In Vivo Assessment of the Midbrain Raphe Nuclear Regulation of Serotonin Release in the Hamster Suprachiasmatic Nucleus

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Evidence from a variety of experimental approaches supports a physiological action of serotonin (5-HT) in the SCN. For example, 5-HT causes a marked inhibition of SCN neuronal firing (Groos et al. 1983; Nishino and Koizumi 1977; Shibata et al. 1983), and 5-HT agonists markedly inhibit photo- tically induced responses of the SCN, including light-activated Fos protein expression in SCN cells (Glass et al. 1994; Selim et al. 1993), electrical activity of light-responsive SCN cells (Miller and Fuller 1990; Ying and Rusak 1994, 1997), and light-induced phase shifts in the free-running circadian activity rhythm (Pickard et al. 1996; Rea et al. 1994). Depletion of 5-HT release in the SCN has been shown to disrupt circadian endocrine and behavioral rhythms (Banky et al. 1988; Levine et al. 1986; Morin and Blanchard 1991; Szafarczyk et al. 1979), and the application of 5-HT agonists to SCN slice preparations (Medanic and Gillette 1992; Prosser et al. 1990, 1993) or directly to the SCN in vivo (Challet et al. 1998) shifts the phase of the SCN clock.

Despite the well-characterized effects of serotonergic agonists and antagonists in the SCN, little is known regarding how endogenous serotonergic activity in the circadian clock is regulated. Notably, studies on the source(s) of serotonergic innervation to the SCN yielded remarkably inconsistent results. Although the midbrain raphe nuclear complex is recognized as the ultimate origin of SCN serotonergic innervation, anatomic tracing and lesioning studies variably ascribed the dorsal and/or the median raphe nuclei (DRN and MRN, respectively) as the source(s) of this innervation (Aghajanian et al. 1969; Azmitia and Segal 1978; Van de Kar and Lorens 1979). Recent mapping studies involving retro- and/or anterograde phaseolus vulgaris leukoagglutinin (PHAL) (Meyer-Bernstein and Morin 1996; Vertes and Kocsis 1994) or biotinylated dextran (BDX) (Moga and Moore 1997) labeling in rodents offered convincing evidence that the MRN provides most if not all of the serotonergic innervation to the SCN. However, observations that electrical stimulation of the DRN induces 5-HT release in the SCN (Dudley and Glass 1996) inhibits light-induced Fos expression in SCN cells (Meyer-Bernstein and Morin 1997) and attenuates light-induced phase shifts (Weber et al. 1998) conflict with this arrangement. In view of these inconsistencies this study was undertaken to directly assess the relative contributions of the MRN and DRN to 5-HT release in the SCN. Brain microdialysis was used to measure acute in vivo changes in SCN 5-HT output after electrical stimulation, localized microinjections of the 5-HT1A somatodendritic autoreceptor agonist (8-OH-DPAT) or antagonist (WAY 100635) into the MRN and DRN, and systemic application of the general 5-HT1,2 antagonist metergoline. This would offer additional perspective into

INTRODUCTION

Circadian rhythms in mammals are controlled by an autonomous clock located within the suprachiasmatic nuclei (SCN) (Klein et al. 1991; Moore 1983; Rusak and Zucker 1979). The timing of this clock is synchronized to the external light–dark cycle primarily by photic information relayed directly from the retina to the SCN via the retinohypothalamic tract (Hendriksen et al. 1972; Johnson et al. 1988; Moore and Lenn 1972; Pickard 1982; Youngstrom and Nunez 1986) and indirectly by a projection from the intergeniculate leaflet (IGL), the geniculohypothalamic tract (Card and Moore 1982; Johnson et al. 1989). A third major input to the SCN is the serotonergic projection from the midbrain raphe nuclei located in the suprachiasmatic nuclei (SCN). By using in vivo microdialysis to measure 5-HT release we demonstrated that electrical or pharmacological stimulations of the dorsal or median raphe nuclei (DRN and MRN, respectively) can alter basal release of 5-HT in the hamster SCN. There were similar increases in SCN 5-HT release after electrical stimulation of either the MRN or DRN, indicating that both could contribute to the serotonergic activity in the SCN. Systemic pretreatment with the 5-HT antagonist metergoline abolished DRN-induced SCN 5-HT release but had little effect on MRN-induced SCN 5-HT release, suggesting different pathways for these nuclei in regulating 5-HT output in the SCN. Microinjections of the 5-HT1A autoreceptor agonist 8-OH-DPAT or antagonist WAY 100635 into the MRN caused significant inhibition and stimulation of SCN 5-HT release, respectively. Both drugs had substantially less effect in the DRN. These differential drug actions indicate that somatodendritic 5-HT1A autoreceptors on MRN neurons provide the prominent raphe autoregulation of 5-HT output in the SCN. Collectively the current results are evidence that DRN as well as MRN neurons can contribute to the regulation of 5-HT release in the hamster SCN. On the basis of the current observations and those from recent anatomic tracing studies of serotonergic projections to SCN it is hypothesized that DRN input to the SCN could be mediated by a DRN → MRN → SCN pathway involving a 5-HT-sensitive multisynaptic interaction between the DRN and MRN neurons.

