Electrical and pharmacological properties of the suprachiasmatic nuclei[1,2]

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ABSTRACT

The suprachiasmatic nuclei (SCN) of the mammalian hypothalamus are an important circadian pacemaker. The electrical activity of these nuclei exhibits an intrinsic circadian rhythm. The rhythmicity of the SCN is also reflected in cyclic glucose consumption and serotonin metabolism. These rhythms are entrained to the light-dark cycle via the retinohypothalamic projection. This pathway, possibly together with a visual projection via the ventral lateral geniculate nuclei, innervates light-responsive SCN cells, which exhibit the functional properties of luminance detectors. The SCN contain various peptides, acetylcholine, and serotonin either intrinsically or in terminals of afferent projections. For acetylcholine it has been demonstrated that the SCN mediate the process of photic entrainment and light suppression of pineal synthetic activity. In the case of serotonin and vasopressin it seems certain that the SCN do not depend on their presence for generating circadian rhythms or for entrainment. Both substances may modulate the intrinsic pacemaker frequency through mechanisms that remain to be established.—**Groos, G.; Mason, R.; Meijer, J.** Electrical and pharmacological properties of the suprachiasmatic nuclei. *Federation Proc.* 42: 2790–2795; 1983.

One of the most fundamental properties of circadian rhythms is their persistence in animals living in a constant, or aperiodic, environment. Under such experimental conditions the free-running rhythmicity exhibits a period that deviates from exactly 24 h. When an animal is exposed to daily environmental cycles its circadian rhythms will entrain to the 24-h period of the external rhythm. These phenomena are most simply explained by postulating that animals possess one or more circadian pacemakers that generate an oscillation with an intrinsic period close to 24 h. These pacemakers, moreover, are susceptible to entrainment by environmental periodicities, particularly the daily alternation between light and darkness. Until recently the concept of a circadian pacemaker was largely hypothetical and its empirical foundation consisted entirely of observations of overt rhythmicity. During the last 2 decades, however, evidence has accumulated demonstrating that parts of the endocrine and nervous systems of various animal species do in fact contain circadian pacemakers (9, 18, 25, 31, 35, 45).

In mammals the suprachiasmatic nuclei (SCN), two small cell groups located in the anterior hypothalamus just dorsal to the optic chiasm, have been identified as a putative circadian pacemaker (25, 33, 35). Although the SCN are probably not the only structures with such a function in mammals (9, 31, 42), the evidence that the SCN constitute a major pacemaker in the mammalian circadian system is compelling. The empirical support for this idea includes numerous demonstrations that many circadian rhythms are abolished by complete bilateral SCN lesions or surgical isolation of the SCN in rodents as well as in primates (9, 18, 25, 31, 35). Furthermore, electrical stimulation of the SCN has been shown to alter the phase of circadian rhythms in rodents (34). The SCN exhibit a circadian rhythm in glucose utilization (38, 39) and they are capable of sustaining a rhythm in electrical activity even when neurally isolated from the rest of the brain (18, 19). All these studies consistently support the notion that the SCN are capable of endogenous production of a circadian pacemaker rhythm. In addition, the retinohypothalamic projection (RHP), which terminates in the SCN, mediates the entrainment of circadian rhythms by light-dark cycles (11, 19, 23, 25, 33). Thus, an anatomical substrate is present for the process of photic entrainment.

CIRCADIAN RHYTHMS OF THE SCN

When electrodes are implanted in various brain regions of freely moving rats, marked circadian rhythms in multiple- and single-unit activity can be recorded (18, 19, 28). The SCN are no exception in this respect. In the rat, SCN multiunit activity is high during the light portion of the day and low during darkness (18). This pattern of rhythmicity corresponds to the circa-

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dian rhythm of glucose consumption of the SCN (38, 39). The SCN appear to be the only brain structure capable of sustaining circadian rhythms in glucose consumption and electrical activity. Thus, in autoradiographic studies the SCN were reported to be the only part of the brain exhibiting a circadian glucose uptake pattern (39). One should be cautious, however, to conclude on the basis of these findings that brain regions outside the SCN are arrhythmic in their glucose uptake. Even in the SCN of rats kept in light-dark cycles, i.e., under conditions where the amplitude of the rhythm in metabolic rate is largest, the ratio of amplitude to mean level of glucose consumption is only approximately 0.25 (38). This suggests that a metabolic activity rhythm may be very difficult to detect in areas with a high metabolic rate and a less homogenous functional organization. Thus, the preoptic area surrounding the SCN exhibits a marked circadian rhythm in electrical activity (18, 19), but autoradiographic studies of glucose utilization have not been able to demonstrate a metabolic rhythm in this region (38, 39). Moreover, there are indications that in addition to the SCN, the cortex may also exhibit a rhythmic glucose uptake pattern (6).

