CHAPTER 16

The anatomical basis for the expression of circadian rhythms: the efferent projections of the suprachiasmatic nucleus

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Introduction

The suprachiasmatic nucleus (SCN) has a key role in the synchronization of a series of behavioral, metabolic and endocrine functions to the daily light-dark cycle (Rusak and Zucker, 1979; Moore, 1982). Each of these activities is synchronized in a distinct temporal pattern of which the peaks are often separated. The role of the SCN as a master synchronizing system in the brain of mammals has been demonstrated in a large number of successive studies, of which the one by Moore and Eichler (1972) was the first to point to the SCN and the one by Ralph et al. (1990) finally completed the evidence for the SCN as a daily synchronizing system. The aim of the present review is to identify the target structures of the SCN in the central nervous system (CNS) where the biological clock may synchronize such a wide variety of activities ranging from temperature regulation and sexual behavior to corticosterone secretion.

Organization of the SCN and its output

The SCN is situated on both sides of the third ventricle at the base of the brain on top of the optic chiasm. Fibers from the optic nerve provide it with the input that serves to synchronize the activity of the SCN with the light-dark cycle (Johnson et al., 1988; Morin, 1994). Under constant dark conditions a free-running rhythm develops. Without light input, food intake, melatonin and possibly the corticosterone peak serve to synchronize the activity of the SCN. The SCN expresses a daily rhythm in electrical activity of neurons (Inouye and Kawamura, 1979; Groos and Hendricks, 1982; Bos and Mirmiran, 1990; Gillette, 1996), synthesis of vasopressin (VP) (Robinson et al., 1988; Uhl and Reppert, 1986), deoxyglucose uptake (Schwarz and Gainer, 1977) and, release of VP (Schwarz et al., 1983; Earnest and Sladek, 1986; Gillette and Reppert, 1987). All these SCN activities take place both in vivo and in vitro (Gillette, 1996), illustrating that the SCN does not depend for these pacemaker functions on other input from the periphery, nor from other areas of the central nervous system.

Under normal physiological circumstances this circadian pattern of SCN activity is translated to the rest of the body and becomes visible in 24-h hormone temperature profiles and activity/sleep patterns. It is hypothesized that a circadian profile in transmitter secretion from SCN terminals is responsible for the 24-h rhythms in hormonal and behavioral patterns (Kalsbeek and Buijs, 1992). Up till now such a secretory pattern of SCN transmitters is only clearly proven for VP and is suspected for a number of other SCN
peptides (Inouye, this volume). The release pattern of these different SCN transmitters might have completely different phases, allowing the hypothesis that such different patterns might serve to control different parts of the activity or hormone profile (see Kalsbeek and Buijs, 1996). This hypothesis formed the basis of a series of studies whereby Kalsbeek provided evidence that a circadian rhythm in VP secretion indeed forms the basis of the circadian surge in corticosterone secretion (Kalsbeek et al., 1992). Consequently it is assumed that under normal physiological conditions the SCN utilizes its projections to the various target areas to enforce its rhythm onto these target structures. Since the discovery of the SCN as 'master clock' this stimulated several attempts to elucidate its neuronal projections. Uptake of tritiated amino acids (Swanson and Cowan, 1975; Berk and Finkelstein, 1981; Stephan et al., 1981), immunocytochemistry for SCN peptides in intact animals and in combination with SCN lesioning (Hoorneman and Buijs, 1982) were initially used to clarify its projections. Only recently, after it became possible to make small localized injections by means of the anterograde tracer phaseolus vulgaris leucoaglutinine (Pha-L), were these projections traced precisely (Watts et al., 1987; Buijs et al., 1993b; Vrang et al., 1995). The Pha-L tracing not only enabled the determination of the exact pathways and terminals areas, but also allowed the chemical identification of the target areas (Buijs et al., 1993b; Vrang et al., 1995). One of the major aims of these tracing studies was to find an anatomical basis for the observed rhythms in melatonin, corticosterone, temperature and activity, which all depend on the integrity of the SCN.

