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CHAPTER 9

The integration of stress by the hypothalamus, amygdala and prefrontal cortex: balance between the autonomic nervous system and the neuroendocrine system

Ruud M. Buijs* and Corbert G. Van Eden

Netherlands Institute for Brain Research, Meibergdreef 33, 1105 AZ Amsterdam, The Netherlands

Introduction

The hypothalamus is the center in the central nervous system (CNS) that is mainly responsible for the organization of homeostasis. This statement can be made, since numerous studies have provided evidence that hypothalamic nuclei not only control basic functions such as food and water intake, but are also essential for the organization of reproduction, body temperature regulation and hormone secretion by the pituitary (see review Swanson, 1987). Carrying out these tasks not only requires that the appropriate behavior is regulated, but also that the adequate hormonal balance and the integration of the correct moment of the day in relation to the sleep wake cycle are taken care of. These three aspects work together with respect to whether the setting of one condition will influence the setting of another. The sensation of an empty stomach, for example, will influence hormonal balance in a way that is completely different from the way it will affect behavior, depending on the moment of the sleep/wake cycle at which this sensation is transmitted to the central nervous system (Iraki et al., 1997).

In this context stress can be seen as a potentially life-threatening situation where external and/or internal influences may seriously disrupt the homeostasis of the body. The hypothalamus thus plays a major role in the registration and integration of stressful information and in orchestrating an adequate response of the organism in order to cope with the stress. In this respect we would like to make a distinction between two types of stress, i.e. *homeostatic stress*, a situation that has already disturbed or will disturb the internal balance of the organism, like severe thirst, hunger, pain, etc. (Aguilera et al., 1993), or *emotional stress*, the perception of a threatening situation that may cause a disturbance in the homeostatic balance (for which memory systems that recognize the stress as such, such as fear, for instance, are essential) (La Bar et al., 1998; Le Doux, 1998). As we will see, the hypothalamus plays a crucial role in the organization of the final response of the animal to both types of stressors. Brain regions other than the hypothalamus, such as the prefrontal cortex and the amygdala, play a major role in mobilizing the emotional stress response (Diorio et al., 1993; Le Doux, 1998), while ascending information from the periphery to the hypothalamus plays a role in homeostatic stress response (Renaud et al., 1988).

In the present review the major brain regions implicated in these two stress systems will be discussed, but special attention will be paid to the role of the hypothalamus in integrating emotional stress information from 'higher' brain structures

*Corresponding author: Tel.: +31 20 5665500; Fax: +31 20 6961006

such as the amygdala, hippocampus, septum and prefrontal cortex. Because the stress response can be measured for a large part as a hormonal response orchestrated by the hypothalamus, recent experimental data will be discussed that shed new light on how hypothalamic nuclei play an essential role in sensitizing organs of the body to the hormones of the pituitary as well.

Sensing the environment

Homeostasis can only be maintained if the organism can adequately register its internal as well as its external environment; in the course of evolution various sensory organs have developed that transmit information about the body and information about the environment to the central nervous system. To us humans the surrounding world is limited to what our sensory organs tell us exists, and we often tend to forget that much more happens in and around us than we consciously realize. Our recently discovered (and still disputed, possibly due to its recent discovery) sixth sense, the vomeronasal organ (Keverne, 1999; Monti-Bloch et al., 1998), only emphasizes that we should not preclude the existence of a seventh sensory organ. For example, the recently discovered sensory structure in the trout brain (Walker et al., 1997) that enables the trout to orient itself using the earth's magnetic field does not necessarily indicate that humans, too, might have such organ, but to exclude such a seventh sense, or any other sense, for that matter, would be preposterous. In fact, most sensory information that reaches our brains does so without reaching our consciousness, a situation that is hardly surprising given the fact that most of this information deals with the homeostatic balance of our organs. More surprisingly, recent evidence shows that a great deal of the visual and acoustic information can affect our homeostatic balance. If we have been taught that a certain visual or acoustic stimulus signals danger, we will subconsciously react to it with an autonomic response (Damasio, 1998; Le Doux, 1998).

It will be clear that even the most primitive organisms use sensory organs – essential not only for pain perception but also for registering the internal environment – and it is therefore not surprising that a large part of the initial integration of sensory information takes place in brain regions that are evolutionary older, such as the spinal cord, brainstem and mid-brain structures that are connected with the hypothalamus as the center where sensory information of body and environment converges. In principle this sensory information (about pain or the internal state of the body) may lead to homeostatic stress if a severe disbalance is noted. This imbalance will be detected in all regions to which this information is sent, leading, initially, at the level of the spinal cord and brain stem, to a reflex response that adjusts, for example, blood pressure, by vasoconstriction or vasodilatation (Tjenalooi et al., 1997). Higher up, in hypothalamus or cortex, similar responses are initiated and will result in proper behavioral and hormonal measures (Hatton et al., 1997). Furthermore, other (visual or acoustic) peripheral information reaches cortical and subcortical structures (e.g. amygdala) and is coupled with memory and other associative processes that come from amygdala and prefrontal cortex (PFC). In coordination with these two regions, the hypothalamus decides what the autonomic output of the organism will be; we call this type of stress emotional stress.