Inouye and Kawamura (18, 19) found that multiunit rhythms in a number of brain structures were abolished by surgical isolation of the SCN from the rest of the brain. Significantly, the electrical activity rhythm of the SCN and the directly adjacent areas in the hypothalamus persisted both in light-dark cycles and in constant light or dark (19). Because the persistence of rhythmicity outside the SCN island was not exhaustively investigated in their study, this result does not warrant the conclusion that the SCN are the only nuclei with intrinsic rhythmicity. These findings are conclusive in another respect, insofar as they demonstrate that the SCN do not require neural input or a light-dark cycle to sustain their circadian rhythm. In contrast, many other parts of the brain appear to be dependent on neural afferents from the SCN for their circadian rhythm in electrical discharge. Therefore, the experiments of Inouye and Kawamura constitute the most compelling evidence presently available that the SCN are indeed a circadian pacemaker. It should be noted that these authors observed the rhythmicity in the SCN after complete neural deafferentation of the anterior medial hypothalamus, with the exception of the RHP. This does not eliminate the possibility that the activity of the SCN is driven rhythmically by humoral factors or by temperature. In view of the recent demonstrations that other circadian pacemakers may be present outside the SCN this possibility should not be dismissed without some consideration. In particular, body temperature and cortisol rhythms of primates persist after destruction of the SCN (9, 31) both in light-dark cycles and in constant conditions. Consequently it is important that rhythmicity be demonstrated in the SCN, not just after neural isolation but also in a constant biochemical and thermal environment. This would require an in vitro study of the SCN. The in vitro approach has proved of great value to demonstrate the capability of endogenous circadian oscillation in the eye of *Aplysia* and the avian pineal gland (45). Unfortunately, similar studies involving long-term (>24 h) recording in SCN explants have not yet been successful. Nevertheless, experiments in our laboratory have shown that single cells in explants of the rat SCN exhibit spontaneous electrical activity that can be recorded for periods of up to 14 h (12). In a large number of such in vitro SCN explants that were incubated at different times during the animals' circadian cycle, we observed that the spontaneous firing rate of SCN cells is significantly higher during the subjective day (corresponding to the light portion of the day) than during the subjective night (12a), as was found for in vivo SCN recordings (18, 19). These experiments are still remote from a demonstration that the SCN can sustain a circadian rhythm after isolation in vitro. Nevertheless, they do support the original interpretation of Inouye and Kawamura's finding, i.e., the electrical activity rhythm of the SCN is most probably intrinsic and does not result from a temperature or humoral rhythm generated elsewhere in the body (9, 31).

VISUAL PROPERTIES OF THE SCN

An essential function of the SCN is to establish a stable phase relationship between its intrinsic rhythm and the light-dark cycle. Consequently, for photic entrainment to occur, relevant information about the illumination cycle should ultimately reach the SCN. In mammals the entrainment pathways originate exclusively in the retina (46). The RHP is of major importance for entrainment of the mammalian circadian system, i.e., lesions of more central parts of the visual system do not result in loss of entrainment (25, 33). Also, it has been demonstrated recently that the entrainment of the electrical activity rhythm of the SCN requires only that their direct retinal input be intact (19). This does not imply, however, that the RHP is the only pathway mediating the effects of light on circadian rhythms. There is evidence that the ventral lateral geniculate nucleus (vLGN), itself a terminal nucleus for retinal fibers, projects to the SCN with avian pancreatic polypeptide-containing fibers (5, 11, 16, 33, 43). Thus a second, multisynaptic visual pathway to the SCN may exist and there are several indications that this indirect projection plays a role in the photic control of circadian rhythms in hamsters (33).