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the roles of the midbrain raphe nuclei in regulating 5-HT release in the SCN.

METHODS

Animals

Adult male Syrian hamsters (Mesocricetus auratus), raised from breeder pairs obtained from Harlan Sprague-Dawley (Madison, IL), were used in these studies. These animals were group housed (2 or 3/cage) in a climate-controlled (20–22°C) vivarium under a 14-h light/10-h dark photoperiod (200-lux illuminance). Preceding experimentation animals were individually housed in a circular polycarbonate cage to facilitate microdialysis procedures. Rodent chow (Prolab 3000, PMI Feeds; St. Louis, MO) and water were provided ad libitum.

Microdialysis–HPLC

Procedures of microdialysis probe construction were described previously (Dudley et al. 1998). Concentrically designed probes were constructed from a 26-gauge stainless steel outer cannula (Small Parts; Miami, FL) into which was inserted a beveled 32-gauge fused silica tube (Polyimicro Technologies; Phoenix, AZ). Hemicylindrical dialysis tubing (12-kDa MW cutoff, 250 μm OD, Spectra-por, Fisher Scientific; Pittsburgh, PA) was inserted ~1.0 mm into the outer cannula and secured with epoxy glue. The distal end of the membrane was cut to a length of 1.5 mm, and the tip was sealed with epoxy, providing an active dialyzing length of 1.0 mm. On the day preceding sampling, animals received a unilateral microdialysis probe implant stereotactically aimed at the lateral margin of the axis of the SCN. Animals were anesthetized with sodium pentobarbital (Nembutal, 50 mg/kg) and positioned with the head level in a stereotaxic frame (David Kopf Instruments). The skull was exposed, and three 1/8-in. stainless steel anchor screws were inserted through pilot holes drilled in the skull. The coordinates for probe implantation were anterior–posterior = 0.0 mm from bregma, lateral = +0.3 mm from midline, and horizontal = −8.0 mm from dura. Microdialysis was performed by continuously perfusing probes with filtered artificial cerebrospinal fluid (ACSF) containing (in mM) 147 NaCl, 4.0 KCl, and 1.8 CaCl2 (pH 7.2) at a flow rate of 1.2 μl/min with a calibrated syringe pump (CMA/100, Bioanalytical Systems; West Lafayette, IN) attached to an overhead liquid swivel (Instech; Plymouth Meeting, PA). 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 20 min.

An additional procedure was undertaken to ensure that the increased 5-HT measured in the microdialysates in response to raphe stimulation was primarily of SCN origin. Electrical stimulations were delivered near the middle of the light-phase at zeitgeber time (ZT) 5–6 (ZT 12 = time of lights off). Beginning at ZT 3, probes were perfused for a 2-h equilibration period followed by a 1-h sample collection to establish baseline 5-HT release levels. Sampling was continued for 100-min period from the onset of electrical stimulation. For evaluation of the effect of the 5-HT receptor antagonist metergoline on electrically stimulated 5-HT release, animals received injection of drug (5.0 mg/kg) or vehicle (DMSO) 20 min preceding the stimulation.

