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The Neurochemical Basis of Photic Entrainment of the Circadian Pacemaker

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Abstract

Circadian rhythmicity in mammals is controlled by the action of a light-entrainable pacemaker located in the basal hypothalamus, in association with two cell clusters known as the suprachiasmatic nuclei (SCN). In the absence of temporal environmental cues, this pacemaker continues to measure time by an endogenous mechanism (clock), driving biochemical, physiological and behavioral rhythms that reflect the natural period of the pacemaker oscillation. This endogenous period usually differs slightly from 24 hours (i.e., circadian). When mammals are maintained under a 24 hour light-dark (LD) cycle, the pacemaker becomes entrained such that the period of the pacemaker oscillation matches that of the LD cycle. Potentially entraining photic information is conveyed to the SCN via a direct retinal projection, the retinohypothalamic tract (RHT). RHT neurotransmission is thought to be mediated by the release of excitatory amino acids (EAA) in the SCN. In support of this hypothesis, recent experiments using nocturnal rodents have shown that EAA antagonists block the effects of light on pacemaker-driven behavioral rhythms, and attenuate light-induced gene expression in SCN cells. An understanding of the neurochemical basis of the photic entrainment process would facilitate the development of pharmacological strategies for maintaining synchrony among shift workers in environments which provide unreliable or conflicting temporal photic cues, such as the proposed space station.

Photic Entrainment of the Circadian Pacemaker

Considerable evidence suggests that a major light-entrainable circadian pacemaker is located in the ventral hypothalamus in association with the suprachiasmatic nuclei (SCN; Rusak and Zucker, 1979; Meijer and Rietveld, 1989). Bilateral destruction or surgical isolation of the SCN results in the permanent disruption of circadian rhythms in mammals (Inouye et al., 1979; Rusak and Zucker, 1979). Furthermore, transplantation of the SCN from a fetal donor into the hypothalamus of an SCN-lesioned host restores rhythmcity (Sawaki et al., 1986; Lehman et al, 1987; Decousey and Buggy, 1989), and the period of the restored rhythm matches that of the donor (Ralph et al., 1990). In addition, the isolated SCN continues to display circadian behavior in vitro. Circadian rhythms in the neuronal activity (Green and Gillette, 1982; Gillette and Reppert, 1987), neuropeptide release (Earnest and Sladek, 1986), and metabolic activity (Newman and Hospod, 1986) have been demonstrated to persist for several days in cultured SCN explants. These observations strongly suggest that the biological mechanism responsible for the generation of physiological circadian oscillations is an intrinsic component of the mammalian SCN.

Photic entrainment of circadian rhythms occurs as a consequence of the phase specific effects of environmental light on the activity of the circadian pacemaker. This relationship is defined by the phase response curve to light pulses administered to animals maintained under constant darkness (Daan and Pittendrigh, 1976; Takahashi et al, 1984). In nocturnal rodents, light pulses administered during the early subjective night cause phase delays of the pacemaker while pulses delivered during the latter half of the subjective night cause phase advances (Daan and Pittendrigh, 1976; Takahashi et al., 1984). Light pulses given during the middle of the subjective day do not cause phase shifts. Light-induced shifts represent long-term alterations in pacemaker function.

Photic information is conveyed to the SCN through at least two visual pathways. The retinohypothalamic tract (RHT) is a direct, bilateral monosynaptic projection from retinal ganglion cells to neurons in the SCN and the surrounding hypothalamus

(Moore and Lenn, 1971; Johnson et al., 1988a). In addition, a second, indirect visual projection, the geniculohypothalamic tract (GHT) has been described. This pathway projects from the retina to relay neurons in the intergeniculate leaflet (IGL) of the thalamus, which, in turn, send their axons to neurons in the SCN (Swanson et al., 1974; Card and Moore, 1982; Pickard, 1982). Lesion studies have shown that the RHT is both necessary and sufficient to support photic entrainment of circadian rhythms in experimental rodents (Johnson et al., 1988b).

