

Sleep deprivation stimulates serotonin release in the suprachiasmatic nucleus

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Received 23 March 2000; accepted 31 March 2000

Recent literature suggests that sleep deprivation has a general stimulatory effect on the central serotonergic system. Herein we report that in hamsters, sleep deprivation induced by gentle handling for 3 h under dim red light at midday stimulates serotonin release in the suprachiasmatic nuclei by as much as 171%. Basal levels of 5-HT release are re-established within 1 h after cessation of treatment. Sleep deprivation also evokes phase advances of the circadian activity rhythm averaging 2 h.

When sleep deprivation is undertaken in bright light, serotonin release is stimulated, but phase-shifting is greatly inhibited. It is therefore proposed that if the phase-resetting response to sleep deprivation is mediated by increased serotonin release, light inhibits the phase-resetting effect by blocking the post-synaptic or other downstream actions of serotonin. *Neuro-Report* 11:1929–1932 © 2000 Lippincott Williams & Wilkins.

Key words: Circadian rhythm; Hamster; Microdialysis; Phase-shift; Serotonin; Sleep deprivation; Suprachiasmatic nuclei

INTRODUCTION

There is a close association between behavioral state and the activity of the central serotonergic system. Changes in behavior associated with feeding [1], locomotor activity [2–4], sleep/arousal [5–8] and the light-dark transition [9,10] are associated with significant adjustments in the release and metabolism of serotonin (5-HT). Experimental manipulations of behavior, including wheel-running [10] and sleep deprivation (SD) [11–14] also have been shown to markedly affect serotonergic activity in multiple brain regions, including the circadian clock located in the suprachiasmatic nuclei (SCN). Notably, these and other related behavioral treatments have pronounced phase-resetting effects on circadian rhythms. The linkage between behavioral state, 5-HT release and circadian phase-resetting has strengthened the view that serotonergic neurotransmission may be important to behavioral entrainment of the circadian clock.

In hamsters, the phase-advancing effect of SD administered at midday is equivalent to that caused by vigorous wheel-running [11,15,16]. Wheel-running strongly stimulates central serotonergic activity [10], and there is evidence from a variety of species that SD may have a similar effect on the serotonergic system. In rats, SD increases the concentration of the 5-HT metabolite 5-hydroxyindoleacetic acid (5-HIAA) in the forebrain and raphe nuclei [14,17] and reduces the inhibitory effect of 5-HT reuptake blocker treatment on raphe activity [18,19]. In cats, SD increases raphe activity and diminishes the inhibition of raphe neuronal firing produced by the 5-HT agonist, 8-OH-DPAT

[12], and in Siberian hamsters SD increases the turnover of 5-HT in the hypothalamus and hippocampus [13]. Despite these findings, however, the effect of SD on the synaptic release of 5-HT has never been directly measured. The present study was therefore conducted to assess the effect of SD on *in vivo* 5-HT release. In view of the suspected involvement of 5-HT in circadian phase-resetting, our assessment was focused on the SCN to determine if the phase-resetting SD procedure directly alters neurochemical activity within the circadian clock.

MATERIALS AND METHODS

Adult male Syrian hamsters (*Mesocricetus auratus*), raised from breeder pairs obtained from Harlan Sprague-Dawley (Madison, IL) were used in the microdialysis studies. The animals were maintained under a light:dark cycle of 14 h light (~500 lux) and 10 h darkness (LD 14:10; lights on at 05:00 h EST) with food (Rodent Chow; Prolab 3000, PMI Feeds, St Louis, MO) and water provided *ad lib*. Two weeks prior to experimentation, animals 8–10 weeks of age were housed individually in polycarbonate cages. The day preceding experimentation, the animals were anesthetized with sodium pentobarbital (Nembutal) and received a unilateral microdialysis probe implant stereotactically aimed at the lateral margin of the SCN. Procedures for the construction and surgical implantation of the microdialysis probes are provided elsewhere [10,20]. The coordinates for probe implantation were: anterior-posterior 0.3 mm from bregma; lateral 0.3 mm from midline; horizontal –8.0 mm from dura. Microdialysis was performed by continuously