RAPHE MICROINJECTION. Microinjections of drugs or vehicle were administered separately near the middle of the light phase at ZT 6 over three consecutive days. Beginning at ZT 3, probes were perfused for a 2-h equilibration period followed by a 1-h sample collection period to establish baseline 5-HT release levels. After microinjection sampling was continued for 2 h. On each experimental day the hamsters received an injection of either the 5-HT1A agonist 8-OH-DPAT (1 μg/500 nl; Research Biochemicals International), the 5-HT3 antagonist WAY 100635 (Wyeth-Ayerst Research; Princeton, NJ; 1 μg/500 nl), or injection vehicle (1:1 ACSF:DMSO, 500 nl) in random order.

Electrical and pharmacological stimulation of the raphe

Electrical stimulation of the raphe was undertaken in animals that received a unilateral bipolar electrode implant (Plastics One; Roanoke, VA) stereotaxically aimed either at the MRN (anterior–posterior = −4.4 from bregma, lateral = +2.3 from midline, horizontal = −6.8 mm from dura at a 20° angle) or at the DRN (anterior–posterior = −4.7 from bregma, lateral = +1.7 from midline, and horizontal = −4.8 mm from dura at a 20° angle). The electrodes were secured to the skull by stainless steel screws and dental acrylic. Constant current stimulation (20-min duration at 150 or 500 μA, 10-Hz stimulus frequency, and 2.0-ms pulse duration) was delivered with a stimulus isolator (World Precision Instruments; Sarasota, FLA) coupled to a Grass S11 stimulator (Grass Instruments; Quincy, MA). The possibility of 5-HT release resultant from passive current spread was assessed in two animals by undertaking stimulation (20-min duration at 150 μA, 10-Hz stimulus frequency, and 2.0-ms pulse duration) at a site adjacent (0.7–0.8 mm lateral) to the MRN.

Pharmacological manipulations of the DRN or MRN were undertaken without anesthetizing the animals with a 31-gauge injection needle inserted into a unilateral 24-gauge stainless steel guide cannula (Plastics One; Roanoke, VA) with its distal end positioned 1.0 mm dorsal to the MRN or DRN using the same stereotaxic coordinates as for the electrode implants. The guide cannula was secured to the skull by stainless steel screws and dental acrylic. The injection needle, connected by polyethylene tubing to a 10-μl Hamilton syringe for injection, was machined such that its tip extended 1.0 mm from the guide cannula. A 31-gauge stylet inserted into the guide cannula was used to maintain patency between injections.

Experimental protocol

ELECTRICAL STIMULATION OF THE RAPHE. Intracranial placements of either the electrode or guide cannula and the microdialysis probe were undertaken in two separate surgeries spaced 3 days apart. Experimentation commenced 24 h after the microdialysis probe implantation. Electrical stimulations were delivered near the middle of the light-phase at zeitgeber time (ZT) 5–6 (ZT 12 = time of lights off). Beginning at ZT 3, probes were perfused for a 2-h equilibration period followed by a 1-h sample collection to establish baseline 5-HT release levels. Sampling was continued for a 100-min period from the onset of electrical stimulation. For evaluation of the effect of the 5-HT receptor antagonist metergoline on electrically stimulated 5-HT release, animals received injection of drug (5.0 mg/kg) or vehicle (DMSO) 20 min preceding the stimulation.

590 mV relative to an AgCl reference electrode was used to measure 5-HT. The lower level of sensitivity (signal × 5 background) was ~500 fg. Electrode output was interfaced with an IBM-compatible computer, which recorded and analyzed the data. Authenticity of the 5-HT peak in SCN microdialysate was verified by predictable changes in its size after electrical and pharmacological stimulations of the raphe (this study) and localized administration of pharmacological agents to the SCN via the dialysis probe (Dudley et al. 1998).
Histological evaluations of intracranial implant sites

After completion of the experiments the locations of the electrode and dialysis implants were verified histologically in each animal. Hamsters were deeply anesthetized with Nembutal and perfused intracardially with 100 ml of buffered 4% paraformaldehyde. The brains were removed and postfixed in 4% paraformaldehyde overnight. Cryostat sections of the SCN region (20 μm) were stained with cresyl violet for verification of microdialysis probe placement. Data from animals whose dialysis probe was located >500 μm from the SCN were excluded from analysis.