The Neuropharmacology of Photic Entrainment

In addition to light, a number of synthetic and natural neuroactive substances have been tested for their ability to reset the circadian pacemaker. Benzodiazepines (Turek and Losee-Olson, 1986), melatonin (Cassone et al., 1986), theophylline (Ehret et al., 1975), and various neuropeptides (Albers et. al., 1984; Albers et al., 1991) have all been shown to alter the phase of circadian oscillations. However, only a few neurotransmitter-specific agents have been systematically investigated for their effects on light-induced phase alterations.

Gamma-amino butyric acid. A large proportion of the neurons in the SCN contain glutamic acid decarboxylase (van den Pol and Tsujimoto, 1985), the enzyme responsible for the synthesis of the inhibitory amino acid neurotransmitter, gamma-amino butyric acid (GABA). Although it is clear that GABA does not play a direct role in RHT neurotransmission, the abundance of GABA-ergic neurons in the SCN raise the possibility that this neurotransmitter may participate in the processing of photic information in the SCN. In fact, neurophysiological evidence suggests that GABA modulates retinal input to the SCN (Shibata et al., 1986), perhaps by acting presynaptically to regulate neurotransmitter release from RHT terminals (Ralph and Menaker, 1989). For this reason, Ralph and coworkers (1985; 1986; 1989) have investigated the effects of specific GABA agonists and antagonists on light-induced phase alterations of the free running activity rhythm in rodents. These investigators reported that (a) GABA A antagonists attenuate light-induced phase delays (Ralph and Menaker, 1985; 1989), (b) benzodiazepines attenuate light-induced phase advances (Ralph and Menaker, 1986; 1989), and (c) GABA B agonists block both light-induced phase advances and delays (Ralph and Menaker, 1989). These results strongly support a role for the SCN GABA-ergic system in modulation of photic input to the circadian pacemaker.

Acetylcholine. Initial investigations into the identity of the RHT neurotransmitter focused on acetylcholine (ACh). Cholinergic agonists have been reported to mimic (Zatz and Herkenham, 1981; Earnest and Turek, 1983), and antagonists to block (Zatz and and Brownstein, 1981; Keefe et al., 1987), the effects of light on the circadian system. Furthermore, Earnest and others (1985) reported that the phase response curve for intraventricular injections of the cholinergic agonist, carbachol, are similar to the phase response curve for light pulses. This similarity was offered as evidence that ACh might be the RHT neurotransmitter. However, neither retinal ganglion cells nor the optic nerves contain measurable quantities of choline acetyltransferase (Hebb and Silver, 1956), the synthetic enzyme for ACh, and bilateral lesions of the optic nerves do not alter the levels of cholinergic markers in the rat SCN (Rea, unpublished observations). On the other hand, the SCN do receive a cholinergic projection, possibly from the basal forebrain (Ichikawa and Hirata, 1986). One interesting possibility is that ACh from another source may modulate RHT neurotransmission by acting presynaptically (Rusak and Bina, 1990).

Excitatory Amino Acids. Excitatory amino acids (EAA) remain the best candidates for the RHT neurotransmitter. EAA antagonists block fast EPSPs (Kim et al., 1989) and field potential responses (Cahill and Menaker, 1987) of SCN neurons to optic nerve stimulation in the hypothalamic slice preparation. Furthermore, using the same preparation, Liou et al. (1986) have reported that optic nerve stimulation causes the release of radioactivity from SCN slices preloaded with radiolabeled EAAs. Recently, Colwell and colleagues (1990) reported that intraperitoneal injections of the non-competitive EAA antagonist MK-801 attenuated light-induced phase shifts of the free-running activity rhythm in the mouse. In order to determine whether EAA antagonists inhibit light-induced phase shifts by acting on the SCN, we determined the effect of direct injections of EAA antagonists into the SCN on light-induced phases advances of the free-running activity rhythm in hamsters.