The hypothalamus

The brains of fish, amphibians and reptiles are organized in such a way that relatively few structures besides the spinal cord, brainstem and hypothalamus are needed to drive essential behavior such as, for instance, food intake, reproduction and body homeostasis. However, food intake and especially behavior associated with reproduction can become quite complex and in mammals the functioning of the cerebral cortex is therefore also required. The increasing complexity of these behaviors and the increasing demands on the memory systems later in evolution, forced structures such as the amygdala and the PFC to evolve more extensively. As we have seen earlier, these brain regions allow the animal to take into account its (recent) past and warn it if its sensory systems activate its memory systems for earlier encountered harmful environments, and initiate a stress response. For this reason amygdala, hippocampus,

septum and PFC have massive connections to the hypothalamus (Swanson, 1987; Moga et al., 1990; Diorio, 1993; Dayas et al., 1999; Van Eden and Buijs, Chapter 4, this volume). One of the interesting aspects of the regulation of the behavioral and hormonal stress response by the 'higher' centers (amygdala; hippocampus; septum and prefrontal cortex) is that recent evidence suggests that these regions may both play either an activating or an inhibiting role in the stress response (Van de Kar et al., 1991; Herman et al., 1995; Lee and Davis, 1997; Van de Kar and Blair, 1999). A stressful learning situation results in sympathetic activation; there is compelling evidence that the PFC and the amygdala are essential for the induction and integration of this autonomic response. Damage of the PFC in humans results in a severe impairment of aversive or reward-reinforced learning (see Chapter 27 by Damasio; Andersen et al., 1999; Bechara et al., 1999). Apparently the PFC in particular detects potentially harmful situations and warns human beings when they encounter such a situation. In this chapter we will pay particular attention to the way in which the PFC and amygdala are involved in the induction of the stress response. In this connection we will discuss our own recent data as well as the data of others, all providing detailed information on the interaction of PFC and amygdala with hypothalamus and autonomic nervous system in relation to, especially, hormonal control. Here we will pay particular attention to the control of adrenal cortex hormones, since this can be considered a reliable means of measuring the stress level of an animal. As we will see, stress-induced corticosterone levels are influenced by many different factors, most of which are associated with one of the above-mentioned brain regions. Furthermore, new insights will be presented that show that the same neuronal structures that organize hormonal secretion also prepare the organs of the body for these oncoming hormones and in this way safeguard an efficient communication with the periphery.

Inevitable for the scope of this review is that considerable attention is paid to the paraventricular nucleus of the hypothalamus (PVN). Within its anatomical boundaries the PVN combines neuro endocrine neurons containing, e.g. corticotropin-releasing hormone (CRH) with 'autonomic' neurons connected to autonomic parasympathetic and sympathetic centers in brainstem and spinal cord (Swanson, 1987). The PVN is of fundamental importance for the control of ACTH secretion and hence for the adrenal cortex and corticosterone secretion (Dallman et al., 1992). How the PVN receives its information from the periphery and the brain and how it organizes an adequate response will be discussed; furthermore, the similarities in organization of the PVN, amygdala and prefrontal cortex with respect to their neuroendocrine and autonomic control will be discussed and related to their function in the response to stress.

The paraventricular nucleus of the hypothalamus

The PVN is centrally situated in the midline of the hypothalamus; the hypothalamus is surrounded by other brain regions that co-organize the stress response (septum, hippocampus, amygdala and suprachiasmatic nucleus (SCN)). The PVN consists of three main parts: (1) A magnocellular part (PVNm) containing vasopressin and oxytocin neurosecretory neurons. (2) A parvocellular part (PVNp) containing mainly anterior pituitary hormone-releasing hormones such as CRH and thyrotrophin-releasing hormone (TRH) and (3) A magnocellular part with neurons projecting to autonomic centers in the brain stem and spinal cord (PVNa) (Swanson, 1987; Renaud et al., 1988). This part extends into the zona incerta, which also contains many autonomic projecting neurons. Anatomical analysis of direct neuron–neuron contacts within the PVN indicates the possibility of a great deal of interaction between these different types of neurons by means of somatic or dendro-dendritic interaction (Van den Pol, 1982; Theodosis and McVicar, 1996) and hence a putative exchange of information between the respective neurons. However, whether this means interactions between different groups of neurons is not clear; in contrast, a differential control of these subdivisions is suggested, since they receive input from quite different sources. This even goes for the two types of magnocellular neurons in the PVNm that contain either vasopressin or oxytocin, since there is physiological evidence that stimuli that activate one