perfusing artificial cerebrospinal fluid (ACSF; flow rate 1.2 μ l/min) through a microdialysis probe constructed from hemicellulose dialysis tubing (12 kDa mol. wt cutoff, 230 μ m o.d., Spectra-por, Fisher Scientific). Measurement of extracellular 5-HT in the SCN was aided by the addition of 4.0 μ M fluoxetine to the perfusate. The sampling interval was 20 min. Probe location was determined histologically at the end of the experiment. Microdialysate was analyzed for 5-HT with HPLC (Bioanalytical Systems, West Lafayette, IN) with amperometric electrochemical detection as described previously [10]. The lower level of sensitivity (signal $\geq 5 \times$ background) was ~ 500 fg. Authenticity of the 5-HT peak has been verified by predictable changes in its size after electrical and pharmacological manipulations of raphe nuclear activity [20].

On the day of experimentation, perfusion began in the home cage at zeitgeber time (ZT) 2, 4 h after lights-on (ZT 12 is designated as the time of lights-off). After a 1 h equilibration period, baseline samples were collected over a 3 h period from ZT 3 to ZT 6. Visual inspection confirmed that the animals slept through the baseline microdialysis sample collection. The animals then were aroused and maintained in the waking state for a 3 h period from ZT 6 to ZT 9 by continuous gentle handling and light puffs of air. In the first experiment, the animals ($n=12$) were released into dim red light (<1.0 lux) at the beginning of the behavioral manipulation, as bright light inhibits non-photic circadian phase-resetting [21]. At the end of the experimental period, the animals were left undisturbed with microdialysis continuing until ZT 14. After 2 days these animals were placed in one of two groups ($n=6$ /group). Animals of the first group underwent the same microdialysis procedures, but underwent SD with the chamber lights left on until ZT 9. Animals of the second group also underwent the same microdialysis sampling procedures, and did not undergo SD (control group). Data were normalized as a percentage of the 3 h pretreatment baseline level. Treatment effects were determined using Dunnett's test procedure for comparing multiple group means or the Tukey mean comparison test. The level of significance was set at $p < 0.05$.

We have reported the circadian phase-resetting effects of SD elsewhere [11,15]. The same procedures for assessing these responses were used in the present experiments, where the phase-shifting was measured using a modified Aschoff Type II procedure. Animals whose wheel-running activity was initially recorded under LD 14:10 were released into dim red light (<1 lux; $n=5$) at the beginning of the 3 h SD procedure beginning at ZT 6. In another group of animals ($n=8$), SD was undertaken under incandescent light (20 lux) and released into dim red light after the procedure. All animals were subsequently maintained under dim red light for 4–7 days to determine the extent of phase-shifting. Wheel-running activity was measured by microswitches connected to an interface and computer.

RESULTS

The effect of a 3 h period of SD in dim red light during the subjective midday on 5-HT release in the SCN is shown in Fig. 1a. This procedure induced a maximal $170 \pm 10\%$ increase in SCN 5-HT release over baseline that occurred within 1 h from the onset of treatment.