A separate tissue block containing the midbrain was serially sectioned (60 μm) on a vibratome and processed immunocytochemically for 5-HT to verify electrode or cannula tip location among 5-HT neurons of the MRN or DRN. Tissue sections were first incubated in 1.0% H₂O₂ in Tris-buffered saline (TBS; pH 7.6) to remove endogenous peroxidase. The tissue was rinsed and incubated in rabbit primary polyclonal anti-5-HT (Eugenotech; Farmendale, NJ) at 1:500 in TBS containing 0.1% BSA and 0.1% Triton X-100 for 24 h at 4°C. Sections were rinsed, incubated in biotinylated anti-rabbit IgG with blocking serum (Vector Laboratories) for 1 h at room temperature, and incubated in an avidin-biotin peroxidase complex (Vector Laboratories, Elite kit) for 1 h at room temperature. Visualization of 5-HT neurons was achieved with 3,3′-diaminobenzidine-HCl as chromogen.

Statistics

Data from the experiments were normalized as percentage of the pretreatment baseline level. These values were analyzed with a one-way repeated measures analysis of variance followed by the Student’s-Newman-Keuls posthoc mean comparison test. For all procedures the level of significance was set at \( P < 0.05 \).

RESULTS

Electrical stimulation of the DRN and the MRN induces 5-HT release in the SCN

Electrode placement within the raphe nuclei was confirmed for each animal with immunocytochemical staining of 5-HT neurons such as those shown in Fig. 1. Sites of implanted stimulating electrodes (and microinjection cannulae) that were effective in altering basal SCN 5-HT release are depicted diagrammatically in Fig. 2. Electrical stimulation of the MRN and DRN with 150 μA at 10 Hz induced significant increases in the output of 5-HT in the SCN region. Excitation–secretion-coupled release of 5-HT was evident within 20 min of stimulation onset, and for both raphe nuclei averaged peak levels of 5-HT release in the SCN attained 40 min after stimulation onset were 156 ± 11% versus 148 ± 9% of baseline for MRN and DRN, respectively (Fig. 3). The maximal stimulated increases over the first 40-min poststimulation period were 147 ± 12% versus 148 ± 10% of baseline for MRN and DRN, respectively (Fig. 4). There was no statistical difference between the two raphe sites of stimulation for each of these parameters (\( P < 0.3 \)). In both groups baseline 5-HT output was reestablished ≤60 min after the cessation of stimulation, although the MRN-stimulated group exhibited a somewhat more rapid decline. The magnitude of response of DRN stimulation with 500 μA at 10 Hz (peak stimulation = 146 ± 6% of baseline; Fig. 5) was not different from that elicited with 150 μA.
The peak increase induced by DRN stimulation measured with hemi-probes (with windows of active dialysis membrane aimed medially within the SCN; 138 ± 7%, n = 3) 40 min after stimulation onset was not significantly different from that assessed with the conventional concentric probes (P > 0.5). In two animals whose stimulating electrodes were placed 0.7–0.8 mm lateral to the MRN to examine the extent of passive current flow from the bipolar electrodes, the release of 5-HT from the SCN was unaffected by stimulation from these implants.

**Metergoline inhibits DRN but not MRN stimulated 5-HT release in the SCN**

Pretreatment of MRN-electrically stimulated animals with an intraperitoneal injection of the general 5-HT1,2 receptor antagonist metergoline did not affect the magnitude or time course of increased 5-HT release in the SCN relative to vehicle-injected controls (peak levels were 132 ± 5% vs. 151 ± 22% of baseline for vehicle and metergoline treatments, respectively; P > 0.4; Fig. 6). In contrast, pretreatment of DRN-stimulated animals with metergoline abolished the excitation-secretion-coupled release of 5-HT in the SCN (peak levels were 156.3 ± 12% vs. 108 ± 9% of baseline for vehicle and metergoline treatments, respectively; P < 0.01; Fig. 7).
tion of metergoline alone had no significant effect on basal release of 5-HT in the SCN over the sampling period (Fig. 8). The suppression of 5-HT release caused by metergoline treatment was not attributable to diminished efficiency of the dialysis probe or stimulating electrode because over sequential days of experimentation stimulated increase in 5-HT release returned on the third day after its suppression by metergoline treatment on the second day (Fig. 9).