A considerable proportion of SCN cells respond to visual stimulation of the retina (11, 13, 14, 19, 27, 37). Figure 1 presents examples of typical light responses of SCN cells. The large majority of light-responsive SCN cells change their firing rate tonically when the ambient luminance level is changed. They do so in either of two ways. Most visual cells increase their discharge when the luminance is increased to a higher level, whereas a smaller proportion responds in the opposite way. Accordingly we have classified these SCN cells as light activated or light suppressed (11, 13, 14). For both classes of neurons the relation between their mean discharge rate and the ambient luminance is monotonic (11). The activated cell type increases its discharge from that in the dark steadily until it saturates at photopic luminance levels ($>10^3$ cd/m^2). The suppressed cells decrease their discharge until, in many cases, at higher luminance levels their firing ceases altogether. Typically, these responses to light are apparent only at relatively high light intensities ($>10^{-1}$ cd/m^2). This high threshold indicates that, under natural conditions, the SCN are primarily sensitive to the large ambient luminance changes that occur between day and night whereas they are relatively unaffected by different intensities of moonlight. The functional specialization of light-responsive

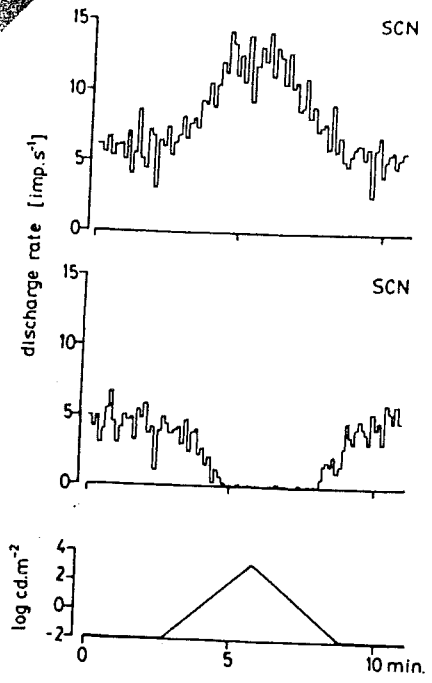


Figure 1. Typical discharge patterns of two neurones in the rat SCN in response to an exponential increase and decrease in overall ambient luminance (in log cd/m²). The activated cell type illustrated in the upper record increases its discharge rate as a monotonic function of luminance. The lower record shows an example of the suppressed cell type, which behaves in the opposite manner. Below the two records the time course of the luminance change is shown.

SCN cells toward coding luminance is further illustrated by the extremely large receptive fields of SCN neuron (Fig. 2) that frequently exceed 40° of arc in diameter (14). The receptive field boundaries are often ill-defined and within the field there is no indication of an antagonistic center-surround organization. In summary, the receptive fields of cat SCN cells are homogenous, large, and well suited for spatial integration of photic energy from large areas of the retina. We have recently confirmed these findings for the rat.

In the vLGN a sizeable subpopulation of cells is found with properties identical to those of the light-activated and light-suppressed SCN cells (11, 15, 32, 40). At least a portion of these vLGN cells terminate in the SCN. Electrical stimulation of their terminals in the SCN in rats results in antidromic activation of their cell bodies in the vLGN (11). Thus it is possible that two separate but functionally equivalent visual pathways project to the SCN. Nevertheless, the integrity of the retinogeniculosuprachiasmatic pathway is not required for the entrainment of circadian rhythms. The RHP alone is sufficient to mediate this function (33). This observation is consistent with the finding that SCN cells respond normally to light after bilateral lesions of the vLGN (11, 13, 37), whereas phase resetting of the SCN rhythm by light pulses is possible with the RHP as the only intact input to the SCN (19).

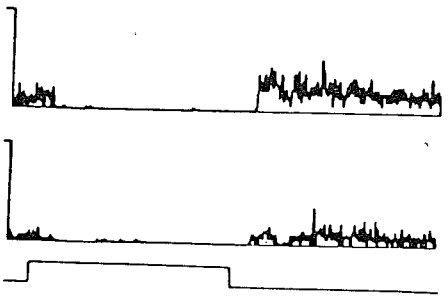
PHARMACOLOGY OF THE SCN

Synaptic interactions between SCN cells as well as between afferent nerve endings and SCN cells are probably important for the generation and entrainment of circadian rhythms. Therefore, neuropharmacological studies of the SCN and their afferents will contribute to the understanding of the central regulation of circadian rhythms. The SCN have high concentrations of various peptides, both in perikarya and in their cell processes, but also in afferent terminals (25a). The ventral portion of the SCN receives a serotonergic input from the midbrain raphe nuclei (29, 41). In contrast there is little evidence that catecholamines are present within the boundaries of the SCN. Catecholaminergic terminals, however, are found in the neuropil surrounding the SCN, which may explain why some SCN cells respond to microionophoretic application of dopamine and norepinephrine (25, 26). Acetylcholine may be one of the SCN transmitters. The SCN have detectable levels of choline acetyltransferase whereas binding sites for α-bungarotoxin have been demonstrated in the SCN (2).