set of magnocellular neurons do not necessarily influence the other set. This has been documented quite extensively, for example by a selective activation of oxytocin neurons by stomach distention or by the activation of vasopressin neurons by a fall in blood pressure; both stimuli failed to elicit a response in the heterologous neurons (see, e.g. Renaud et. al., 1988; Renaud and Bourque, 1991). There are no similar electrophysiological data for the specific classes of parvocellular neurons, although the different daily secretion patterns of TSH, ACTH and GH suggest that the corresponding neurons in the PVN that produce the releasing factors are selectively activated. Tanimura and Watts (1998) demonstrated that PVN neurons have a different sensitivity for the feedback of corticosterone; no data are available for the 'autonomic' PVN neurons that suggest selective activation or inhibition. In order to evaluate which input is essential for the various outputs of the PVN, over the past decades numerous laboratories have invested tremendous efforts in unraveling the anatomical organization of the PVN afferents. Initially little attention was paid to deciphering this input in relation with the functional subdivisions of the PVN, but as the work progressed the need for this knowledge became self-evident. The anatomical data suggest that the majority of the direct input to PVN cell bodies arises from noradrenergic A1 and A 2 cell groups in the lower brainstem and from adrenergic neurons in the C1 area and serotonergic neurons in the dorsal raphe nucleus (for a detailed review on these direct afferents see Sawchenko and Swanson, 1981; Swanson, 1987).

All these ascending systems have a direct functional activating synaptic access to the cell body and therefore play a major role in the acute activation of the neurons in the PVN (Day and Renaud, 1984).

Most activational stimuli are very powerful stimulators of the secretion of one or more hormones of the PVN. In general these stimuli (nausea, dehydration, hypotension, pain, etc.) can be considered homeostatic stressors since they pose an acute threat to the homeostatic balance of the animal. Interestingly, it seems that the majority of the inputs from other (higher) brain regions, such as hippocampus, septum, amygdala and PFC, in the PVN that may represent the anatomical substrate for emotional stress seem to be organized around the PVN rather than aimed directly at the cell bodies of the PVN. As we will see, such an orientation of the (sub)cortical inputs allows more differentiation in the control of the PVN neurons.

Organization of the forebrain input to the paraventricular nucleus

Direct input to the neuroendocrine PVN

In this chapter we will pay attention not so much to the above-described ascending (activating) input to the PVN, which is mainly derived from brainstem and spinal cord centers receiving direct autonomic information, but rather to the (modulatory) descending input from other brain regions. Tracing and functional studies have indicated that, in addition to the ascending direct input, structures in the immediate vicinity of the PVN, too, have a direct input to this central hypothalamic nucleus (Roland and Sawchenko, 1993; Herman et al., 1994; Boudaba et al., 1995, 1996). We will mainly pay attention to the input in the parvocellular and autonomic neurons because this is the most relevant for the control of the emotional stress response.

Since the direct monosynaptic input to PVN neurons, and especially to the cell body, is physiologically the most relevant, we would first like to discuss the brain regions that, in addition to giving such input to brain stem autonomic disorders, also provide input to the PVN. The dorsomedial nucleus of the hypothalamus (DMH) was shown to have a massive input in the PVN, whereby especially the PVNp receives the strongest innervation (Levin et al., 1987; Roland and Sawchenko, 1993). Both GABA and galanin are neurotransmitters used in this pathway. Furthermore the bed nucleus of the stria terminalis (BNST) also projects to the parvocellular PVN, mainly utilizing GABA as inhibitory neurotransmitter (Herman et al., 1994). The medial preoptic nucleus; the anterior periventricular region; the ventromedial hypothalamus, the lateral hypothalamus and the arcuate nucleus all project to the medial parvocellular PVN and thus in principle to the CRH neurons. Many hypothalamic regions in the immediate vicinity of the PVN contain GABA as a

neurotransmitter (Boudaba et al., 1996), often in addition to a certain peptide. The circumventricular organs, such as the subfornical organ, project to the parvocellular and magnocellular PVN and have a special role in the control of circulation and directly innervate and activate (!) the hypothalamic CRH neurons involved in the control of the hypothalamo-pituitary-axis (HPA), although autonomic projecting PVN neurons are also activated (Bains and Ferguson, 1995). However, the main function of circumventricular organs is generally also thought to mobilize the PVNm in homeostatic, mainly circulation-related stress responses (Li and Ferguson, 1993). In short, the PVN is literally surrounded by nuclei that project directly into the PVN.