MRN and DRN 5-HT1A autoreceptors differentially regulate 5-HT release in the SCN

Microinjection of 8-OH-DPAT into the MRN caused a suppression of basal SCN 5-HT release to 59 ± 8% of pretreatment levels (Fig. 10; $P < 0.05$). Maximal suppression of 5-HT release occurred ≤40–60 min postinjection, and this release was still significantly suppressed at 2 h postinjection. Similar treatment in the DRN induced a small but statistically insignificant suppression of SCN 5-HT release (Fig. 10; $P > 0.30$). Microinjection of the somatodendritic autoreceptor antagonist WAY 100635 into the MRN acutely increased SCN 5-HT release to 170 ± 13% of baseline (Fig. 11; $P < 0.05$). Maximal stimulation of SCN 5-HT release occurred within 20 min of drug injection, and this declined to levels not statistically different from baseline by 40 min postinjection. In contrast, local application of WAY 100635 to the DRN increased SCN 5-HT release to only 126 ± 13% of baseline (Fig. 11; $P < 0.05$). Maximal changes in SCN 5-HT release induced by 8-OH-DPAT and WAY 100635 are shown in Fig. 12.

DISCUSSION

By using microdialysis in the unanesthetized hamster it was shown that electrical or pharmacological stimulations of either the DRN or the MRN can significantly affect the release of 5-HT in the hamster SCN. The similar degree of response evoked by electrical stimulation of either of these raphe nuclei suggests that both could contribute to and/or modulate the release of 5-HT in the SCN. The respective inhibitory and stimulatory responses to intra-MRN applications of the 5-HT1A autoreceptor agonist 8-OH-DPAT and selective 5-HT1A antagonist WAY 100635 strongly implicate somatodendritic 5-HT1A autoreceptors in the regulation of 5-HT output in the SCN, as was demonstrated for other brain regions (for review see Jacobs and Fornal 1997). Importantly, the substantially greater effects of these pharmacological agents when administered to the MRN compared with the DRN suggests that the MRN may be the principal locus for 5-HT1A autoreceptor-mediated regulation of 5-HT output in the SCN circadian clock.

This study represents the first direct in vivo physiological assessment of the contributions of the DRN and MRN to SCN 5-HT output. The finding that 5-HT release in the SCN can be evoked by electrical stimulation of either of these nuclei is consistent with autoradiographic (Azmitia and Segal 1978) electrolytic lesioning (Aghajanian et al. 1969) and retrograde labeling (Pickard 1982) examinations of midbrain raphe projections in the rat, where it was reported that the SCN receives projections from the DRN and MRN. However, these findings
differ from those of other studies involving the effects of raphe lesions on SCN 5-HT content (Van De Kar and Lorens 1979) or more recent anterograde and/or retrograde tracings of raphe neurons with PHAL (Meyer-Bernstein and Morin 1996; Vertes and Kocsis 1994) or BDX (Moga and Moore 1997), which strongly suggest that the MRN provides most if not all of the serotonergic input to the rat and hamster SCN. A possible explanation for the contradiction between these and the earlier anatomic tracing studies is greater degree of methodological variability inherent to the older autoradiographic procedures. The newer tracing techniques, especially when augmented with specific 5-HT neurotoxic lesioning, potentially offer greater specificity and interpretive reliability (Meyer-Bernstein and Morin 1997). However, it is important to recognize that these tracing methods would not have delineated multisynaptic efferent or afferent pathways of the raphe neuronal system.

