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BRIEF CONSTANT LIGHT ACCELERATES SEROTONERGIC RE-ENTRAINMENT TO LARGE SHIFTS OF THE DAILY LIGHT/DARK CYCLE

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Abstract—Brief (~2 day) constant light exposure (LL_b) in hamsters dramatically enhances circadian phase-resetting induced by the 5-HT receptor agonist, (±)-2-dipropyl-amino-8-hydroxyl-1,2,3,4-tetrahydronapthalene (8-OH-DPAT) and other nonphotic stimuli. The present study was undertaken to determine if LL_b can also amplify phase-resetting responses to endogenous 5-HT and accelerate re-entrainment to large-magnitude advance and delay shifts of the light/dark (LD) cycle. First, central serotonergic activity was increased by i.p. injection of L-tryptophan±the 5-HT reuptake inhibitor fluoxetine. Hamsters under LD or exposed to LL_b received vehicle or drugs during the early morning, and phase-shifts of the locomotor activity rhythm were measured after release to constant darkness. Neither drug phase-shifted animals not exposed to LL_b ($P>0.5$ vs. vehicle); however in animals receiving LL_b, L-tryptophan with and without fluoxetine produced large phase-advance shifts (means=2.5±0.4 h and 2.6±0.2 h, respectively; both $P<0.035$ vs. vehicle). Next, the effects of LL_b combined with 8-OH-DPAT or L-tryptophan+fluoxetine on serotonergic re-entrainment to 10 h phase-advance and phase-delay shifts of the LD cycle were assessed. In groups not exposed to LL_b, vehicle controls re-entrained slowly to the advance and delay shifts (means=16±1 and 24±4 days, respectively), but those treated with 8-OH-DPAT re-entrained faster (means=11±2 and 9±2 days, respectively; both $P<0.05$ vs. vehicle). In groups exposed to LL_b, vehicle controls re-entrained slowly to the advance and delay shifts (means=15±2 and 25±3 days, respectively); however those receiving 8-OH-DPAT rapidly re-entrained to the delay and advance shifts, with the majority (75%) requiring only 1–2 days (means=2±1 and 4±2 days, respectively; both $P<0.05$ vs. vehicle). Animals exposed to LL_b and treated with L-tryptophan+fluoxetine also exhibited accelerated re-entrainment to a 10 h advance shift (mean=5±2 days; $P<0.05$ vs. vehicle). Thus through enhancing serotonergic phase-resetting, LL_b facilitates rapid re-entrainment to large shifts of the LD cycle which offers a potential approach for treating circadian-related desynchronies.

Key words: Syrian hamster, suprachiasmatic nucleus, tryptophan, fluoxetine.

The master circadian clock in mammals is located in the suprachiasmatic nucleus (SCN) of the anterior hypothalamus (Inouye and Kawamura, 1979; Klein et al., 1991; Rusak, 1979; Stephan and Zucker, 1972). The SCN clock is entrained by photic input received directly from the retina via the retinohypothalamic tract (RHT; Hendrickson et al., 1972; Moore and Lenn, 1972; Pickard, 1982; Youngstrom and Nunez, 1986; Johnson et al., 1988), and indirectly via a projection from the intergeniculate leaflet (IGL), the geniculohypothalamic tract (GHT; Card and Moore, 1982; Johnson et al., 1989). The IGL also provides nonphotic (behavior-related) entraining input to the SCN mediated by neuropeptide Y (NPY) release from GHT terminals (Albers and Ferris, 1984; Biello et al., 1994; Marchant et al., 1997). Serotonergic input from the midbrain raphe nuclei is also believed to be another important source of nonphotic signaling to the SCN (Glass et al., 2003; Meyer-Bernstein and Morin, 1996). It is now apparent that nonphotic influences, in the form of behavioral manipulations (eg. sleep deprivation, social interaction, cage changing, or novel wheel exposure) or pharmacological interventions (eg. benzodiazepines or 5-HT agonists), can be as potent as photic cues for resetting the clock (~1-2 h; Ellis et al., 1982; Mistlberger et al., 2000; Mrosovsky, 1988).