Interestingly, none of the forebrain regions endowed with a high concentration of glucocorticoid receptors (hippocampus, septum, prefrontal cortex) and known to be implicated in the inhibition of the HPA axis has direct extensive projections to the medial parvocellular part of the PVN, the location of the CRH neurons. Instead, ventral hippocampus, septum and prefrontal cortex seem to concentrate their projections to the hypothalamic brain regions in the immediate vicinity of the PVN, such as the DMH and the BNST, that contain such a high density of GABA-ergic interneurons. The high density of GABA-ergic inhibitory input into the PVN stems from the BNST and DMH and the presumed activation of BNST and DMH neurons by glucocorticoid receptor-containing neurons forms the firm anatomical basis for the inhibitory action of hippocampus, septum and PFC on the neuroendocrine PVN (Boudaba et al., 1996; Herman and Cullinan, 1997).

Interestingly, the pattern of projections from ventral hippocampus, amygdala, septum and PFC around the PVN closely resembles the pattern of projections of the suprachiasmatic nucleus (SCN), known to be responsible for the daily rhythm in ACTH and corticosterone secretion (Fig. 1). Since antrograde tracing has demonstrated that projections of this nucleus also largely avoid the body of the PVN, it was investigated on what basis our biological clock could influence the HPA axis; the observed interaction of the SCN with the PVN may illustrate how forebrain regions influence the HPA axis. Therefore we will pay particular attention to the anatomical, physiological and electrophysiological experiments that revealed the basis of the control of the HPA axis by the SCN; furthermore we will illustrate that this interaction may also hold for the way the ventral hippocampus, septum, amygdala and PFC may influence the HPA axis.

Forebrain control of the hypothalamo-pituitary axis

Light microscopical evidence shows SCN terminals in many midline regions of the hypothalamus, with the exception of the PVN, which seems to be largely avoided by SCN projections. Only close to the ventricle in the periventricular zone of the PVN and in the dorsal cap, the autonomic part of the PVN can SCN fibers be visualized, whereas in the so-called sub-PVN, an area just ventral from the PVN, dense termination is seen (Watts et al., 1987; Buijs et al., 1994). Other SCN terminations around the PVN are observed in the medial preoptic area (MPO), just rostral of the PVN, while just caudal of the PVN another dense termination zone is seen in the DMH (Buijs et al., 1993; 1994). As we have seen above, the SCN seems to share these three areas of termination with other forebrain regions that also influence the HPA axis, such as the ventral hippocampus (subiculum), the amygdala (the medial (Ame) and central part (Ace)), the lateral septum and the PFC (Figs. 1 and 2). The only exception is that all these other brain regions also have the BNST as important zone of termination, whereas the SCN only gives sparse input and only to the medial part of the BNST.

As discussed above, the regions directly surrounding the PVN provide a substantial (GABA-ergic) input to the neuroendocrine PVN; consequently the hypothalamic projections of the SCN, PFC and amygdala may influence hormone secretion without a major direct innervation of the neuroendocrine neurons.

Like the PFC and amygdala, the SCN plays a major modulatory role in the process of corticosterone secretion; in the case of the SCN, the factor time-of-day is the variable, and in the case of the PFC or amygdala the variable is the motivation or the emotional condition.

Initially projections of the SCN were revealed in combination with CRH staining, demonstrating that SCN projections largely failed to contact CRH cell bodies (Fig. 1).

In order to further identify where SCN projections may interact in their zone of termination with neurons involved in the regulation of the stress response, we subjected rats to stress and subsequently identified, by means of immunocytochemical staining, *c-fos* as marker for neuronal activation simultaneously with SCN projections. It was demonstrated that SCN terminals synaptically impinged on neurons showing *c-fos* in the DMH and MPO (Buijs et al., 1993). In view of the direct GABA-ergic inhibitory input to the parvocellular PVN from DMH and MPO, this observation illustrates the possible indirect pathways to the PVN via which the SCN affects the stress response. Apart from these neurons around the PVN, logically also the parvocellular neurons in the CRH containing part of the PVN show *c-fos* as an indication of activation; this observation indicates that almost simultaneous with the activation of the CRH neurons, the inhibitory inputs of DMH are activated as well.