Methodological considerations could account for the apparent discrepancy between the current finding that electrical stimulation of the DRN evokes 5-HT release in the SCN and the apparent lack of direct DRN innervation to the SCN as determined from the tracing experiments discussed previously. The first consideration in this regard is the possibility that nonspecific excitation of neural elements by passive current spread from the site of stimulation could affect 5-HT release in the SCN. This could also explain the observed suppression of light-induced SCN Fos expression (Meyer-Bernstein and Morin 1997) or behavioral phase shifts (Weber et al. 1998) induced by electrical stimulation of the DRN. We believe, however, that the induction of 5-HT release in the SCN induced by electrical DRN stimulation is an anatomically discrete response. This contention is based on our findings that 1) stimulation with electrodes positioned ~0.8 mm lateral to the MRN had no effect on SCN 5-HT release, indicating that passive current flow from electrodes in the DRN likely would not have a stimulatory effect on MRN neurons located ~2.0 mm ventral from the point of stimulation, and 2) systemic pretreatment with the general 5-HT antagonist metergoline totally abolished DRN electrically stimulated 5-HT release in the SCN but had no effect on MRN electrically stimulated SCN 5-HT release. Notably, this differential effect of metergoline points to the existence of a 5-HT-responsive neural target for DRN neurons, which when stimulated promotes SCN 5-HT release. The existence of a prominent DRN-to-MRN projection (Vertes and Kocsis 1994) together with the apparent absence of a direct DRN-to-SCN projection but demonstrated MRN-to-SCN projection (Meyer-Bernstein and Morin 1996; Moga and Moore 1997; Vertes and Kocsis 1994) suggests that this target may be anatomically associated with the MRN.

An additional methodological consideration concerns the...
presence of a direct projection from the DRN to anterior hypothalamic regions lateral to the SCN (Moga and Moore 1997). The possibility exists that the increased 5-HT release measured in response to DRN stimulation could have come from DRN fibers lateral to the SCN rather than from the SCN itself. This issue was approached in a separate experiment with probes with a small window of active dialysis membrane aimed medially in the SCN to limit contamination by 5-HT from sources external to the SCN. This method provided equivalent estimations of peak DRN-stimulated 5-HT release as obtained with the conventional concentric probes, confirming that the majority of 5-HT measured during DRN stimulation comes from terminals in the SCN.

This demonstration that intra-DRN microinjection of the selective 5-HT1A antagonist WAY 100635 acutely increased 5-HT output in the SCN is complementary evidence supporting the involvement of the DRN in SCN 5-HT release. Possible diffusion of the injected drug to the MRN could have accounted for this effect; however, it is unlikely that it could have diffused from the DRN to the MRN in sufficient time and concentration to act with a pharmacokinetic pattern similar to that produced by intra-MRN injection. The stimulatory effect of WAY 100635 on SCN 5-HT release is consistent with the stimulatory action of this 5-HT1A autoreceptor antagonist on DRN raphe neuronal firing (Jacobs and Fornal 1997). This effect is also internally consistent with the induction of SCN 5-HT release after electrical stimulation of the DRN. Despite these stimulatory effects, however, failure of DRN microinjections of 8-OH-DPAT to elicit a significant inhibition of 5-HT release in the SCN argues that the DRN may contribute relatively little to basal levels of 5-HT output in the SCN.

As microinjections of WAY 100635 or 8-OH-DPAT in the MRN caused a significantly greater stimulation and suppression of SCN 5-HT release, respectively, compared with similar treatments in the DRN, it is likely that the MRN is the major raphe site for the autoregulation of serotonergic activity in the SCN. In a previous study we reported that systemic treatment with the mixed 5-HT1A autoreceptor agonist BMY 7378 had a substantial greater suppressive effect on SCN 5-HT release during the night compared with midday. Evidence from this study suggests that this action may have been mediated primarily via the activation of MRN autoreceptors. This in turn indicates that a daily variation in MRN 5-HT1A autoreceptor-mediated response may be involved in regulating the timing of the circadian rhythm in 5-HT output in the SCN (Dudley et al. 1998).

There are numerous lines of evidence supporting direct circadian-related actions of 5-HT in the SCN, which include regulation of photic as well as nonphotic entrainment of circadian activity. Convincing evidence for a role in photic entrainment has come from numerous studies that demonstrated a marked suppressive effect of 5-HT receptor agonists on RHT-mediated signaling events, including light-induced Fos expression of SCN cells and circadian phase resetting (Bradbury et al. 1997; Glass et al. 1994; Pickard and Rea 1997; Rea et al. 1994; Selim et al. 1993). Consistent with these effects are preliminary findings that the (presumed) release of SCN 5-HT induced by electrical stimulation of the raphe attenuates light-induced SCN Fos expression (Meyer-Bernstein and Morin 1997) and phase shifting of the circadian activity rhythm (Weber et al. 1998). Also, treatment with the 5-HT antagonist WAY 190-190 (Rea et al. 1995) or lesions of 5-HT terminals in the SCN (Bradbury et al. 1997) potentiate photic signaling events in the SCN.

