 µM dissolved in artificial cerebrospinal fluid) were undertaken from ZT 12 to 15 over the two nights of the LL procedure to re-establish the peaks in extracellular 5-HT that occur under LD. Controls received artificial cerebrospinal fluid perfusion. Both groups were then treated with an i.p. injection of 8-OH-DPAT (5 mg/kg) the next day at ZT 6 (15 h after the end of the second microdialysis perfusion) and released to DD. Phase-resetting responses were measured as described above.

Constant light exposure and neuropeptide Y-induced phase resetting

It is possible that LL exposure potentiates nonphotic phase-resetting responses by also enhancing sensitivity to neurotransmitters other than 5-HT. To test this, the effects of intra-SCN microinjection of NPY (0.5 µL, 0.5 µg; Phoenix Pharmaceuticals, Belmont, CA, USA) or vehicle were assessed at ZT 6 and 18 in animals receiving 2 days of LL. Neuropeptide Y was also administered at ZT 6 in a separate group maintained under LD. Injections were administered directly into the SCN in unanesthetized animals under dim light (25 lx) using a microinjection needle with tip extending 1 mm ventral from a re-entry cannula. Animals were released to DD immediately after receiving the injection. The re-entry cannula was implanted using pentobarbital anesthesia (Nembutal, 50 mg/kg) a minimum of 5 days prior to experimentation using the following stereotaxic coordinates: anterior-posterior, 0.3 mm anterior to bregma; lateral, 0.3 mm from midline; horizontal, 7.0 mm below dura.

Constant light exposure and sleep deprivation-induced phase resetting

Sleep deprivation is a behavioral stimulus that potently resets the circadian clock and enhances SCN 5-HT release (Antle & Mistlberger, 2000; Grossman *et al.*, 2000). Trials were, therefore, undertaken to determine if the brief LL exposure affects behavioral PRCs in the same manner as it does the 8-OH-DPAT PRC. The SD procedures used to induce phase resetting here are similar to those described in our previous studies (Antle & Mistlberger, 2000; Grossman *et al.*, 2000). Wheel-running activity was monitored under LD for a minimum of 10 days prior to experimentation. Animals were then subjected to 2 days of LL beginning the morning of the last day of LD. At ZT 6 or 18 of the second day of LL, the animals were placed in a novel cage under dim red light (<1 lx). They were aroused and maintained in the waking state for 3 h using intermittent gentle air puffs. At the end of the experimental period, the animals were returned to their home cage and released to DD. Controls consisted of groups maintained under LD that received or did not receive the behavioral manipulation at ZT 6 or 18 or groups that received 2 days of LL but were not behaviorally stimulated. All control groups received the 3-h exposure to dim red light and were subsequently released to DD.

Constant light exposure and novel wheel-induced phase resetting: activity dependence

It is unknown if LL-potentiated behavioral phase-resetting responses are a direct consequence of suppressed locomotor activity or some other effect on the circadian time-keeping mechanism. To approach this question, food deprivation was used to maintain locomotor activity over the period of LL exposure. Adult hamsters were initially housed individually in standard polypropylene cages equipped with 17-cm running wheels monitored continuously by computer. The hamsters were entrained to a 14:10 LD cycle for 10 days and then subjected to several tests in LL and DD, as follows. The hamsters first received two counterbalanced tests in which they were exposed to 3 days of DD, beginning at ZT 6, followed by 10 days in LD for re-entrainment. During one test, the hamsters were sleep deprived for 3 h beginning at DD onset (i.e. from ZT 6 to 9) by confinement to a novel 33-cm diameter running wheel, after which time they were returned to their home cages and left undisturbed for the remaining days in DD. The novel wheel procedure stimulates running and has proven to be a convenient procedure for keeping hamsters awake without additional intervention. During the other test, the hamsters were left undisturbed from the onset of DD. Following re-entrainment to LD, the hamsters received two additional tests, separated by 12 days in LD and counterbalanced for order, during which they were exposed to LL for 56 h

beginning at ZT 0. Lights were turned off at ZT 6 and the hamsters confined to a novel wheel for 3 h. The hamsters were then returned to their home cages and left undisturbed for 3 days in DD. During one test, the hamsters were deprived of food for 27 h to maintain locomotor activity (Mistlberger *et al.*, 1997), beginning at ZT 6 on the second day in LL. During the other test food was available *ad libitum*. Light intensity during LD and LL averaged 16 lx in the middle of each cage and a maximum of 96 lx at the side of the cage closest to the incandescent light source.

Histology

The locations of the SCN microdialysis and microinjection implant sites were verified histologically after experimentation. The animals were killed with an overdose of Nembutal, and the brain was removed and placed in 4% paraformaldehyde for 2 days at 4 °C. After washing with phosphate-buffered saline, 20- μ m-thick frozen sections were prepared by cryostat. Implant tract locations were analysed in sections mounted on glass slides and stained with cresyl violet. Data from animals with an implant located further than 500 μ m lateral from the SCN were not included.

Statistics

Drug treatment and LL treatment effects on circadian phase resetting were analysed using a randomized ANOVA and the Student-Newman-Keuls posthoc mean comparison test was performed. An unpaired *t*-test was used to compare 5-HT levels during day to the mean 5-HT level during night. The level set for statistical significance was $P < 0.05$.

Results

Constant light exposure suppresses locomotor activity and suprachiasmatic nucleus serotonin release

Locomotor activity

The 1–3-day LL exposures significantly suppressed general locomotor and wheel-running activity. As measured on the second day of LL, general locomotor and wheel-running activity were suppressed by 65.6 ± 3.6 and $81.5 \pm 6.0\%$, respectively, vs. pretreatment levels measured in LD immediately prior to the LL exposure ($n = 8$ for both groups). For the majority of LL-exposed animals, there was an absence of the activity peak usually associated with the time of lights-off.

Suprachiasmatic nucleus serotonin release

Continuous 48-h microdialysis sampling of SCN 5-HT release over one LD cycle and the succeeding day after release to LL confirmed that the nocturnal elevation of SCN 5-HT release normally occurring during the early night was eliminated by LL exposure (Fig. 2; $n = 4$). The night-time levels of 5-HT release during the LL exposure were equivalent to basal levels observed during the day. This suppression was not a procedural artifact of the long-duration microdialysis sampling, as we have previously measured two consecutive nocturnal peaks of SCN 5-HT release using similar 48-h microdialysis sampling procedures conducted over two LD cycles (Dudley *et al.*, 1998).