For most of these substances it has not been established if and how they are involved in circadian timekeeping. However, some information is available on the functional importance of the acetylcholine, vasopressin, and serotonin (5-HT) systems. Carbachol, a cholinergic agonist, acutely suppresses the high nocturnal level of pineal serotonin-*N*-acetyltransferase (SNAT) activity when administered intraventricularly or in the vicinity of the SCN of blinded rats (48, 49). This effect can be blocked by nicotinic but not by muscarinic antagonists (49). In intact animals a suppression of SNAT can be induced by nocturnal exposure to light, a response that involves the RHP, the SCN, and the sympathetic innervation of the pineal gland (27, 35, 48). Thus at the level of the SCN, a nicotinic cholinergic mechanism is implicated in the transmission of photic information to the pineal. Similar injections of carbachol are capable of phase shifting the free-running pineal SNAT activity rhythm in rats and the locomotor activity rhythms of mice (48, 50). Interestingly, delay shifts are observed after injections in the early subjective night, whereas advance shifts occur after late subjective night injections. The phase-shifting effects of carbachol therefore qualitatively parallel the phase response curve for light pulses (7, 50). These studies demonstrate that photic entrainment as well as the immediate effects of light on pineal function may involve acetylcholine and its nicotinic cholinergic receptors in the SCN. If so, it is still unclear whether acetylcholine is a transmitter between RHP terminals and SCN cells or between intrinsic SCN cells involved in the visual functions of these nuclei. It seems unlikely that acetylcholine plays an important role in the mechanism of rhythm generation. Local injection of α-bungarotoxin, a potent and irreversible cholinergic antagonist, does not prevent the circadian rise in pineal SNAT (49).

The Brattleboro rat has been used as an experimental model to study the role of the vasopressin SCN cells in circadian timekeeping (10, 30). This animal is genetically deficient for vasopressin in the SCN as well as in the hypothalamopituitary system. From these studies it is clear that vasopressin is not necessary for the generation or for the entrainment of the drinking

Figure 2. Poststimulus time histograms computed for a light-suppressed SCN cell in the cat. The cell had a large receptive field with partly undetermined boundaries. Stimulation of the central portion of the receptive field with a flashing spot of 3° produces the response illustrated in the upper histogram; the response to a 9° spot is shown in the lower histogram. It can be seen from these responses that an antagonistic surround mechanisms are absent. The time base of the records is 840 ms; the ordinate calibration indicates 50 impulses/bin. From ref 14.



and locomotor activity of rats (10, 30). Vasopressin administration does not alter the period or phase of the free-running activity of the Brattleboro rat (30). The estrous cycle, which is critically dependent on a circadian timing system in the SCN (35), is apparently normal in vasopressin-deficient animals (30). The only abnormality observed in these rats is their comparatively long free-running period. It remains to be established whether this results from an altered photic responsiveness or from an actual difference in the rhythm-generating mechanisms of the SCN.

Of the identified and putative transmitters in the SCN, the serotonergic system has been studied most extensively; therefore we shall discuss the role of 5-HT separately.

SEROTONERGIC INNERVATION OF THE SCN

It is well established that the SCN have a high 5-HT content. The 5-HT in the SCN is contained in terminals, the perikarya of which are located in the midbrain raphe complex (29, 41). 5-HT, tryptophan hydroxylase, and monoamine oxidase are all present in the SCN (36, 47). The 5-HT terminals in the SCN are capable of 5-HT uptake. Interestingly, this process shows a circadian rhythm (24) as does the concentration of 5-hydroxyindole acetic acid in these nuclei (8). These biochemical observations indicate that 5-HT may act as a transmitter between raphe and SCN cells. Microionophoretic studies confirm this conclusion (4, 26). In our experiments microionophoretically applied 5-HT elicited a dose-dependent inhibition of discharge in 71% of the cells in the rat SCN. As in the case for the cat (4), focal stimulation of the raphe resulted in suppression of spontaneous or glutamate-induced discharge of SCN cells responsive to 5-HT (Fig. 3). Ionophoresis of 5-HT with simultaneous application of imipramine resulted in an enhanced and prolonged inhibition (Fig. 3). Application of imipramine alone was characterized by an inhibitory response in the majority of SCN cells. Imipramine ejection in the SCN also augmented the inhibition caused by raphe stimulation. These effects of imipramine are thought to reflect blocking of presynaptic 5-HT uptake by imipramine in the SCN (21, 47) which would result in increased levels of 5-HT. Clorgyline, a monoamine oxidase inhibitor (1), produced a prolonged and dose-dependent suppression of discharge in a large proportion (90%) of SCN neurons, when applied by ionophoresis. These and previous studies (4, 26) demonstrate that SCN neurons are responsive to 5-HT and its release via activation of the raphe projection to the SCN.