Various lines of evidence also exist for a role of 5-HT in mediating nonphotic (behavioral) phase shifting of the circadian system. Such a role for 5-HT is heuristically attractive because the intrinsic pattern of activity of 5-HT neurons, which is highly correlated with circadian changes in behavioral state (Jacobs and Fornal 1997), could provide direct behavioral feedback to the circadian system in the form of changing 5-HT release in brain regions such as the IGL and/or SCN. The finding that wheel running has a pronounced phase-dependent effect on 5-HT release in the hamster SCN is consistent with this contention (Dudley et al. 1998). Pharmacological evidence for a role of 5-HT in nonphotic clock resetting includes observations that increases in behavioral activity induced by wheel running, cage changing (Mrosovsky et al. 1989; Reesbs and Mrosovsky 1989), or treatment with the GABAA agonist triazolam (Peney et al. 1995; Tominaga et al. 1992; Turek and Van Reeth 1988) phase advance the circadian activity rhythm in a similar manner as does systemic administration of 5-HT1A agonists, with maximal effects around midday (Bobrzynska et al. 1996a; Tominaga et al. 1992). Depletion of 5-HT in whole brain by intraperitoneal injection of p-chloroamphetamine (Peney et al. 1995) or blockade of 5-HT action with 5-HT7/2 receptor antagonists (Sumova et al. 1996) attenuates triazolam- or arousal-induced phase advances. It is not clear from these studies, however, whether activity/5-HT–associated shifts in clock activity are mediated by the direct action of 5-HT in the SCN and/or at other relevant sites.

A direct nonphotic phase-shifting action of 5-HT in the SCN is supported by numerous studies, including reports that application of 5-HT receptor agonists to SCN slice preparations causes marked shifts in the circadian rhythm of spontaneous neuronal activity (Medanic and Gillette 1992; Prosser et al. 1990, 1993; Shibata et al. 1992), with phase advances occurring near the time when serotonergic and behavioral advances are observed in vivo. Phase advances of the circadian activity rhythm during the mid-subjective day were also observed after administration of 8-OH-DPAT into the third ventricle immediately upstream from the SCN in rats (Edgar et al. 1993) or after bilateral microinjection of 8-OH-DPAT into the SCN region of hamsters (Challet et al. 1998). In addition, depletion of 5-HT in the SCN by neurotoxic lesioning prevents entrainment to daily schedules of voluntary or forced activity in mice (Edgar et al. 1997; Marchant et al. 1997) and triazolam-induced phase advances of the circadian activity rhythm (Cutler et al. 1994). Despite these results, however, the case for direct phase-resetting effects of 5-HT in the SCN is hindered by observations in the Syrian hamster that 1) depletion of 5-HT terminals from the SCN does not attenuate behavioral phase shifting (Bobrzynska et al. 1996b), 2) unilateral injection of 8-OH-DPAT into the raphe but not the SCN (or the IGL) induces behavioral phase shifting (Mintz et al. 1997), 3) the degree of behavioral phase shifting attained by direct bilateral in vivo application of 8-OH-DPAT to the SCN (Challet et al. 1998) is small compared with that induced by systemic application of the drug (34 vs. 75 min) (Bobrzynska et al. 1996a; Tominaga et al. 1992), and 4) permanent destruction of MRN neurons supplying 5-HT innervation to the SCN does not prevent wheel-running–induced phase shifting (Meyer-Bern-
stein and Morin 1998). Thus the SCN may indeed not represent an important target for the nonphotic clock resetting action of 5-HT, at least in hamsters. Studies involving timed perfusions of selected 5-HT1A agonists to the SCN, raphe, and IGL standardized between species may offer insight into this question.

In summary, the current findings are evidence that the DRN and the MRN differentially contribute to the regulation of serotonergic activity in the Syrian hamster SCN. The greater degree of response elicited by microinjection of the 5-HT1A autoreceptor agonist 8-OH-DPAT and antagonist WAY 100635 into the MRN compared with the DRN suggests that the MRN is the principal raphe site for 5-HT1A autoreceptor-mediated regulation of SCN 5-HT output.

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