Taken together all animal tracing studies indicate that the observed SCN projections are mainly restricted to hypothalamic target areas with the exception of its projections to the paraventricular thalamus (PVT), and the lateral geniculate nucleus (Fig. 1). On the basis of these anatomical data alone, it is clear that these hypothalamic projections of the SCN will serve to synchronize homeostatic functions with the daily light/dark cycle. On the basis of what is already known of these hypothalamic targets with respect to projections, transmitter content and physiological experiments a further subdivision can be made with respect to specific SCN functions. We therefore attempted to indicate in Fig. 1 which functions might be influenced by the SCN in these different areas. It seems evident that the SCN projections to the various sites in the medial hypothalamus will serve to modulate the release of hormones into a pattern which expresses a circadian rhythm. Most adenohypophysal controlling neuroendocrine neurons are situated in the medial hypothalamus. However, anatomical evidence that the SCN has a direct interaction with all neuroendocrine neurons in these areas is not available yet. Consequently, closer examination of the precise SCN projections in combination with target identification will have to provide the picture that reveals where exactly the SCN may influence these releasing factor containing neurons. In relation to the control of general (motor) activity it is not apparent yet what target area is involved. It is possible that this message is transmitted to all SCN target areas, and that all these target areas control to some extent motor activity for a particular kind of behavior. On the other hand, it is possible that one or more target areas, such as the PVT or LGN, serve to modulate motor activity more precisely (Berendse et al., 1988; Johnson et al., 1988, 1989). Consequently, it is of importance to also collect knowledge on the projections and functions of the target sites of the SCN, thus enabling conclusions on the processes that may be influenced by these target areas. In Figs. 2–4 an attempt has been made to indicate on the basis of literature data to which regions several target areas of the SCN may project to. Consequently this also provides an indication as to which functions may be modulated by the SCN in that particular target area.

In order to acquire more insight into the manner in which the SCN influences corticosterone secretion, considerable attention was paid in our anatomical and physiological studies to elucidate sites and action of SCN transmitters. Initially a
great deal of effort was directed to the question whether the observed anatomical network of SCN terminals in the dorso-medial hypothalamus can indeed provide an explanation for the diurnal peak in corticosterone.

Anatomical basis for diurnal corticosterone rhythm

Lesions studies indicated that the SCN has a profound inhibitory influence on corticosterone secretion, i.e. an SCN lesion results in highly elevated plasma corticosterone levels after a novel environment stimulus as compared with intact animals which only show a moderate increase in corticosterone levels irrespective of the circadian time at which they are subjected to a novel environment (Buijs et al., 1993a; Fig. 5). Since the corticotrophin releasing hormone (CRH) producing neurons in the parvo-cellular part of the PVN (PVNP) control for a large part the adrenocorticotropic releasing hormone (ACTH) release from the adenohypophysis, which in turn stimulates the adrenal cortex to secrete corticosterone (Dallman et al., 1987, 1992; Swanson et al., 1988), much attention has been directed to the question whether the SCN is able to influence these CRH neurons directly. Thus far, in spite of the observed circadian rise in CRH mRNA immediately preceding the corticosterone peak (Kwak et al., 1992), all anatomical studies have indicated the absence of any substantial direct projection to these neurons (Buijs et al., 1993b; Vrang et al., 1995). Instead, Watts et al. (1987) demonstrated a massive projection of the SCN to an area just ventral of the PVN, which they called the sub-PVN zone.
In their paper they suggested that this region may project into the PVN. Furthermore, using Pha-L tracing, Buijs et al. (1993) showed that not only the sub-PVN but also the DMH receives a substantial SCN input. Subsequently Roland and Sawchenko (1993) provided evidence that these peri-PVN areas contain GABAergic neurons projecting to parvocellular PVN neurons. This also holds for the DMH, which has an established direct connection with the PVNp; one of the peptide transmitters of this projection is galanin (Ter Horst and Luiten, 1986; Levin et al., 1987). Moreover, Kalsbeek et al. (1992) provided substantial evidence that the DMH is the site, or one of the sites, where VP of SCN origin serves to inhibit corticosterone and ACTH secretion. This corroborates with the observation that the SCN projects extensively to the DMH with VP fibers (Hoorneman and Buijs, 1982).