Microinjection of EAA Antagonists into the SCN Attenuate Light-Induced Phase Advances

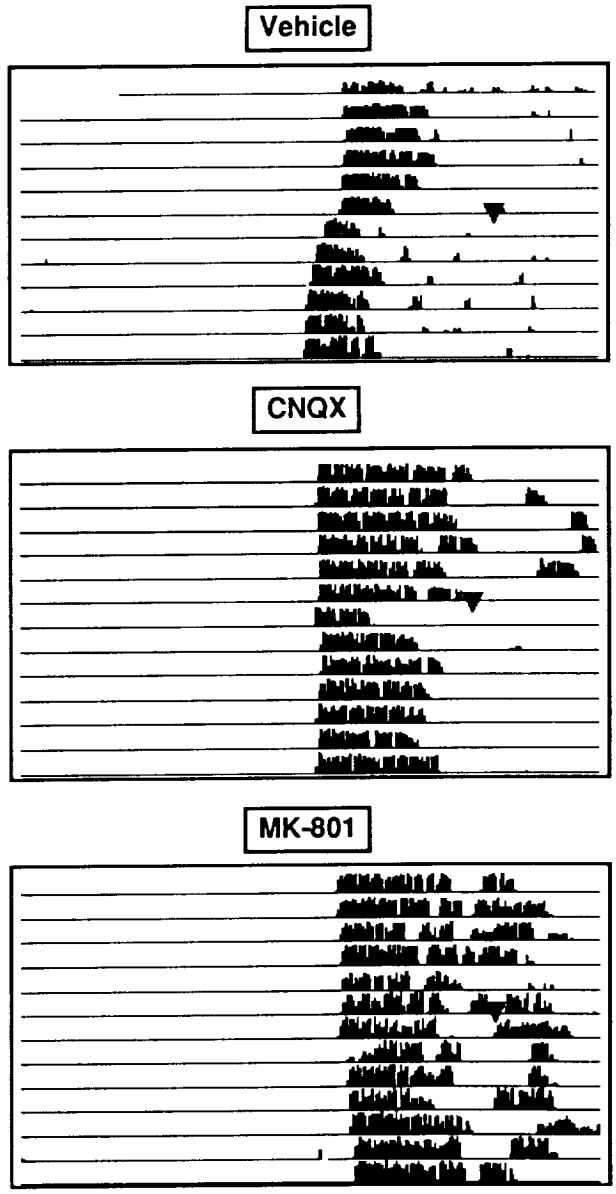
Syrian hamsters were implanted with 26 gauge guide cannulae stereotaxically aimed at the SCN. The cannulae were fixed in place with dental cement and the animals were allowed to recover for 7 -

10 days under LD 14:10. After the recovery period, the hamsters were transferred to individual cages equipped with computer-monitored running wheels and maintained under constant darkness (DD). Only animals with robust free running activity rhythms and stable periods were used in the study. Hamsters remained in DD for 7 - 10 days before treatment. At mid subjective night (CT18.5), hamsters received an injection of 300 nl of artificial CSF (aCSF) containing either 1 mM CNQX (a competitive, non-NMDA type EAA antagonist), 1 mM MK-801 (a non-competitive, NMDA type antagonist) or no drug (vehicle control) directly into the suprachiasmatic hypothalamus using a 33 gauge infusion cannula. Five minutes after injection, each animal was exposed to light (30 lux) for 10 minutes. After light exposure, the hamsters were returned to their cages and maintained under DD for an additional 10 days. The effect of treatment on the phase of the free-running activity rhythm was determined as described previously (Daan and Pittendrigh, 1976) using the onset of wheel running activity as a phase reference point. Injection sites were verified histologically and only data collected from animals with injection sites within 0.5 mm of the SCN were included in the analysis.

In the absence of drug injection, light treatment at this time results in a phase advance of the free running activity rhythm of approximately 81 $\pm$ 8 minutes. Both drugs attenuated light-induced phase advances by more than 85%. This result suggests that EAA antagonists inhibit light-induced phase shifts of the circadian pacemaker by acting directly on the SCN, possibly at the RHT synapse, and support a role for EAA as the RHT neurotransmitter.