Constant light potentiates serotonergic circadian phase-resetting responses

Time-course of constant light effects on 8-OH-DPAT phase resetting

Daytime. Animals exposed to 1, 2 or 3 days of LL exhibited significantly larger phase-advance shifts to 8-OH-DPAT at ZT 6 compared with animals maintained under LD (Figs 3 and 4). The 2-day treatment with LL had the most robust enhancing effect on

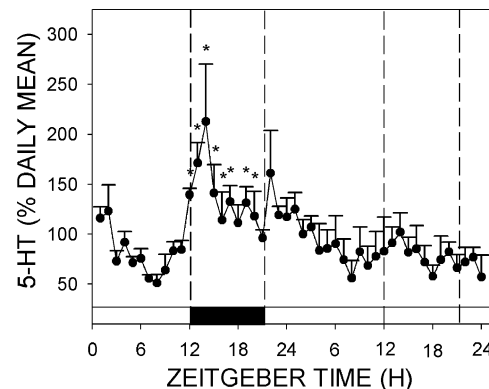


FIG. 2. Exposure to constant light (LL) suppresses the circadian rhythm of serotonin (5-HT) release in the suprachiasmatic nucleus measured by microdialysis in freely behaving hamsters. Note the absence of the nocturnal peak of 5-HT during the second subjective night after release from light/dark cycle (LD) to LL. *Significant difference from the averaged daytime level. Black bar shows dark phase of the LD cycle. Vertical lines represent the SEM.

8-OH-DPAT shifting (4.9 ± 0.4 h; $P < 0.05$ vs. LD; $n = 6$). In comparison, the average 8-OH-DPAT-induced phase advance under LD at ZT 6 was less than half of that of the LL-exposed group (2.1 ± 0.3 h; $n = 6$).

Night-time. Animals exposed to 2 or 3 days of LL exhibited large phase-delay shifts to 8-OH-DPAT at ZT 18. The extent of these shifts differed greatly from the negligible phase-resetting effects of 8-OH-DPAT under LD (Figs 3 and 4). Similar to the ZT 6 trials, the phase-advancing effect of 8-OH-DPAT was maximal after 2 days of LL (-12.1 ± 2.4 h; $n = 7$; $P < 0.05$ vs. LD). In contrast, the 8-OH-

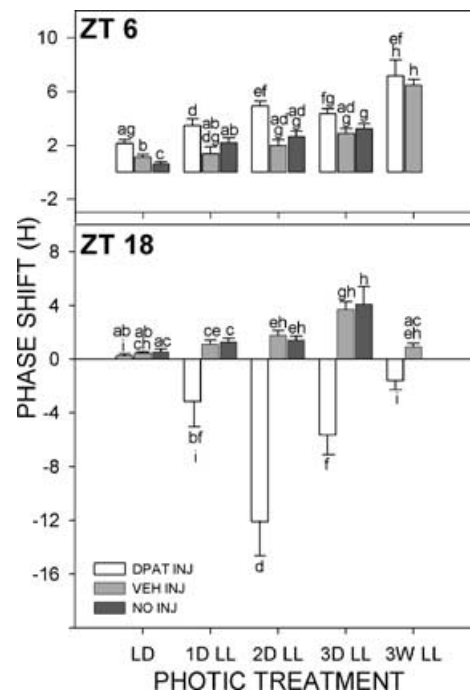


FIG. 3. The potentiating effects of 1–3 days and 3 weeks of constant light (LL) exposure on the phase-resetting responses to i.p. ($\pm$)-2-dipropyl-amino-8-hydroxyl-1,2,3,4-tetrahydronaphthalene injection (DPAT INJ), vehicle injection (VEH INJ) or no injection (NO INJ) during the mid subjective day [zeitgeber time (ZT) 6, upper panel] and late subjective night (ZT 18, lower panel). For each time-point, bars with different letters are significantly different ($P < 0.05$). Bars represent the mean $\pm$ SEM. LD, 14 h light: 10 h dark cycle.

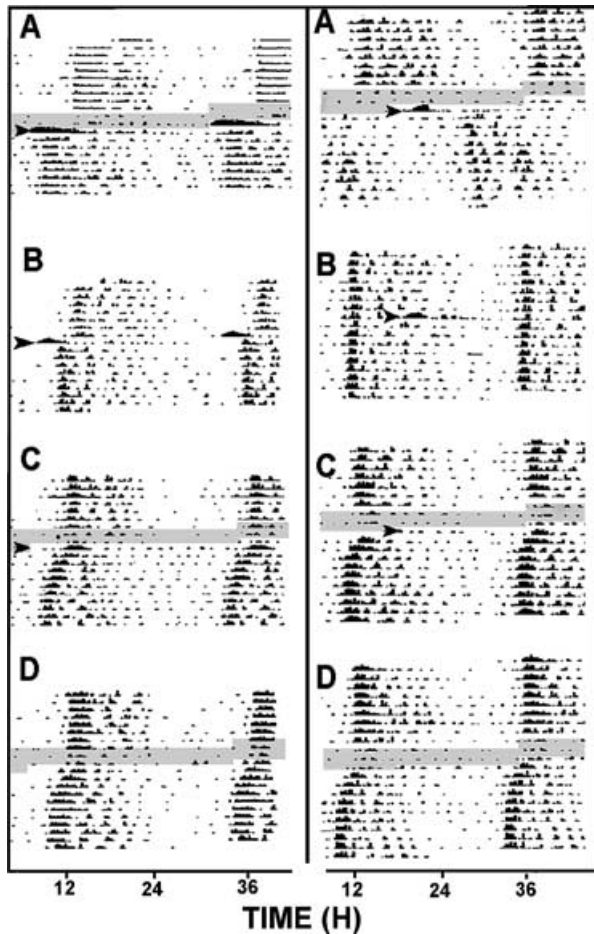


FIG. 4. Representative double-plotted actograms of general locomotor activity showing the potentiating effects of 2 days of constant light (LL) exposure on the phase-resetting responses to i.p. ($\pm$)-2-dipropyl-amino-8-hydroxyl-1,2,3,4-tetrahydronaphthalene (8-OH-DPAT) injection during the mid subjective day [circadian time (CT) 6; left panel] or late subjective night (CT 18; right panel). Arrows denote the time of injection. Shading represents the LL treatment and animals are released to constant darkness thereafter. (A) 8-OH-DPAT after 2 days of LL exposure; (B) 8-OH-DPAT under 14 h light:10 h dark cycle; (C) vehicle injection after 2 days of LL exposure; (D) no injection after 2 days of LL exposure. In all cases note the diminished level of general locomotor activity during the period of LL.

DPAT-induced phase-resetting response under LD at ZT 18 was an advance of $+0.2 \pm 0.1$ h ($n = 7$). After 3 weeks of exposure to LL, a phase-delaying effect of 8-OH-DPAT was still evident (1.6 ± 0.7 h; $P < 0.05$ vs. LD; $n = 3$) but significantly reduced compared with that in the 2-day LL group (Fig. 3).