Because of the lack of direct contacts between SCN efferent and PVN neurons and in order to investigate which (other) putative sites in the hypothalamus might be influenced by the SCN and may change the HPA axis, we decided to label SCN efferents in combination with identification of neurons implicated in the stress response by fos immunocytochemistry (Buijs et al., 1993b). A 15-min restraint stress resulted in the presence of numerous fos-positive neurons in the medial hypothalamus. Apart from the CRH neurons in the parvocellular part of the PVN (PVNp), fos positive neurons were also present in the DMH, periventricular PVN (PVNpe), dorsal cap of the PVN (PVNdca) and rostral PVN (PVNr). All these areas with fos-positive neurons receive a dense input from the SCN except for the PVNr, where only a few Pha-L-labeled SCN efferents could be detected suggesting no or very limited output to CRH neurons. Similarly, when we employed a CRH-immunocytochemical staining to identify the PVN neurons involved in the control of the ACTH cells of the hypophysis, we were
also unable to demonstrate extensive interaction between SCN efferents and CRH neurons. This observation was corroborated by Vrang et al. (1995), who used the same procedure combined with CRH immunocytochemistry. In view of (1) the elaborate SCN terminals in the DMH region on 'after stress fos-positive' neurons and the extensive projections of the DMH to the parvo cellular PVN (Ter Horst and Luiten, 1986; Levin et al., 1987), (2) the physiological studies pointing to the DMH as an important target for the SCN to control corticosterone secretion (see Kalsbeek et al., 1996b), and (3) the 'after stress fos-positive' neurons that receive SCN input are located around the PVN in areas that project into the PVN; all anatomical and physiological evidence so far points to an important indirect action of the SCN onto CRH neurons. The disadvantage of the anatomical approach thus far is that it does not allow the determination of contacts between SCN fibers and dendrites of PVN neurons. That this might be a serious disadvantage is illustrated by Hermes et al. (1996), who employed an in vitro slice preparation. Electrical stimulation of the SCN resulting in monosynaptic responses in most PVN neurons projecting to the median eminence, which suggests that direct contacts between SCN and CRH neurons do exist. In combination with the anatomical data, this finding probably indicates that the SCN does not project directly to the cell bodies of CRH neurons, but reaches their dendrites. The dendritic field of PVNp neurons as revealed by the intracellular injection of biocytin (Hermes et al., 1996) extends at least into
the PVNpe, which allows contacts between CRH neurons and the SCN to take place outside the main body of the PVN. Consequently, the proof for the existence of a direct interaction of SCN and putative PVN-CRH neurons as provided by the experiments of Hermes and Renaud (1993; Hermes et al., 1996), both in vivo and in vitro, in combination with the anatomical data suggests that both direct SCN-CRH and indirect SCN-CRH interaction exists, whereby the direct interaction mainly takes place on the dendrites of CRH neurons and the indirect interaction via neurons located in the DMH, PVNpe and PVNr (Fig. 6).

Taken together, these findings illustrate an elaborate mechanism by which the SCN is able to control the HPA axis. Hereby it should be taken into consideration that the attention cannot only be focused on the CRH neurons controlling ACTH secretion from the pituitary. In addition to the control of the HPA axis, a series of studies indicated that the SCN is also able to directly influence the sensitivity of the adrenal to ACTH. Already more than 15 years ago Kaneko et al. (1980, 1981) demonstrated that the adrenal responds to the same level of ACTH in the blood with more corticosterone secretion in the p.m. than in the a.m. Recently, Jasper and Engeland (1994) demonstrated that corticosterone secretion from the adrenal, as measured by direct microdialysis, showed low levels during the daytime in intact animals. In another group of animals the splanchnic nerve, which provides the adrenal with sympathetic innervation, was cut. Microdialysis of the adrenal in splanchnic nerve lesioned animals resulted in more elevated levels during the day.
period, suggesting that an inhibitory neural input had been removed (Jasper and Engeland, 1994). In view of the results of Kalsbeek et al. (1992) and Kalsbeek and Buijs (1996), who demonstrated that the inhibition of corticosterone secretion is largest during the light period and is provided by the SCN, we propose that this direct neural inhibition of the adrenal cortex originates from the SCN. Moreover, our recent experiments, conducted in collaboration with Dr. G.J. Ter Horst, demonstrated labelled neurons in the SCN using transneuronal tracing after virus injection into the adrenal. This finding supports the existence of a direct (multisynaptic) interaction between the biological clock and the adrenal (Telemariam-Mesbah et al., in prep.). Consequently the SCN has at least three different means to modulate the output of the adrenal cortex: (1) a direct innervation of the CRH neurons, probably to the CRH dendrite, (2) an indirect innervation of the CRH neurons via intermediate neurons located in the DMH, PVNpe, PVNr and possibly the MPO, and (3) via a neural control of the adrenal cortex by a direct or indirect input on neurons located in the PVN projecting to spinal cord preganglionic neurons (SPN) (Fig. 6).