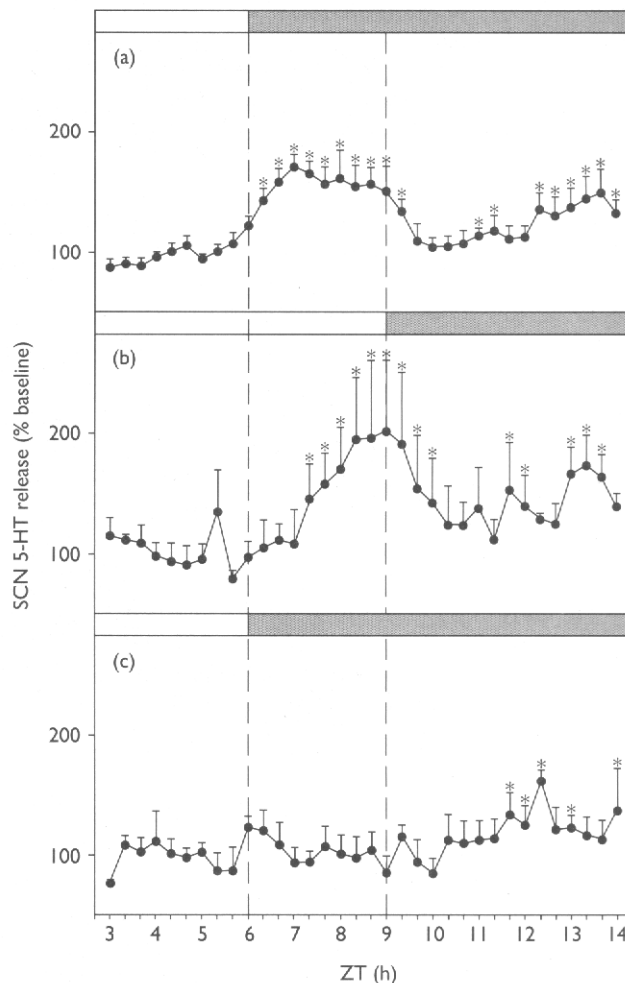


Fig. 1. Stimulatory effect of sleep deprivation from ZT 6–9 (broken lines) on 5-HT release in the SCN under dim red light (a; $n=12$) or bright light (b; $n=6$). Note the lack of a significant increase in 5-HT release in the non-sleep-deprived control group (c; $n=6$). Shaded horizontal bars denote the period of exposure to dim red light. * $p < 0.05$ vs averaged pretreatment baseline release.

The average increase in 5-HT output over the 3 h treatment period was $158 \pm 5\%$ of baseline (Fig. 2). The rate of 5-HT release returned to baseline levels within 40 min after the end of treatment. SD undertaken in the presence of normal room illumination (500 lux) was as effective in stimulating SCN 5-HT release as assessed under dim light, although there was greater variability in response. There also was an increased latency of serotonergic stimulatory response to the SD procedure. The maximal increase was $201 \pm 60\%$ of baseline (Fig. 1b), and the average increase in 5-HT output over the 3 h treatment $149 \pm 12\%$ of baseline (Fig. 2). In the control experiment, the dim light exposure alone had little effect on SCN 5-HT release (Fig. 1c, Fig. 2).

Animals in all of the experiments exhibited increases in SCN 5-HT release associated with the onset of behavioral activity shortly before and following the onset of subjective night at ZT 12. Behavioral phase-advancing responses to SD under dim red light and brighter incandescent light conditions are depicted in Fig. 3.

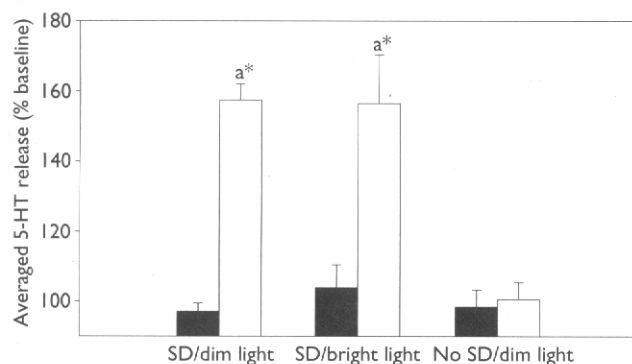


Fig. 2. Graphical representation of the integrated rate of release of 5-HT in the SCN averaged over the 3 h sleep deprivation treatment period (ZT 6–9) designated by open bars, and the 3 h pretreatment period (ZT 3–6) designated by filled bars. * $p < 0.05$ vs pretreatment period; ^a $p < 0.05$ vs non-sleep-deprived controls.