It should be noted that the averaged ≥ 12 h 8-OH-DPAT-induced shifts of the 2-day LL group at ZT 18 and 21 were deemed delay rather than advance shifts for two reasons: first, within this treatment group, there was a continuum of phase-delaying response with some animals clearly exhibiting smaller delays of 3–4 h with no evidence of advance shifts like those of the control groups and second, the 1 and 3-day LL groups exhibited delays of lesser magnitude, also with no evidence of advance shifting.

Constant light exposure alters the shape of the 8-OH-DPAT phase response curve

The PRCs for 8-OH-DPAT, various control treatments under LD and after 2 days of LL exposure are shown in Fig. 5. The LL treatment dramatically increased the amplitude and extended the phase of the

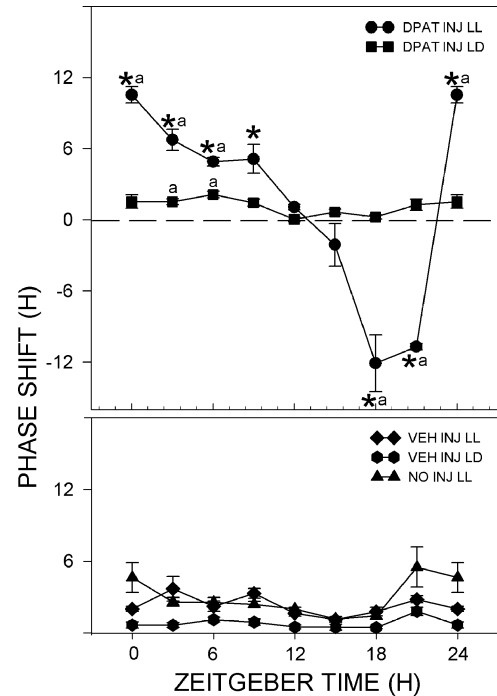


FIG. 5. The phase response curves (PRCs) for i.p. injection of ($\pm$)-2-dipropyl-amino-8-hydroxyl-1,2,3,4-tetrahydronaphthalene (8-OH-DPAT) (DPAT INJ) and control treatments under 14 h light:10 h dark cycle (LD) and after 2-day constant light (LL) exposure. The LL exposure markedly enhanced the phase-advancing portion and generated a large phase-delaying portion of the 8-OH-DPAT PRC (top panel). * $P < 0.05$ LL vs. LD 8-OH-DPAT effects; ^a $P < 0.05$ vs. respective vehicle injection controls. Vertical lines represent the SEM. VEH INJ, vehicle injection; NO INJ, no injection.

advancing portion of the 8-OH-DPAT PRC during the day. In particular, phase advances during the early morning ZT 0 (2 h after lights-on) and ZT 3 (10.5 ± 0.7 h, $n = 5$ and 6.8 ± 0.9 h, $n = 6$, respectively) were several-fold greater than those obtained with time-matched 8-OH-DPAT treatment groups under LD (1.5 ± 0.6 h, $n = 6$ and 1.1 ± 0.2 h, $n = 6$, respectively; $P < 0.01$ for LL vs. LD effects for either time point). The 2-day LL exposure also added a large phase-delaying portion to the 8-OH-DPAT PRC during the late night at ZT 18 (see above) and 21 (-10.7 ± 0.3 h, $n = 7$) compared with time-matched 8-OH-DPAT treatment groups under LD where there was no significant shifting effect ($+0.2 \pm 0.1$ h, $n = 7$ and $+1.3 \pm 0.5$ h, $n = 7$, respectively; Fig. 5). The 2-day LL exposure alone or with vehicle injection produced phase-advance shifts throughout the 24-h circadian day.

8-OH-DPAT phase transition curve

Replotting 8-OH-DPAT-induced phase shifts against the initial phase of drug application to produce a phase transition curve (PTC) (Winfree, 1970) for animals maintained under LD produced a line with a slope of 1.02. This value is characteristic of Type 1 phase resetting (Fig. 6). A PTC plot of the larger 8-OH-DPAT phase-resetting responses for groups that received 2 days of LL had a slope of 0.00 denoting a Type 0 phase-resetting response.

Serotonin perfusion of the suprachiasmatic nucleus reduces constant light-potentiated 8-OH-DPAT phase resetting

The 3-h reverse microdialysis perfusions of the SCN with 5-HT caused a 30% reduction of the LL-potentiated phase-advancing response to i.p. 8-OH-DPAT injection compared with artificial cerebrospinal fluid-perfused controls (3.0 ± 0.2 vs. 4.2 ± 0.3 h, respectively;

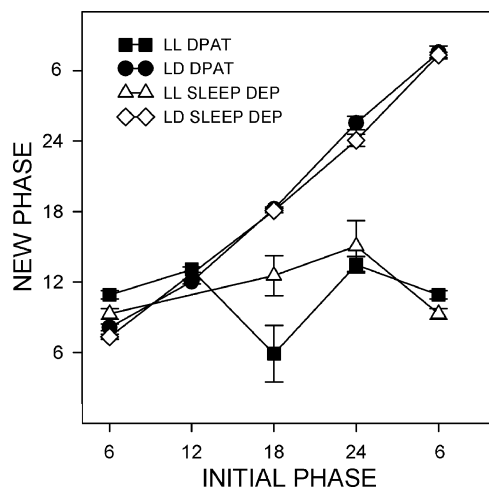


FIG. 6. Phase transition curve analysis of phase-shifting responses to ($\pm$)-2-dipropyl-amino-8-hydroxyl-1,2,3,4-tetrahydronaphthalene (DPAT) and sleep deprivation (SLEEP DEP) in animals maintained under 14 h light : 10 h dark cycle (LD) or that received 2-day constant light (LL) exposure. Under LD, the slopes of the lines approximated 1 (indicative of Type 1 resetting), while the LL treatment produced lines with slopes approximating 0 (indicative of Type 0 resetting).

$P < 0.05$; Fig. 7; $n = 4$ /group). Actograms showed no phase-resetting effect of the 5-HT perfusions on the circadian locomotor activity rhythm, which is consistent with the present PRC analyses showing negligible 8-OH-DPAT phase resetting between ZT 12 and 15.