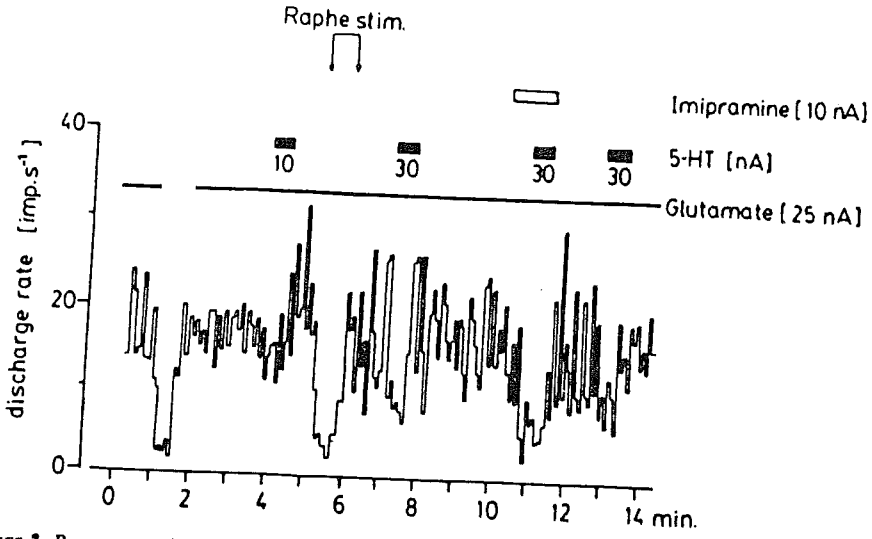


Figure 3. Responses of a nonvisual SCN cell in the rat to microionophoretically applied 5-HT and imipramine and to electrical stimulation of the dorsal raphe nucleus. Inasmuch as the spontaneous discharge rate of the SCN cells is generally low in some of our experiments, an increased firing rate was achieved by continuous ionophoresis of glutamate. The effect of discontinuing the glutamate ejection in the discharge is shown early in the record. By this procedure it could be demonstrated that ionophoresis of 5-HT inhibits this cell. The inhibitory response wad enhanced by ionophoresis of imipramine before and during 5-HT ejection. Electrical stimulation through a carbon fiber microelectrode positioned in the dorsal raphe nucleus resulted in suppression of firing of this SCN cell.

In spite of the demonstrated transmitter function of 5-HT in the SCN, raphe lesion or local injection of 5,7-dihydroxytryptamine in the SCN has remarkably little effect on either entrained or free-running rhythms (3, 17, 20, 35, 44). Therefore it must be concluded that the serotonergic innervation of the SCN is not essential for these functions. This is further demonstrated by the observation that the SCN sustain entrained and free-running rhythms after transection of their raphe afferents (18, 19). Inhibition of 5-HT synthesis by systemic administration of *p*-chlorophenylalanine or by feeding animals on a tryptophan-free diet results in circadian arrhythmicity during the time that brain 5-HT levels are significantly reduced (17, 20, 22, 44). In view of the raphe lesion experiments it seems most likely that this finding is dependent on suppression of serotonergic mechanisms involved in the expression rather than the generation of circadian rhythms. Recently it was demonstrated that chronic systematic administration of clorgyline delays the activity rhythms of hamsters with respect to the light-dark cycle (45a). This delay could result from a change in the phase response curve or from a lengthening of the pacemaker's period. In either case the SCN would be implicated as a site of action of clorgyline. Preliminary experiments indicate that this might indeed be the case. Local implantation of a clorgyline-containing cannula near the SCN lengthens the period of the free-running food intake rhythm of blinded rats. This effect is not observed when blank cannulas are placed near the SCN or when clorgyline cannulas are implanted 1 mm away from the SCN (47). Thus, inasmuch as clorgyline will increase the level of locally available 5-HT in the SCN, it may be that increased 5-HT concentration in the SCN can modulate the free-running period of the pacemaker. One should be very cautious, however, in drawing this conclusion. In particular, there are many questions about the local and pharmacological specificity of the clorgyline effect. Moreover, if increased 5-HT levels in the SCN can indeed modulate pacemaker frequency, it is surprising that 5-HT injection in the vicinity of the SCN is ineffective in phase shifting the pineal SNAT rhythm (48).

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