Light-induced Gene Expression

Light-induced resetting of the circadian pacemaker represents a permanent alteration in pacemaker function. The c-fos protooncogene has been implicated in the process of stimulus-transcription coupling in the CNS (Curran and Morgan, 1987; Sagar et al., 1988) and appears to mediate long-term adaptive changes in neuronal function (Berridge, 1986; Goelet et al., 1986). Recent work in our own laboratory (Rea, 1989; 1992; Rea and Michel, 1990) and elsewhere (Kornhauser, et al., 1990; Rusak et al., 1990; Aronin et al., 1990) has demonstrated that light exposure during the subjective night induces the expression of certain immediate-early genes, including c-fos, among a population of SCN cells. Furthermore, light-induced c-fos expression only occurs when the light is administered at a circadian time at which



Actograms showing the effects of microinjections of vehicle (top), 1 mM CNQX (middle), or 1 mM MK-801 (bottom) on the light-induced phase advance of the free-running activity rhythm. In all cases, a brief light pulse (30 lux for 15 minutes) was given at CT18.5 (inverted triangle).

a phase shift of the pacemaker results (Kornhauser et al., 1990; Rea, and Michel, 1990; Rea, 1992). These findings suggest that c-fos expression may represent a transcriptional event in the response cascade leading to light-induced phase alterations of the pacemaker. If this is the case, then ould expect that light-induced phase shifts and c-fos expression would share similar pharmacology.

This hypothesis was tested using our cannulated hamster model. Hamsters were housed in LD 14:10 for at least 7 days after surgery. At lights-out on the day before the experiment the animals were transferred to DD. Thiry hours after transfer (i.e., mid subjective night) the animals received microinjections of either aCSF or a solution of EAA antagonist in aCSF (300 nl at 1 mM) directly into the SCN. Five minutes later, each animal was exposed to 30 lux of white light for 10 minutes and returned to their cages under DD. Two hours after the onset of the light pulse, animals were deeply anesthetized, perfused transcardially with 0.4% paraformaldehyde, and the brains were processed for c-fos immunocytochemistry as described previously (Rea, 1989).

Light stimulation induces c-fos expression among a population of approximately 1055 $\pm$ 110 cells in the SCN. Both CNQX and MK-801 reduced the number of SCN cells that expressed c-fos in response to photic stimulation by about 45%. This result strenthens our proposal that c-fos expression represents an early transcriptional event mediating the effects of light on the SCN circadian pacemaker.

Significance of this Work to Aerospace Operations

The global mission of the U. S. Air Force demands that its personnel remain prepared at all times to participate in activities which are vital to the national security and likely to involve transmeridian air travel and irregular work schedules for sustained periods. In responding to this challenge, Air Force personnel are uniquely vulnerable to the performance limitations imposed upon them by the circadian timing system.

Knowledge gained from an investigation of the neurobiological basis of circadian rhythmicity will provide the database necessary for the development of photic and/or pharmacological strategies for alleviating the performance decrements associated with work schedules and practices which are incompatable with the circadian timing system. The elucidation of the mechanism of photic entrainment is an important step toward a detailed understanding of circadian processes.

Pharmacological agents with specific and predictable effects on the circadian pacemaker could serve as useful tools for the control of circadian rhythmicity. For example, it may be possible to develop a specific antagonist against the RHT neurotransmitter. Such an agent could be used to selectively "blind" the circadian clock to the entraining influence of environmental light. This would alleviate the potential conflict between a shift worker's work-rest cycle and the environmental LD cycle. Similarly, pharmacological aids could be developed which would permit the rapid resetting of the circadian clock, accelerate rates of reentrainment of overt rhythms after rapid transmeridian travel, and maintain synchrony among shift workers in environments which provide unreliable or conflicting temporal photic cues, such as the proposed space station.

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