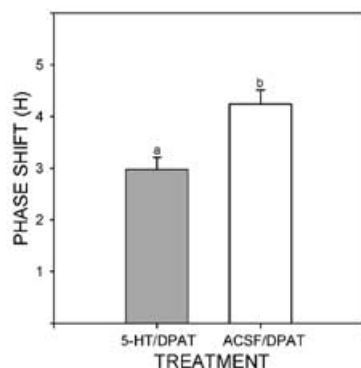
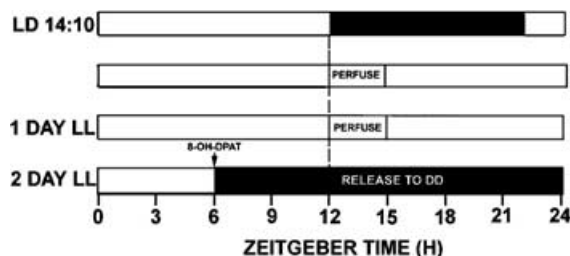


FIG. 7. Mimicking the night-time peak of serotonin (5-HT) release in the suprachiasmatic nucleus (SCN) under constant light (LL) attenuates the potentiating effect of LL on serotonergic phase resetting. Top: Protocol for delivery of the two 3-h intra-SCN perfusions of 5-HT or vehicle initiated at the onset of two consecutive subjective nights [zeitgeber time (ZT) 12] during LL. An i.p. injection of ($\pm$)-2-dipropyl-amino-8-hydroxyl-1,2,3,4-tetrahydronaphthalene (8-OH-DPAT) (DPAT) was subsequently administered at ZT 6 of the second day of LL exposure, accompanied by immediate release to constant darkness. Bottom: The intra-SCN 5-HT perfusion suppressed the LL-potentiating phase-resetting response to i.p. 8-OH-DPAT administered 15 h after the end of the second 5-HT perfusion vs. intra-SCN vehicle-perfused controls; $P < 0.05$ (bars with different letters above are significantly different). ACSF, artificial cerebrospinal fluid; LD, 14 h light : 10 h dark cycle.

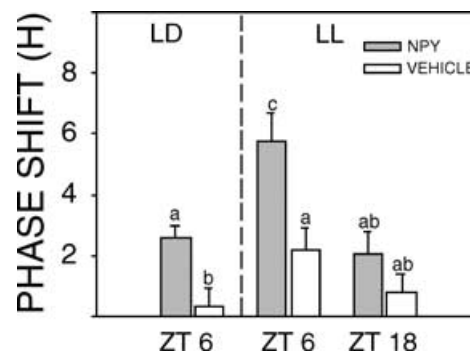


FIG. 8. Two days of constant light (LL) exposure significantly enhances the phase-advancing effect of intra-suprachiasmatic nucleus microinjection of neuropeptide Y (NPY) during the subjective day compared with controls maintained under 14 h light : 10 h dark cycle (LD). For each time point, bars with different letters are significantly different, $P < 0.05$. Bars represent the mean $\pm$ SEM. ZT, zeitgeber time.

Constant light potentiates phase-advance shifts to neuropeptide Y

Daytime

Animals exposed to LL for 2 days exhibited large (5.8 ± 0.9 h) phase-advance shifts to intra-SCN injection of NPY administered at ZT 6 (Fig. 8; $n = 6$). These shifts differed significantly from the phase-advancing effects of intra-SCN vehicle injection (2.2 ± 0.7 h; $n = 5$) or intra-SCN injection of NPY administered to animals maintained under LD at this time (2.1 ± 0.4 h, $n = 4$; both treatments $P < 0.05$ vs. LL NPY).

Night-time

Intra-SCN NPY injection administered at ZT 18 to animals exposed to LL for 2 days caused phase-advance shifts of 2.1 ± 0.7 h ($n = 4$). Intra-SCN vehicle injection at this time induced phase-advance shifts of 0.8 ± 0.6 h ($n = 3$) which did not differ significantly from the NPY shifts ($P > 0.05$; Fig. 8).

Constant light potentiates phase shifts to sleep deprivation

Constant light exposure and sleep deprivation-induced phase resetting

Daytime. In the LL time-course trials, all groups exposed to 1, 2 or 3 days of LL exposure exhibited significantly larger phase-advance shifts to SD at ZT 6 compared with animals maintained under LD (Figs 9 and 10), with potentiating effects of LL manifest after the first day of LL exposure (3.4 ± 0.5 h). In comparison, the average SD-induced phase advance under LD at ZT 6 was 1.3 ± 0.2 h ($P < 0.05$ vs. each LL group, $n = 6$ and 5, respectively). Similar to the 8-OH-DPAT trials, maximal SD-induced phase advances were observed at ZT 0 (8.9 ± 2.2 h, $n = 9$; Fig. 11). Animals receiving no SD treatment at this time had phase advances of 4.6 ± 1.3 h ($P < 0.05$ vs. the SD group). Also, similar to the phase-advancing effects of LL alone on the general circadian locomotor activity rhythm described above, LL caused significant phase-advance shifts in the circadian wheel-running rhythm.

Night-time. Animals exposed to LL for 2 or 3 days exhibited large phase-delay shifts to SD at ZT 18. These shifts contrasted markedly with the negligible phase-resetting effects of SD under LD at this time (Fig. 9, bottom panel). The phase-delaying effect of SD was maximal after 2 days of LL exposure (-5.5 ± 1.2 h; $P < 0.05$ vs. LD, $n = 5$) and SD-induced phase shifts under LD at ZT 18 averaged $+0.2 \pm 0.1$ h ($n = 5$).

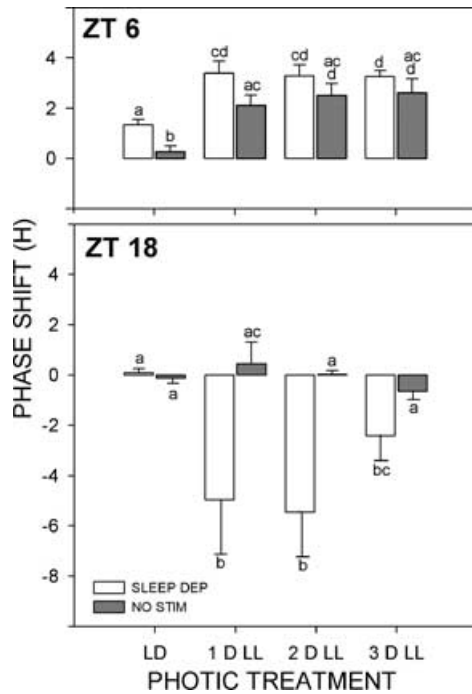


FIG. 9. The potentiating effects of 1–3 days of constant light (LL) exposure on the phase-resetting responses to 3 h of sleep deprivation (SLEEP DEP) or no behavioral stimulation (NO STIM) during the mid subjective day [zeitgeber time (ZT) 6–9, upper panel] and late subjective night (ZT 18–21, lower panel). For each time-point, bars with different letters are significantly different ($P < 0.05$). Bars represent the mean $\pm$ SEM. LD, 14 h light : 10 h dark cycle.

Sleep deprivation phase transition curve

Similar to the 8-OH-DPAT trials, PTC analysis of SD-induced phase shifts for animals maintained under LD revealed a line with a slope of 1.03, characteristic of Type 1 phase resetting (Fig. 6). The PTC plot of the SD phase-resetting responses for groups that received 2 days of LL had a slope of 0.10, denoting Type 0 phase resetting.