Lesioning the SCN resulted in an enhanced secretion of corticosterone during the day and after stress, suggesting an inhibitory role for the SCN on the HPA axis (Fig. 3) (Buijs et al., 1997). In order to further investigate this SCN action on corticosterone secretion, a series of physiological experiments was undertaken whereby probes were

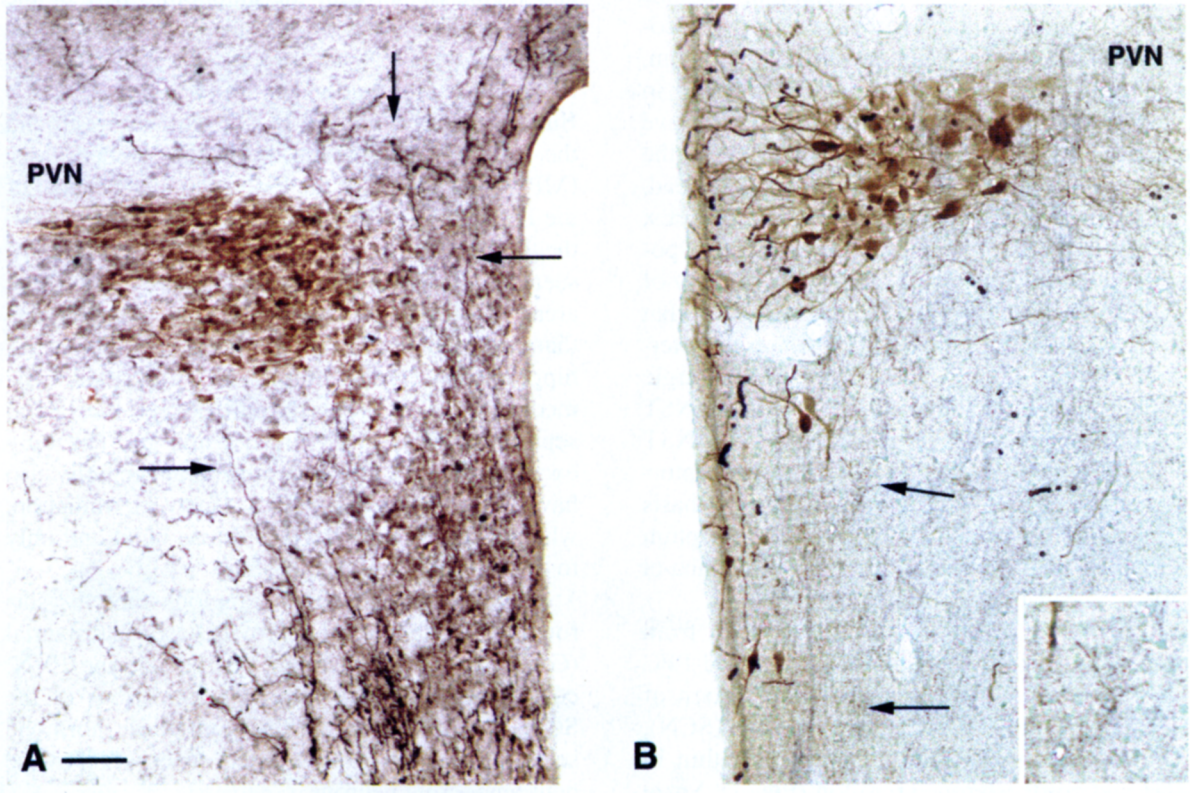

Fig. 1. A, B illustrates a transversal section of the hypothalamus in the region of the paraventricular nucleus (PVN). Bar = 100 μm. (A) illustrates fibers arising from the suprachiasmatic nucleus (SCN) that penetrate (arrows) the boundaries of the PVN in which cell bodies stained for corticotrophin-releasing factor are stained (dark brown). Branching and putative termination of SCN fibers are visible just ventral of the PVN close to the ventricle and in the periventricular and dorsal part of the PVN (two arrows at the top). (B) illustrates fibers arising from the ventral hippocampus that show very fine but dense termination in the area just ventral of the PVN (arrows). Inset is a higher magnification of the area indicated by the upper arrow. PVN is stained for oxytocin (dark brown) to show the outline of the PVN (rostral part).

placed in the vicinity of the DMH in SCN-intact and SCN-lesioned animals, through which SCN transmitters or antagonists were infused. The results showed that vasopressin as a transmitter of the SCN was able to inhibit ACTH and corticosterone secretion strongly when infused in the DMH. Furthermore, a vasopressin antagonist increased blood ACTH and corticosterone levels quite strongly, especially during periods of high endogenous VP secretion, which occurs during the light period (Kalsbeek et al., 1996a, b). Since plasma corticosterone levels are quite low during the early