Modulation and unity of functions

It will be clear that although the SCN may influence corticosterone secretion by (anatomically) a number of different pathways, its influence on
corticosterone secretion will be to set a certain baseline during the 24-h period. This type of modulation of corticosterone secretion is only logical for a biological clock. It ‘only’ serves to control at the right moment the right setting of this ‘stress system’ to prevent the HPA response from becoming inappropriate for a particular time of the day. From the above paragraph it can be concluded that this modulation is largely inhibitory, which seems to be the logical approach for a system setting baseline levels. This may hold not only for corticosterone secretion but also for the control of activity. For example, SCN-lesioned animals do not show the inhibition of activity that is normally seen during the light period, which suggests that an inhibition on activity has been removed. Intact animals may, in spite of this SCN inhibition, respond to a very mild stress as a novel environment with increased activity or elevated plasma corticosterone levels at any moment of the circadian cycle (Buijs et al., 1993a). This indicates that the SCN inhibition on corticosterone and activity is not absolute and only modulatory and can be overruled at any moment of the circadian cycle. Interestingly this observation agrees quite well with the anatomical data, i.e. no direct contacts with output neurons at the level of the cell body (e.g. the CRH neurons). The input at the level of the dendrite as suggested by electrophysiology (Hermes et al., 1996) allows a lot of space for a hierarchically more powerful input on the cell body. This could be the brainstem-originating ascending stress input from the noradrenergic cell bodies in the A1 and A2 region (Petrov et al., 1993). Several studies have indicated that noradrenergic terminals do form synaptic contacts with CRH cell bodies illustrating the anatomical basis for the hierarchy of stress response overruling the input of the biological clock. Another function of the direct SCN input to PVN neurons may be to prime these neurons for their expected diurnal activity. The existence of such an activating mechanism without concomitant hormone secretion might be concluded from the results of Kwak et al. (1992), who observed an increase in CRH mRNA preceding the circadian corticosterone surge.

This system of direct and indirect control of neurosecretory neurons may also hold for other neuroendocrine systems, of which the hormone secretion is synchronized by the SCN. Currently we are investigating whether the SCN directly innervates somatostatin, dopamine or TRH neurons in the hypothalamus, in order to provide, at least partially, the anatomical basis for hormonal rhythms in growth hormone, prolactin and thyroid stimulating hormone. For example, the studies of Van der Beek et al. (1993) on the SCN control of the gonadotrophin releasing hormone (GnRH) system illustrate that the GnRH system also receives a small direct input from the SCN as a VIP-containing innervation. The main input of the SCN into the GnRH system most probably employs the medial preoptic nucleus as a system in between the SCN and the GnRH neurons (see Van der Beek, 1996). Such a dual (or triple) control system may be a general rule for the way in which the SCN expresses its function and is possibly in place for the regulation of other hormonal rhythms as well. The advantage of engaging mainly intermediate neurons instead of output neurons is that the message of the SCN is not ‘lost’ in a simple output hormone but is multiplied instead by the intermediate neurons that also project to other structures. Consequently, special emphasis should be put on describing the output and putative functions of these ‘intermediate’ target areas of the SCN. For example, the projections of the DMH to the VMH and caudal brain regions make it likely that the SCN message to this area not only serves to drive the CRH neurons, but may also influence, respectively, food intake and autonomic functions by activating these other projections (Fig. 2). Likewise, the MPN, which may serve as ‘intermediate’ area between the SCN and GnRH system, may serve to express the influence of the SCN on sexual activity and temperature regulation (Fig. 3).

Provided that one SCN neuron has projections
to different target areas, for which no anatomical evidence exists so far but which seems quite logical with regard to the small number of SCN neurons and the large density of SCN terminals, it seems quite logical that an SCN neuron receiving a particular input and possibly containing (a) particular transmitter serves to enforce and set the right conditions for certain sets of functions that are expressed at the right moment of the circadian cycle. For example, already in 1983 Södersten et al. demonstrated that VP, probably of SCN origin, inhibits sexual behavior, whereas Kalsbeek showed that VP, of SCN origin, inhibits corticosterone secretion (see chapter Kalsbeek and Buijs). These observations concur completely with the observed secretion of VP from the SCN during the beginning of the light period. Consequently extrapolation of these data may suggest that other functions of VP of SCN origin may be inhibition of general activity (e.g. in the thalamus), sexual behavior (e.g. in the MPN), and food intake (e.g. in the DMH). In other words, there should be at least some unity in the function of the same SCN transmitter released in different target areas of the SCN, i.e. its action in one target area should not counteract its action in another target area. Then it will depend on the homeostatic demands of the animal and its environment which behavior will be chosen. One of the challenges in the future period will be to unravel the functions of the different SCN neurotransmitters and thus to clarify the mechanisms employed by the SCN to 'enforce' its rhythm onto the central nervous system.