Phase shifting and locomotor activity suppression by constant light

Degree of activity suppression under constant light and phase-resetting response

8-OH-DPAT. Two indices of general locomotor behavior were measured over the 2-day period of LL: (i) intensity (integrated activity counts under LL normalized as a percentage of the same individual's counts integrated over an equivalent period of LD) and (ii) duration (total time of activity normalized as a percentage of that individual's time of activity under an equivalent period of LD; Fig. 12). For both day (ZT6) and night (ZT18), correlation analysis revealed no significant relationship between the extent of suppressed activity intensity or duration caused by LL exposure and the magnitude of 8-OH-DPAT-induced advance or delay shifts (all groups $P > 0.5$).

Sleep deprivation. Similar analyses undertaken for wheel-running activity in the SD trials also revealed no significant relationships between the LL-related suppression of activity intensity (number of wheel revolutions) or running duration and the magnitude of phase-resetting responses at ZT6 or 18 (for all groups, $P > 0.1$; Fig. 12).

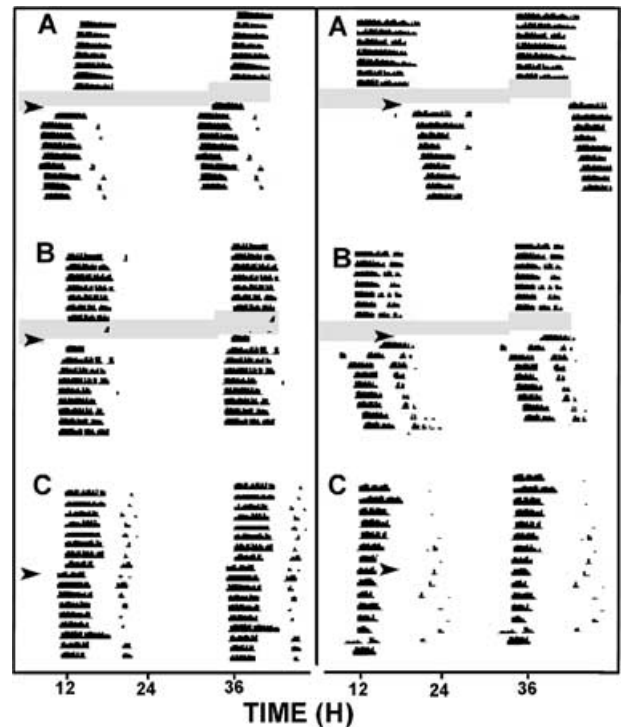


FIG. 10. Representative double-plotted actograms of wheel-running activity showing the potentiating effects of 2 days of constant light (LL) exposure on the phase-resetting responses to 3 h of sleep deprivation (SD) during the mid subjective day [beginning at zeitgeber time (ZT) 6; left panel] or late subjective night (beginning at ZT18; right panel). Arrows denote the time of SD stimulation. The shading represents the LL treatment and animals are released to constant darkness thereafter. (A) SD after 2 days of LL; (B) no SD stimulation after 2 days of LL; (C) SD in animals maintained under 14 h light : 10 h dark cycle. Note the lack of wheel running throughout the period of LL exposure.

Constant light-potentiates phase shifting induced by novel wheel access: effect of food deprivation-induced activity

Novel wheel-induced phase advances. Eleven hamsters provided usable data in all tests and were included in the analysis. When hamsters maintained under LD were confined to a novel wheel for 3 h from ZT6 to 9 at DD onset, the phase of activity onset was

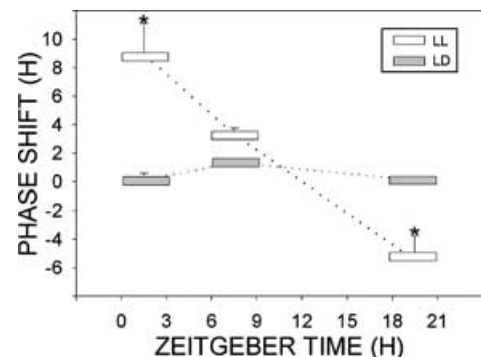


FIG. 11. Sleep deprivation (SD) phase response curves (PRCs) for animals receiving 2 days of constant light (LL) exposure or maintained under 14 h light : 10 h dark cycle (LD). The LL exposure markedly increased the phase-advancing and phase-delaying portions of the SD PRC. * $P < 0.05$ LL vs. LD effects. The bars span the 3-h period of each SD treatment and represent the mean $\pm$ SEM.

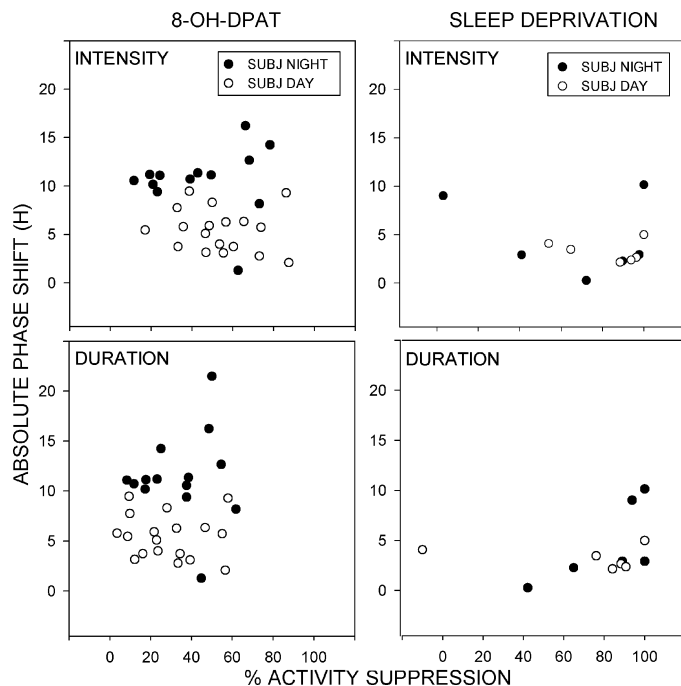


FIG. 12. Suppression of locomotor activity under constant light (LL) [wheel-running for the sleep deprivation trials or general locomotor activity for the ($\pm$)-2-dipropyl-amino-8-hydroxyl-1,2,3,4-tetrahydronaphthalene (8-OH-DPAT) trials] is not correlated with the phase-resetting response. No significant correlations existed for intensity (number of wheel revolutions or activity episodes) or duration (integrated bouts of wheel running or general activity) under LL expressed as a percentage of equivalent periods immediately preceding the LL exposure.

advanced by 91 ± 28 min ($P < 0.02$; Fig. 13). When hamsters were confined to a novel wheel for 3 h after 56 h of LL, however, phase-advance shifts were markedly potentiated (235 ± 22 min; $P < 0.01$). As phase shifts exhibit a sigmoidal relationship to the amount of running stimulated by wheel confinement, statistical comparisons between the LD-DD and LL-DD conditions were also conducted using data from the subset of hamsters ($n = 7$) that ran continuously on both tests. These hamsters showed no difference between conditions in the amount of running in the novel wheels (5245 ± 277 vs. 5485 ± 117 revolutions; $P > 0.3$) but did show significantly larger phase-advance shifts in the LL condition (167 ± 11 vs. 238 ± 13 min; $P < 0.04$, respectively).