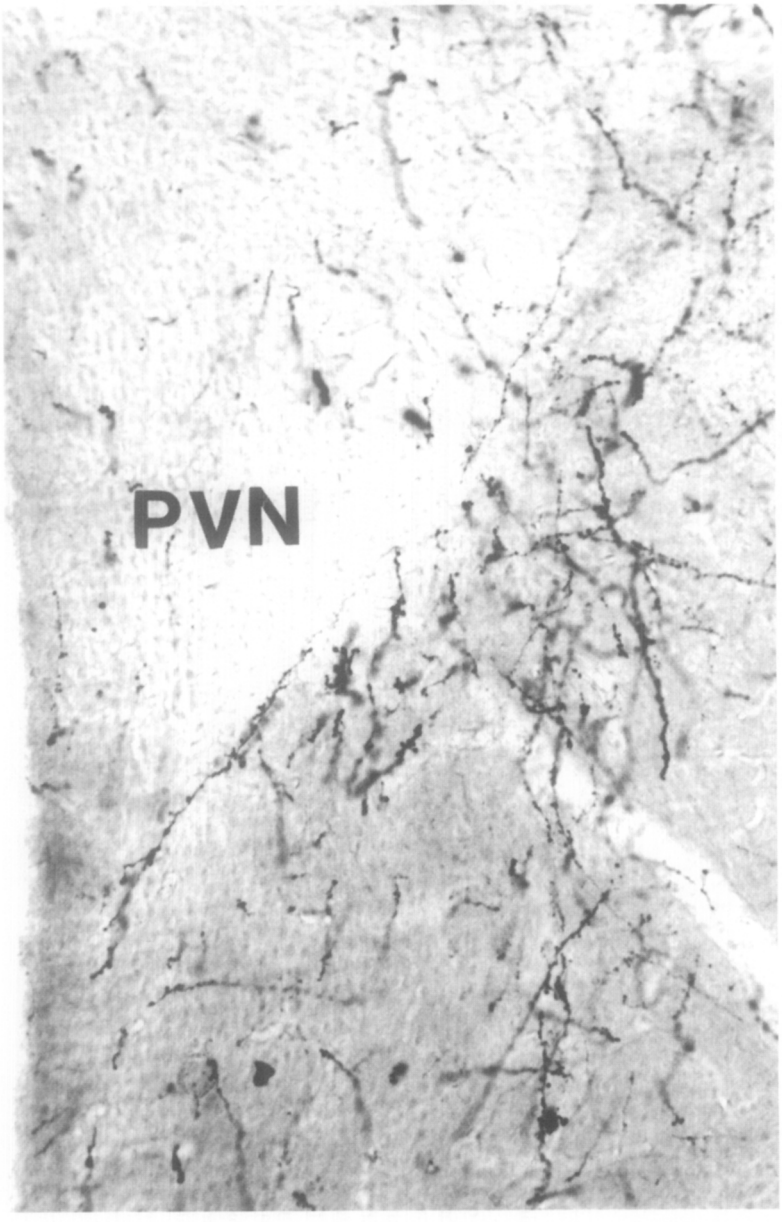

Fig. 2. illustrates a transversal section of the hypothalamus in the region of the paraventricular nucleus (PVN). Fibers arising from the prefrontal cortex show terminations in the area just ventral of the PVN where in Fig. 1 termination of suprachiasmatic nucleus and ventral hippocampus is present.