Transmitters of the SCN

SCN neurons may be identified in three different ways: (1) classification depending on their input, e.g. one can define the target neurons of the retina or raphe nucleus, etc. (see chapter by Moore et al.); (2) classification depending on their membrane properties; and (3) classification depending on their transmitter content. The output targets of these neurons may also determine a certain class, but at present it seems that there is hardly any specialization in target structures (Buijs et al., 1993b), except that somatostatin neurons do not seem to project outside the SCN (Buijs, unpublished observations).

The input of the SCN is dealt with in the chapter of Moore et al. and this description shows that the input from different areas of the central nervous system reaches separate targets in the SCN. For example, it is assumed that the retinal input reaches mainly VIP neurons (Shibata and Moore, 1993; Shibata et al., 1994). However, recently Romijn et al. (1996) demonstrated that fos induced after a light stimulus at CT14 or CT19 appears in at least three different sets of SCN neurons containing GRP, PHI or VIP. Thus, these three peptides should play an essential role in the transmission of the light signal within and outside the SCN. This example illustrates that with regard to possible functions of SCN neurons, knowledge of their transmitter content is essential.

So far several peptidergic transmitters have been identified in the SCN of which VP and VIP seem to be present in the SCN of every species. Recently both anatomical (Moore and Speh, 1993; Buijs et al., 1994) and functional evidence (Hermes et al., 1993; Kalsbeek et al., 1996a) for the presence of GABA in SCN cell bodies and projections was provided as well. In addition, Hermes recently demonstrated that glutamate may also serve as an SCN neurotransmitter (see chapter by Hermes). The presence of these amino acid neurotransmitters opens up new possibilities for the way the SCN expresses its circadian functions. Recently we demonstrated that GABA is co-localized in approximately 30% of the peptidergic terminals that we examined (Buijs et al., 1995). One of the main questions that needs to be resolved in this matter, is whether individual SCN neurons are able to select for the secretion of peptides or amino acids or for both. Peptide secretion is facilitated with bursts of activity and a higher spiking frequency than classical transmitters that are already released after a single discharge (Lundberg et al., 1981, 1986, 1994; Cropper et al., 1990). This observation suggests that during the light period, when SCN neurons have
the highest electrical activity (Groos and Hendriks, 1982; Gillette, 1996), more peptide is released than during the dark period. The high levels of VP that are released from the SCN during this period seem to support this idea, which would mean, however, that VP neurons may switch their message during the circadian cycle; from VP/GABAergic (during the light period) to mostly GABAergic (during the dark period). How this relates to the message delivered by these SCN neurons is unclear at present, but clearly will have to be investigated in the future. Cellular electrophysiological approaches may solve these questions (see chapters Van den Pol and Dudek, and Hermes et al., 1996). At present the available data indicate that in most structures VP is excitatory and GABA mainly inhibitory. Consequently, a neuron containing VP and GABA in its terminal might be able to change its impact on the target neuron completely depending on its firing frequency. For VIP the result might be the reverse. Since it has been demonstrated in the retina that VIP potentiates the action of GABA (Veruki and Yeh, 1994), it is possible that at the moment the SCN neurons release more VIP, the neuron becomes more inhibitory. This also indicates that it is of crucial importance to obtain a more precise wiring diagram, i.e. with which target neurons do the VP, VIP, GRP etc. SCN neurons connect? Information on their electrical properties and putative colocalization with GABA or glutamate is essential too. Consequently, it is clear that the organization of the circadian cycle in hormone secretion, cardiovascular regulation and behavior depends on a delicate balance of the secretion of peptidergic and amino acid neurotransmitters targeted to a particular set of neurons in the hypothalamus and adjoining areas.

References


sponses of identified rat hypothalamic paraventricular neurons to suprachiasmatic nucleus stimulation. Neuroscience, 56: 823–832.