Recently we showed that the nonphotic phase-resetting effects of various stimuli, including treatment with the $5\text{-HT}_{1A,7}$ receptor agonist, (±)-2-dipropyl-amino-8-hydroxyl-1,2,3,4-tetrahydronapthalene (8-OH-DPAT), can be dramatically increased by brief prior exposure to constant light (1–2 days [LL_b] Knoch et al., 2004). Notably, immediate phase-advance and phase-delay shifts of ~12 h are produced using this combined treatment. The neurologic mechanism underlying the potentiating effects of LL_b on 8-OH-DPAT-induced phase-resetting is not known, but possibly could involve an upregulation of 5-HT-mediated postsynaptic responses centered in the SCN clock, as phase-advance shifts caused by intra-SCN microinjection of 8-OH-DPAT are potentiated by LL_b (Knoch et al., 2006). It should be noted that while the circadian phase-resetting effects of serotonergic agonists including 8-OH-DPAT are well documented (summarized in Ehlen et al., 2001; Mistlberger et al., 2000; Morin, 1999; Sprouse et al., 2005), the role for 5-HT as a player in nonphotic phase resetting remains controversial, and it is therefore uncertain how the potentiated phase-resetting action of LL_b on behavior-induced shifting may be linked to (endogenous) 5-HT signaling.

This uncertainty is related in part to observations that raphe lesions (Meyer-Bernstein and Morin, 1998), 5-HT antagonist treatments (Antle et al., 1998) and SCN 5-HT depletion (Bobrzynska et al., 1996) do not block activity-

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Abbreviations: CT, circadian time; DD, constant darkness; GHT, geniculohypothalamic tract; IGL, intergeniculate leaflet; LD, 14-h light/10-h dark photocycle; LL_b, brief constant light; NPY, neuropeptide Y; PRC, phase-response curve; SCN, suprachiasmatic nucleus; ZT, Zeitgeber time; 8-OH-DPAT, (±)-2-dipropyl-amino-8-hydroxyl-1,2,3,4-tetrahydronapthalene.

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induced phase-shifts and that the phase-resetting effect of direct injection of 8-OH-DPAT into the SCN is absent or small (Challet et al., 1998; Ehlen et al., 2001; Mintz et al., 1997). Also, approaches used to increase endogenous serotonergic activity have yielded inconsistent phase-resetting results. For example, while electrical stimulation of the midbrain raphe nucleus which acutely enhances 5-HT release in the SCN (Dudley et al., 1999) induces phase-advances comparable to those induced by behavioral activation (Glass et al., 2000; Meyer-Bernstein and Morin, 1999), acute administration of 5-HT reuptake blockers (including fluoxetine and clomipramine), which also increases central extracellular 5-HT levels, has weak or no circadian phase-resetting effects *in vivo* (Klemfuss and Kripke, 1994; Yannielli et al., 1998) or *in vitro* (Sprouse et al., 2006) (but does attenuate photic phase-shifts [Gannon and Millan, 2007]).

The present study was thus undertaken to exploit the potentiating effect of LL_b on nonphotic clock resetting as a means to assess the potential actions of 5-HT in nonphotic clock-resetting and rhythm re-entrainment. Two experiments were undertaken to: (1) explore the *in vivo* circadian phase-shifting effects of increased endogenous 5-HT activity stimulated by the 5-HT precursor, L-tryptophan, and/or fluoxetine under LL_b-sensitized phase-shifting conditions; and (2) determine if the large serotonergic (8-OH-DPAT- and L-tryptophan-induced) phase-shifts potentiated by LL_b exposure could accelerate rhythm re-entrainment to simulated jet-lag involving large (10 h) advance and delay shifts of the light/dark cycle. Results from these experiments would strengthen the case for participation of endogenous serotonergic in nonphotic phase-resetting, and also could help in the design of therapeutic strategies for more efficient re-synchronization of the circadian clock during periods of circadian phase disruption.

EXPERIMENTAL PROCEDURES

Animals

Adult male Syrian hamsters obtained from Harlan (Indianapolis, IN, USA) were housed in a light- and temperature-controlled (22 °C) environmental chamber. Animals were individually housed in polystyrene cages and kept under a 14-h light/10-h dark photocycle (LD) with light intensity of approximately 250 lx. Food (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. The experiments were conducted using the National Institutes of Health Guidelines for the Care and Use of Laboratory Animals, and were approved by the Kent State Institutional Animal Care and Use Committee.