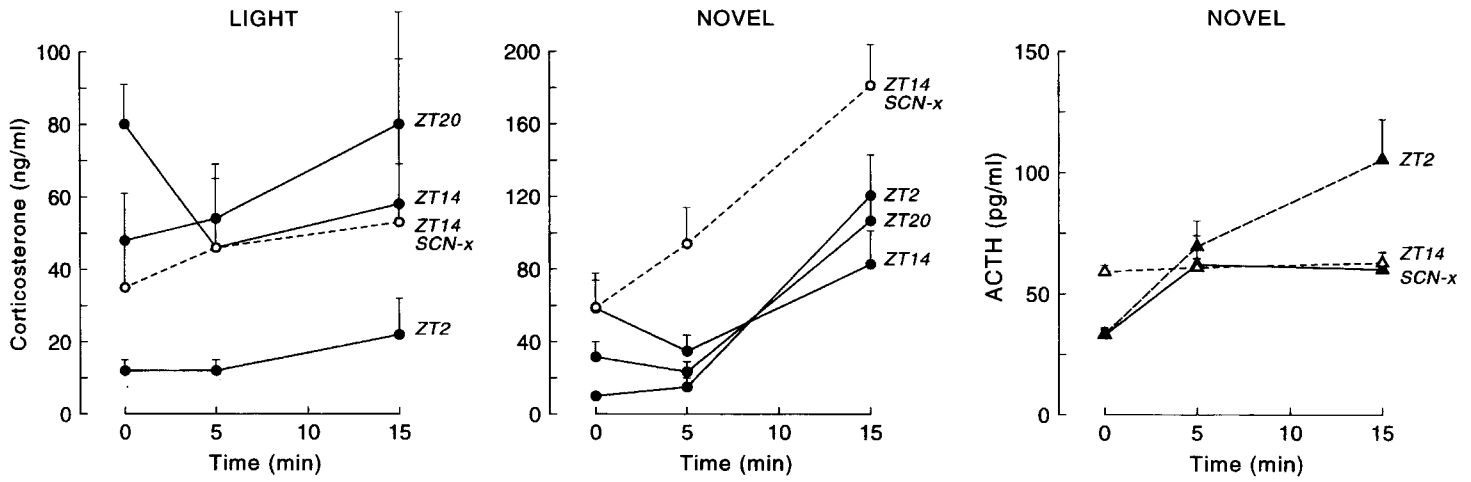

Fig. 3. illustrates the effect of plasma corticosterone and ACTH after subjecting animals to a new environment or to light. Both stimuli show a strong similarity in their response, especially the first five minutes, indicating the direct involvement of the SCN. Novel: Plasma corticosterone and ACTH values after intact rats had been placed in a new cage at three different circadian times. 2 h after light onset (ZT2, $n=8$), 2 h after dark (ZT14, $n=15$). Single-factor ANOVA revealed time-dependent significant effects for all curves $p<0.001$, except for ACTH release during a ZT14 new environment exposure $p>0.52$. Novel corticosterone: Student-Newman-Keuls indicated significant differences at $t=0$ between ZT2 and ZT14. The paired Student's t-test indicated a significant difference between $t=0$ and $t=5$ only at ZT14 when the new cage resulted in a highly significant decrease in plasma corticosterone values ($p<0.0025$). Novel plasma ACTH. The paired Student's t-test revealed no differences at ZT14: At ZT2 all time periods differed significantly from $t=0$, $p<0.0025$. The SCN-lesioned animal (SCN-x), in contrast to the intact animals, shows an immediate increase in plasma corticosterone without any decrease at $t=5$. Student-Newman-Keuls indicated that at $t=5$ SCN-x differed significantly from all other corticosterone curves at that time point. LIGHT: The effect of a 15-min light exposure at $t=0$ at three different Zeitgeber times, ZT2, ZT14 and ZT20. The first two panels show the effect of light on basal corticosterone values. ANOVA revealed significant time-dependent effects only at ZT14, and only in intact animals. $F_{28.2}=5.59$, $p=0.009$ with a significant decrease at 5 min, $p<0.001$, and no significant changes at the other Zeitgeber times. ZT14 SCN-X: $F_{16.2}=0.54$, $p=0.595$; ZT2: $F_{14.2}=1.23$, $p=0.322$; ZT20: $F_{10.2}=3.52$, $P=0.070$. The suprachiasmatic nucleus-lesioned animals (SCN-X) do not show a slight increase instead of a decrease. The rapid decrease in plasma corticosterone is not reflected in a change in plasma ACTH. The last panel shows unchanging ACTH values after light exposure, both in intact ($F_{12.2}=3.06$, $p=0.084$) and SCN-X ($F_{16.2}=0.39$, $p=0.686$) animals at ZT14.

moments of the day period this is taken as evidence that the task of vasopressin secreted from SCN terminals in the DMH area is to inhibit the HPA axis. It is interesting to note that the very same peptide, but this time secreted as a hormone from PVN terminals in the median eminence, functions as a corticotrophin liberating factor; apparently a new role for this peptide has developed somewhere during evolution and the neurotransmitter role for the biological clock might be another adaptation. This observation fits the fact that vasopressin is generally excitatory to postsynaptic neurons and to the GABA-ergic inhibitory projections from DMH to PVN. These observations provide physiological evidence for the idea that the DMH is an important relay station for central information to the neuroendocrine PVN. It is clear that by means of this projection to the DMH the SCN but also the PFC and amygdala are put in a position to modulate the inhibitory input to the neuroendocrine PVN (Fig. 4).

Next, electrophysiological studies were undertaken to understand more precisely the possible circuits involved; the SCN was stimulated and electrical activity recorded in the PVN. Intracellular and patch clamp recording revealed that stimulation of SCN neurons induced both monosynaptic and bi- or multisynaptic activation and inhibition of PVN neurons (Hermes et al., 1996). It was demonstrated by using specific antagonists that both GABA and glutamate serve as aminoacid transmitters of the SCN whereby PVN neurons are monosynaptically inhibited or activated; apparently this monosynaptic connection between SCN and PVN neurons could not be revealed by neuroanatomical techniques. Most probably therefore the site of contact is on the dendrites of the PVN neurons that extend into the periventricular or subPVN (Fig. 1).