Food deprivation-restored activity and phase shifting. During LD prior to the LL tests, the 11 hamsters averaged 7435 ± 1683 revolutions/day in the home wheel (Fig. 13). Daily running was strongly suppressed in LL, averaging 1490 ± 486 and 1271 ± 427 revolutions during the first and second days, respectively, when food was available *ad libitum*. When LL was combined with 27 h of food deprivation, total running activity on day 2 of LL was markedly increased to LD levels (11696 ± 2683 revolutions). Despite this ninefold difference in wheel running under LL between the *ad libitum* and deprived groups, there was no significant difference between the magnitude of the phase shifts induced by novel wheel confinement (238 ± 13 vs. 298 ± 28 min, respectively; $P = 0.09$). The amount of running during wheel confinement also did not differ between food access conditions (5137 ± 399 vs. 4969 ± 248 revolutions/3 h in *ad libitum* and deprivation conditions, respectively; $P > 0.1$).

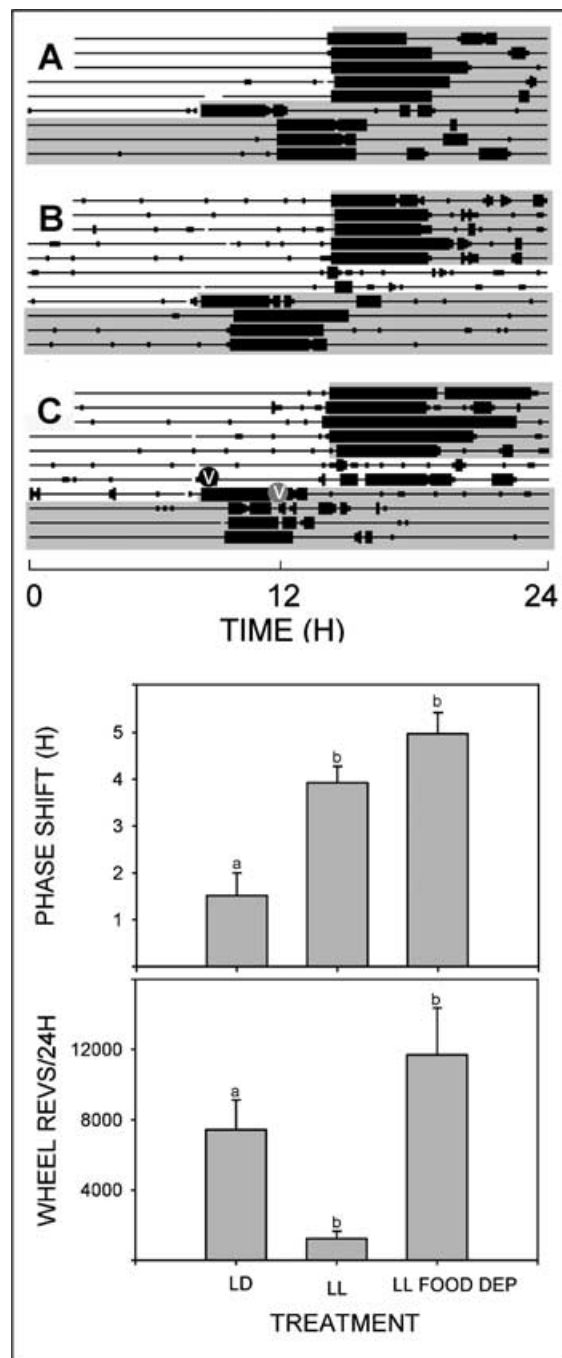


FIG. 13. Food deprivation-enhanced wheel running under constant light (LL) does not attenuate the phase-resetting effect of novel wheel exposure. Top: Representative actograms showing the phase-advancing effects of 3 h novel wheel exposure from zeitgeber time 6 to 9 in animals that (A) were maintained under 14 h light: 10 h dark cycle (LD); (B) received 2-day LL exposure or (C) received 2-day LL exposure and 27 h of food exposure to induce running (V indicates onset and offset of food deprivation). Middle and bottom: Effects of the three manipulations on phase resetting and running intensity, respectively. Bars with different letters are significantly different, $P < 0.05$. Bars represent the mean $\pm$ SEM.

Discussion

Here we report that short-term exposure to LL hypersensitizes nonphotic circadian clock-resetting responses. A salient feature of this treatment is its promotion of strong Type 0 phase shifts [PTCs

with slopes approximating 0 (Winfree, 1970)] from stimuli normally eliciting weaker Type 1 shifts. This attribute permitted comprehensive analyses of nonphotic Type 0 phase resetting by a variety of stimuli [systemic 8-OH-DPAT injection, intra-SCN NPY microinjection and behavior (SD and novel wheel exposure)], expanding initial observations (J.D. Glass, unpublished observations; Knoch *et al.*, 2002; Mistlberger *et al.*, 2002). In animals under LD, these stimuli induce phase advances of ~1–2 h during the day but have little phase-delaying effect at night. In marked contrast, animals exposed to ~250 lx LL for 1 or 2 days exhibit phase-advance and/or phase-delay shifts averaging 8–12 h in response to these stimuli. The present results suggest possible mechanisms for these Type 0 phase-resetting responses. For example, the elimination of the nocturnal peak of SCN serotonergic activity by LL may up-regulate SCN 5-HT postsynaptic receptor sensitivity, thus hypersensitizing serotonergic phase resetting. The LL-potentiated phase-advancing effect of intra-SCN NPY injection indicates that an up-regulation of this transmitter's action may also occur. One or both of these effects could underlie LL-potentiated phase-advancing responses to SD and novel wheel exposure. Another possibility, based on the suppressive effects of LL on circadian rhythmicity, is that LL could attenuate circadian pacemaker amplitude, thus boosting the effective strength of stimuli to levels sufficient for Type 0 resetting.