Constant light protocol and phase-shift analyses

The method for administering the LL_b regimen is the same as that described by Knoch et al. (2004). This photic treatment begins at Zeitgeber time 12 (ZT 12; designated as the time of lights off under an LD cycle), and is maintained over 2 consecutive days by keeping the ambient room lighting [~250 lx] on, thus eliminating two dark-phase periods (Fig. 1). This constituted 50 h of continuous light extending from the last dark phase for treatments delivered at ZT 0. In the L-tryptophan/fluoxetine phase-resetting trials, the animals were released to constant darkness (DD) immediately after drug injection for a minimum of 14 days to assess phase-resetting response using a modified Aschoff type II procedure (Aschoff, 1965). For clarity of data presentation, ZT rather than the circadian time (CT) convention for free-running conditions with no Zeitgeber, was used to designate the circadian phase of treatments delivered during the ~2 day LL_b exposures. There were no perceptible changes in phase or period of the free-running circadian activity rhythm during these brief exposures, so under these conditions ZT is considered equivalent to CT as a phase marker.

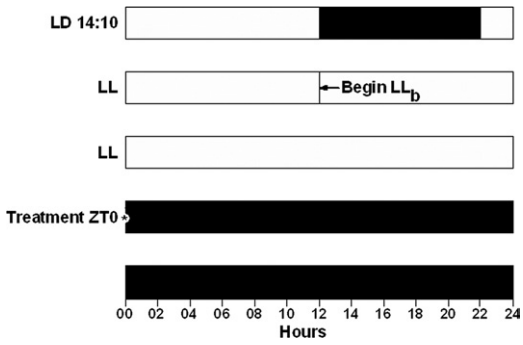

Fig. 1. Protocol for assessing the effects of 2 days of brief constant light (LL_b) exposure on serotonergic (L-tryptophan±fluoxetine) phase-resetting responses. Shown is a treatment delivered at ZT 0 on the second day of constant light (LL) exposure. Animals are released from LL_b to DD at the outset of the drug treatment. The top black bar represents the dark phase of the initial LD.

For all experiments, the circadian rhythm of general locomotor activity was recorded using overhead infrared sensors interfaced with a computerized data acquisition system (ClockLab; Coulbourn Instruments, Allentown, PA, USA). Phase-shifts were calculated as follows: A line based on general locomotor activity onsets for the 7 preceding days of LD was extrapolated to the day of treatment under LL_b. Then a regression line based on days 3–14 post-treatment activity onsets was back-extrapolated to the same day of treatment. The difference between these two extrapolated lines on the day of treatment was considered the phase-shift. Activity onset was defined as the first bout of activity sustained for at least 30 min. Rates of re-entrainment to 10 h advance or delay shifts of the LD cycle were measured by counting the number of days required for the stable adjustment of the locomotor activity rhythm to the new LD cycle. Stable adjustment was characterized as the first 10 days of activity onset temporally aligned with the beginning of dark phase of the new LD cycle, with tau=24 h.

Experimental protocols

Effects of brief constant light on L-tryptophan/fluoxetine phase-resetting. Potential endogenous 5-HT phase-resetting effects were explored using LL_b in combination with various treatments to enhance serotonergic activity, including L-tryptophan loading (50 mg/kg i.p.), fluoxetine (10 mg/kg i.p.) and L-tryptophan+fluoxetine. Controls received i.p. vehicle injection (DMSO; n=5–6/treatment group). Drugs were administered at ZT 0, the phase of the 8-OH-DPAT phase-response curve (PRC) when robust phase-advance shifts to 8-OH-DPAT (>10 h) occur in LL_b-treated animals (Knoch et al., 2004). All hamsters were initially housed under LD with general locomotor activity sensors and were exposed to LL_b or maintained under LD prior to drug or vehicle treatment. Immediately after each treatment, the animals were released into DD for 3 weeks to assess phase-resetting responses using the Aschoff type II procedure.

Effects of brief constant light together with 8-OH-DPAT or L-tryptophan and/or fluoxetine on re-entrainment to new LD cycles. Hamsters exposed to LL_b exhibit large and immediate phase-