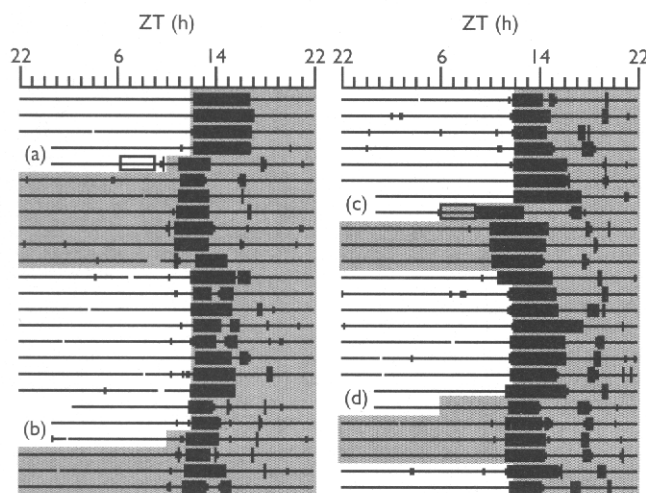


Fig. 3. Representative actograms of wheel-running activity (solid horizontal bars) over 25 days demonstrating the circadian phase-resetting effects of 3 h of sleep deprivation from ZT 6 to ZT 9 (designated by rectangle) under incandescent light (20 lux; left panel) and dim red light (<1 lux; right panel). Shading represents dim red light exposure. (a) Sleep deprivation under incandescent light resulted in a phase-advance of 60 min; (c) sleep deprivation under dim red light resulted in a phase-advance of 140 min; (b,d) no sleep deprivation under equivalent photic conditions did not induce phase-shifting.

Consistent with our earlier unpublished observations, SD under dim red light caused phase-shifts averaging 109 ± 27 min (range 30–153 min). In contrast, SD under incandescent light produced phase-advances averaging only 47 ± 18 (range 0–60 min; $p < 0.05$ vs dim red light).

DISCUSSION

The present experiments demonstrate that SD treatment can acutely stimulate the release of 5-HT in the brain. Our findings also confirm a direct link between SD-related behavior/arousal and increased serotonergic activity in the SCN circadian clock. Notably, the peak increase in SCN 5-HT release caused by SD (170% of baseline) is equivalent

to the maximal 5-HT output induced in the SCN by electrical stimulation of the raphe (~160%) [20], intra-raphé microinjection of the 5-HT_{1A} antagonist, WAY 100635 (170%) [20], or by continuous wheel-running (152%) [10]. This comparison underscores the substantial stimulatory impact of SD on the activity midbrain raphe neurons projecting to the SCN and possibly other regions. This stimulating effect of SD on 5-HT is consistent with earlier reports in Siberian hamsters, where SD significantly increased the ratio of 5-HIAA/5-HT tissue content (estimate of 5-HT turnover) in the hypothalamus, hippocampus and brain stem [13]. The SD procedure also increased tissue levels of 5-HIAA in the rat dorsal raphe nucleus (DRN), thalamus and hypothalamus [17] and forebrain [14].

Related to these findings is evidence that SD may increase the activity of midbrain raphe neurons, at least in part, by diminishing the inhibitory response of these neurons to raphe somatodendritic 5-HT_{1A} autoreceptor activation. SD has been found to significantly reduce the degree of raphe neuronal inhibition produced by systemic administration of the 5-HT_{1A} agonist, 8-OH-DPAT in cats [12], and attenuate the suppressive effect of peripheral administration of 5-HT reuptake blockers on raphe neuronal activity in rats [18,19]. Such a diminution of raphe 5-HT_{1A} autoreceptor negative feedback response could strongly potentiate the release and metabolism of 5-HT in response to SD as discussed above. In line with this autoreceptor-mediated action is our previous finding that the suppressive effect of the partial 5-HT_{1A} autoreceptor agonist, BMY 7376, on *in vivo* SCN 5-HT release is low at midday, compared to the late dark-phase [10]. This result implies that the 5-HT_{1A} autoreceptor response is normally reduced during the light-phase and thus could contribute to the stimulated 5-HT output caused by SD (or other forms of behavioral arousal) during the subjective day.