Constant light inhibits serotonin release and potentiates serotonergic phase resetting

Brief exposure to LL differentially affects pre- and postsynaptic components of circadian-related serotonergic activity. A strong inhibitory impact on presynaptic activity is evident from the microdialysis measurements revealing that the daily rhythm of SCN 5-HT output is lost under LL. Serotonergic activity remains at basal levels throughout the 24-h day, without the nocturnal peak of 5-HT release exhibited under LD (Dudley *et al.*, 1998). As the release and metabolism of 5-HT are driven by locomotor behavior and arousal (Levine & Jacobs, 1992; Jacobs & Fornal, 1997; Dudley *et al.*, 1998), the elimination of this daily 5-HT rhythm may be a consequence of attenuated behavioral activity under LL. This fits with our data showing that LL strongly suppresses circadian locomotor activities, including the robust bout of nocturnal activity that is normally associated with peak SCN and intergeniculate leaflet 5-HT release (Dudley *et al.*, 1998; Grossman *et al.*, 2003).

Postsynaptic influences of LL on serotonergic transmission are suggested by the enhanced phase-resetting response to 5-HT_{1,7} receptor activation by 8-OH-DPAT. Daytime phase-advance shifts induced by i.p. 8-OH-DPAT injection after 2 days of LL were several-fold greater than those seen after similar treatments in hamsters under LD (Bobrzynska *et al.*, 1996a; Mintz *et al.*, 1997; present results). Notably, LL exposure has been shown to hypersensitize postsynaptic responses to 5-HT in rat lateral geniculate neurons (Mason, 1988). Enhancement of the postsynaptic mechanism is also shown by *in vitro* (Prosser *et al.*, 1992) and *in vivo* (Ehlen *et al.*, 2001) experiments, which showed that 5-HT agonists can act directly on SCN pacemaker cells (in a TTX-insensitive manner) to elicit phase resetting. It should be noted that intra-raphé 8-OH-DPAT can induce small phase advances (Mintz *et al.*, 1997) raising the possibility of a potentiating effect of LL on the presynaptic response related to raphe autoreceptor activation. Nevertheless, we have recent data showing that LL potentiates the phase-resetting action of intra-SCN microinjection of 8-OH DPAT (Knoch and Glass, unpublished observations), which substantiates an SCN-related mode of LL postsynaptic receptor hypersensitization. The present data suggest that such receptor hypersensitization may be a consequence of the eliminated peak of 5-HT release under LL. This is

supported by two lines of evidence. First, 8-OH-DPAT phase advances are potentiated by inhibiting 5-HT release with para-chlorophenylalanine (*p*-CPA) (Ehlen *et al.*, 2001), an effect attributed to depletion-induced hypersensitization of the 5-HT postsynaptic response. Second, increasing the levels of extracellular 5-HT in the SCN under LL using reverse microdialysis 5-HT perfusion dampens the potentiating effect of LL on 8-OH-DPAT phase advances, presumably by limiting hypersensitization. A complete removal of the LL potentiating effect was not achieved by this procedure, possibly because of the unilateral mode of 5-HT application.

While the hypothesis that LL hypersensitizes the serotonergic postsynaptic response is consistent with the aforementioned observations, little is known of the physiological ramifications of altered sensitivity of SCN neurons to 5-HT. Inferences may be drawn from related studies in this and other brain regions, however. For example, depletion-induced 5-HT hypersensitization by 5,7-dihydroxytryptamine (5,7-DHT) lesioning of 5-HT neurons enhances hypothalamic neuroendocrine responses to 5-HT agonists (Van de Kar *et al.*, 1998) and up-regulates 5-HT binding in the SCN (Manrique *et al.*, 1993). A relationship between the physiological change in 5-HT release and postsynaptic responsiveness to 5-HT is also suggested by observations that circadian fluctuations in postsynaptic neurophysiological and behavioral responses to 8-OH-DPAT may be keyed to rhythmic daily changes in 5-HT release modulating changes in 5-HT_{1A} receptor sensitivity (Mason, 1986; Currie & Coscina, 1993; Lu & Nagayama, 1996).

A related aspect of LL treatment was the manifestation of a large phase-delaying portion of the 8-OH-DPAT PRC during the night. This is significant because a phase-delaying action of this drug has never been reported. In the rat, intra-cerebroventricular injection of 8-OH-DPAT induces phase advances during the subjective day but has no effect at night (Edgar *et al.*, 1993). Similarly, in the rat SCN slice, where 8-OH-DPAT application induces large phase advances during the subjective day, there is no effect during the night (Prosser *et al.*, 1990; Medanic & Gillette, 1992; Shibata *et al.*, 1992). In the only other study of *in vivo* 8-OH-DPAT phase shifting during the subjective night in hamsters under LL, there was also no phase-resetting response (Tominaga *et al.*, 1992), which was probably attributable to longer-term exposure to LL prior to drug treatment negating the inductive effect of LL on 8-OH-DPAT-mediated phase delays.

Phase resetting and the constant light to constant darkness transition

A notable aspect of the LL potentiation unrelated to drug or behavioral treatment was the phase-advancing effect of the LL to DD transition in itself, as observed in the untreated control groups (see Figs 3 and 9). Phase advances ranging from ~2 to 3 h were observed in all of these control groups after release to DD during the day (ZT 6) and to an overall smaller extent during the night. Notably, in the SD trials, the sizes of these advances were not significantly different from those of animals receiving the SD treatment. This effect is similar to that seen in a previous study, where hamsters kept under LL for 10 days and then released to DD exhibited phase advances of similar magnitude (Albers, 1986). We determined if these LL-induced advances were mediated by a serotonergic mechanism by treating animals exposed to LL for 2 days with an i.p. injection of metergoline immediately before release into DD at ZT 6. As the metergoline treatment did not affect LL-induced phase advances ($P > 0.4$ vs. vehicle controls; see Fig. 14), these advances were probably not serotonergic in nature.

It should be noted that the potentiated responses to 8-OH-DPAT and SD in LL-exposed animals at ZT 6 may have resulted from the phase-resetting effect of the LL to DD transition in itself (seen in the control

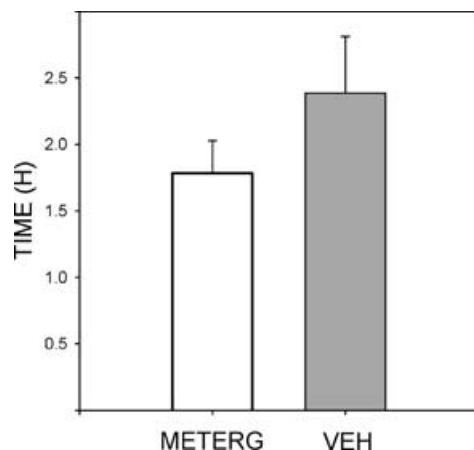


FIG. 14. Lack of effect of the serotonin_{1,2,7} receptor antagonist metergoline (METERG) on vehicle (VEH) injection-induced phase advances at zeitgeber time 6 ($P > 0.4$). Bars represent the mean $\pm$ SEM.

groups; Figs 3 and 9) added to the LD shifting effects of 8-OH-DPAT or SD treatments produced without the LL exposure. Thus, the larger shifts may, in fact, not be due to potentiating effects of the LL exposure on drug or behavioral shifting responses. This is consistent with results from the 2-day LL trials at ZT 6, where 8-OH-DPAT and SD advance shifts under LD added to the advance shifts of the respective 2-day LL control groups approximate the larger treatment-induced shifts under LL. Nevertheless, such an additive effect does not account for the much larger phase-advance shifts produced by 8-OH-DPAT or SD earlier during the day (ZT 0), where the sum of control shifts under LL added to the LD treatment shifts is several hours less than the size of the treatment shifts under LL. The same is true for the large night-time 8-OH-DPAT and SD delay shifts induced by 2 days of LL. These data confirm the potentiating effect of LL on phase resetting at the largest phase-delaying and phase-advancing portions of the 8-OH-DPAT and SD PRCs under the brief LL conditions.