Our observations support the current view that increased activity of the serotonergic system is associated with wakefulness, and not sleep [22]. In cats, the electrical activity of the DRN is increased during waking and decreased during sleep [23]. Correspondingly, 5-HT release in the hypothalamus, caudate, DRN and medial pontine reticular formation is highest during waking and lowered during sleep [4,6,7,12]. In rats, 5-HIAA levels are higher during waking than sleep in the hypothalamus and caudate nucleus [4,5,8], but not in the DRN [5]. Our continuous microdialysis measurements undertaken before, during and after the SD procedure confirmed that the lowest levels of 5-HT release were associated with sleep during the pretreatment period and after the SD treatment during the late subjective day. Output of 5-HT was high during SD and also near time of subjective lights-off (ZT 12) when the animals entered the active phase of their circadian sleep-activity cycle. It is important in this regard to note that the SD procedure did not disrupt the normal daily increase in SCN 5-HT release associated with the subjective light–dark transition [10]. It is also noteworthy that this previous characterization of the circadian profile of 5-HT release in the hamster SCN revealed that serotonergic activity in the SCN was lowest during much of the subjective day when the animals were asleep, and highest at the light–dark transition, when the animals became active.

Another pronounced effect of SD is its phase-resetting action on the SCN circadian clock [11,15]. In the present study, and also in our previous experiments, SD under dim red light from ZT 6–9 elicited phase-advances of ~2 h. Interestingly, when SD is undertaken in the light, phase-shifting is greatly diminished, with phase-advances averaging only about 0.5 h. The blocking effect of light on behavioral phase-shifting has previously been demonstrated in hamsters, where light exposure during an activity pulse blocked the phase-shifting response to the activity pulse [21]. The similar blocking actions of light on SD- and activity-induced phase-resetting points to a functional and/or anatomical convergence of pathways mediating the effects of these stimuli in the circadian system. As light also blocks the phase-advancing effect of systemic [24] and intra-SCN (Glass and Ehlen, unpublished observations) administration of 8-OH-DPAT, it is plausible that the phase-resetting effects of SD and activity are both mediated by 5-HT, and that light blocks the postsynaptic or other downstream actions of the 5-HT. Our demonstration that SD-induced 5-HT release is not blocked by light is consistent with this hypothesis, in that the inhibitory action of light could not be due to a presynaptic blockade of 5-HT release. However, it is conceivable that SD-induced phase-shifting could be mediated via neuropeptide Y (NPY) neurotransmission, as the behavioral phase-shifting action of NPY microinjection in the SCN is also suppressed by light [25]. Thus, the functional significance of the behavioral and SD-induced 5-HT release in the SCN during the subjective day remains conjectural.

CONCLUSION

The present results constitute the first direct *in vivo* evidence that SD reversibly stimulates the central release of 5-HT. These experiments also confirm that the SD under dim red light during the subjective midday stimulates 5-HT release in the SCN in association with a phase-advancement of the circadian activity rhythm. When the SD treatment is conducted under bright light SCN 5-HT release is also stimulated, but the phase-shifting response

is significantly impaired. If an increase in 5-HT release mediates the phase-advancing effect of SD, then the inhibitory affect of light on this phase-shifting must be registered on the postsynaptic targets of the 5-HT. Finally, our collective *in vivo* data support the contention that raised levels of serotonergic neurotransmission are associated with waking, and not sleep.

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Acknowledgements: Supported by grants from the National Institutes of Health NS35229 (J.D.G.) and NSERC (R.E.M.) and an NSERC Graduate Fellowship (M.C.A.).