Constant light hypersensitizes suprachiasmatic nucleus neuropeptide Y phase-advance shifts

The NPY released from geniculohypothalamic tract terminals in the SCN is critical for mediating certain nonphotic clock-resetting responses (Albers & Ferris, 1984; Biello *et al.*, 1994; Marchant *et al.*, 1997). Intra-SCN microinjection of NPY produces phase shifts similar to those induced by wheel running, SD and electrical stimulation of the geniculohypothalamic tract (present results; Rusak *et al.*, 1989). Conversely, blocking NPY action in the SCN by intergeniculate leaflet lesioning (Janik & Mrosovsky, 1994) or intra-SCN injection of antibody to NPY (Biello *et al.*, 1994) prevents wheel-running-induced phase resetting. Previous reports have shown phase-advance shifts of 1–2 h induced by intra-SCN injection of NPY during the subjective day in hamsters (Albers & Ferris, 1984; Huhman & Albers, 1994), which is consistent with the present results. In animals receiving LL, however, intra-SCN NPY induced up to ~8 h phase-advance shifts. Thus, LL hypersensitizes NPY postsynaptic response. However, unlike the case for 8-OH-DPAT, LL did not promote a phase-delaying effect of NPY during the night. This suggests that the NPY PRC may lack a phase-delaying region, at least under LL conditions. A small or negligible phase-delay region for NPY is consistent with results from studies in the sensitized SCN slice (Medanic & Gillette, 1993; Golombek *et al.*, 1996) and with *in vivo* observations of the shifting effects of SCN application of NPY (Albers & Ferris, 1984; Huhman & Albers, 1994;

Biello & Mrosovsky, 1996; Weber & Rea, 1997), which argues against NPY playing a significant role in mediating the large LL-potentiated phase delays induced by behavioral procedures in the night. It is worth noting that one of these studies (Albers & Ferris, 1984) was in long-term LL, suggesting that lengthy exposure to LL attenuates the potentiating effect of LL on NPY shifts as it does with 8-OH-DPAT shifts (present observations).

Constant light hypersensitizes behavioral phase-resetting responses

In hamsters under LD, the size of phase-advance shifts induced by SD or novel wheel exposure at midday are equivalent to those induced by intra-SCN NPY injection or systemic 8-OH-DPAT administration (see Discussion above). During the late night, however, SD has negligible shifting effects. This differs markedly from those of animals treated with LL, where SD produced maximal advance and delay shifts averaging approximately 9 and –6 h, respectively, and novel wheel exposure produced advance shifts of 5 h (delays were not evaluated but have been observed up to –8 h; Mistlberger, unpublished). Large phase-advance shifts have also been reported in a Syrian hamster group housed under LD with no running wheels and then transferred individually during the light phase to a cage outfitted with a running wheel under DD (Gannon & Rea, 1995). These phase advances (~12 h) occurred early (ZT 23) as well as later in the day. We also observed SD-induced phase advances of a similar size in the early morning (ZT 24) after LL exposure. As the large-magnitude responses in both studies are of similar magnitude and phase, they may share a common mechanism, possibly related to summation of multiple concurrent phase-shifting influences.

Constant light exposure, circadian amplitude and Type 0 resetting

For limit cycle oscillators, such as the circadian pacemaker, amplitude is considered to be an important determinant of phase-resetting responses (Winfree, 1970; Kronauer, 1990; Jewett *et al.*, 1991). According to the limit cycle formulation, the magnitude of phase resetting to a standard stimulus is inversely related to the amplitude of oscillation, such that a stimulus producing weak Type 1 shifts can theoretically produce Type 0 shifts (~12 h) if amplitude is sufficiently small. This prediction is empirically supported by demonstrations that treatments appearing to suppress circadian amplitude can produce Type 0 shifts of circadian rhythms (see Jewett *et al.*, 1991). From the present findings, it is possible that the brief LL exposure promotes Type 0 PRCs via its suppression of circadian pacemaker amplitude. This is consistent with the attenuated circadian rhythms under LL, including those of wheel running, general locomotor activity, SCN 5-HT release (present results), SCN electrical activity (Shibata *et al.*, 1984) and mPer 2 mRNA expression (Sudo *et al.*, 2003). Very large phase shifts to 3 h of novelty-induced running have also been reported in Syrian hamsters homozygous for the ‘tau’ mutation of casein kinase 1 epsilon (Mrosovsky *et al.*, 1992), an enzyme that regulates phosphorylation of per circadian clock genes (Lowery *et al.*, 2000). It would thus be of interest to know whether LL also inhibits this enzyme, concomitant with suppression of the circadian cycling of this and other canonical clock genes. Given that very large phase shifts to nonphotic stimuli are evident in wildtype hamsters after 2 days in LL, but generally not after a week or more in LL, it may be possible to identify the cellular correlates of potentiated shifting by time-course studies correlating phase-shift magnitude with cellular variables over time in LL.

It is notable that suppression of locomotor activity under LL was not correlated with the magnitude of phase-resetting responses. This is

consistent with the observations that blocking wheel access for 2 days in DD does not potentiate phase shifts to SD (Mistlberger *et al.*, 2002) and that restoring wheel running in LL by food deprivation does not prevent potentiation of phase shifts to SD (present results). Thus, it appears that changes in running activity are neither necessary nor sufficient to mediate the switch from Type 1 to Type 0 phase resetting. The nature of this switch remains to be more fully elucidated but warrants attention as it may provide a target for developing interventions to enhance circadian clock resetting in humans.

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Abbreviations

DD, constant darkness; 5-HT, serotonin; LD, 14 h light: 10 h dark cycle; LL, constant light; NPY, neuropeptide Y; 8-OH-DPAT, ($\pm$)-2-dipropyl-amino-8-hydroxyl-1,2,3,4-tetrahydronaphthalene; PRC, phase response curve; PTC, phase transition curve; SCN, suprachiasmatic nucleus; SD, sleep deprivation; ZT, zeitgeber time.

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