Insight in the multisynaptic inhibitory connection was revealed when application of vasopressin to hypothalamic brain slices resulted in an inhibi-

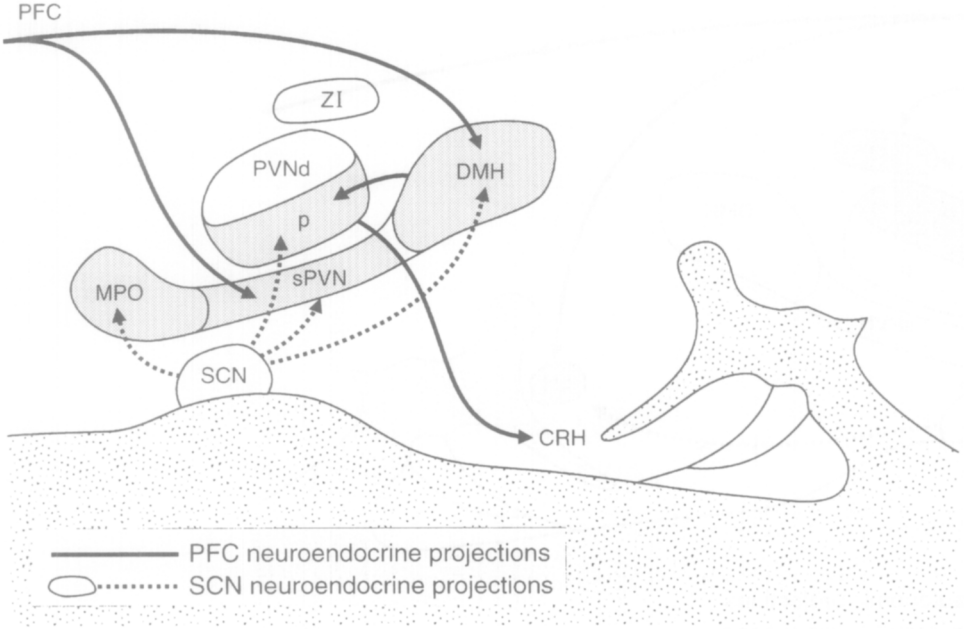

Fig. 4. Sagittal scheme of the hypothalamus illustrating the projections of the suprachiasmatic nucleus (SCN) and the prefrontal cortex (PFC) to neuroendocrine centers in the medial hypothalamus. The SCN reaches the medial preoptic nucleus (MPO), the subPVN (sPVN) and the dorsomedial nucleus of the hypothalamus (DMH). These three regions give rise to input to the neuroendocrine parvocellular (p) and magnocellular parts of the paraventricular nucleus (PVN). The PVNp gives rise to the corticotrophin-releasing hormone (CRH) output of the PVN to the median eminence. It is not clear whether the dorsal cap of the PVN (PVNd) or zona incerta (ZI), where the autonomic projecting neurons are located, also receives an input from the MPO, sPVN and DMH.

tion of a selected population of parvocellular PVN neurons and oxytocin PVN neurons that could be blocked by the GABA antagonist bicuculline. Since vasopressin also activates neurons in the sub-PVN area, which could be blocked by vasopressin antagonists, we take this as evidence that vasopressin inhibits PVN neurons via an indirect route by activating GABA-ergic interneurons in the immediate vicinity of the PVN (Hermes et al., 2000). Consequently a dual mode of controlling the functioning of the neuroendocrine PVN seems to be in effect: a direct as well as an indirect input to the PVN neuron (Figs. 4 and 5). Moreover, both the direct and the indirect input may exercise an inhibitory as well as a stimulatory influence. The paucity or absence of contacts on the cell body in the PVN seems to indicate that the direct inputs of the SCN have a merely modulatory character that can be overruled by information that reaches the neuron more closely to the cell body. As is clear from the results of the anterograde tracing from the amygdala and PFC, both brain regions have extensive projections to the DMH and subPVN, suggesting a similar arrangement as has just been described for the SCN (see chapter Van Eden and Buijs; Coolen and Wood, 1998; Prewitt and Herman, 1998) (Figs. 1, 2, 4 and 5). The consequence of this interaction seems clear; it gives each brain region limited but direct access to the neuroendocrine motor neurons of the PVN; at the same time, by means of access to the neurons around the PVN, the activity of main inhibitory centers that feed into the PVN is changed. The duration and magnitude of the change, however, will depend on the information from the other brain regions; consequently, emotional state, day/night conditions, and other circumstances are integrated in the areas around and within the PVN. Interestingly, lesioning the PFC results in a similar response as lesioning the SCN as far as corticosterone secretion is concerned, i.e. enhanced secretion after a stressful stimulus (see, e.g. Van Eden and Buijs, this

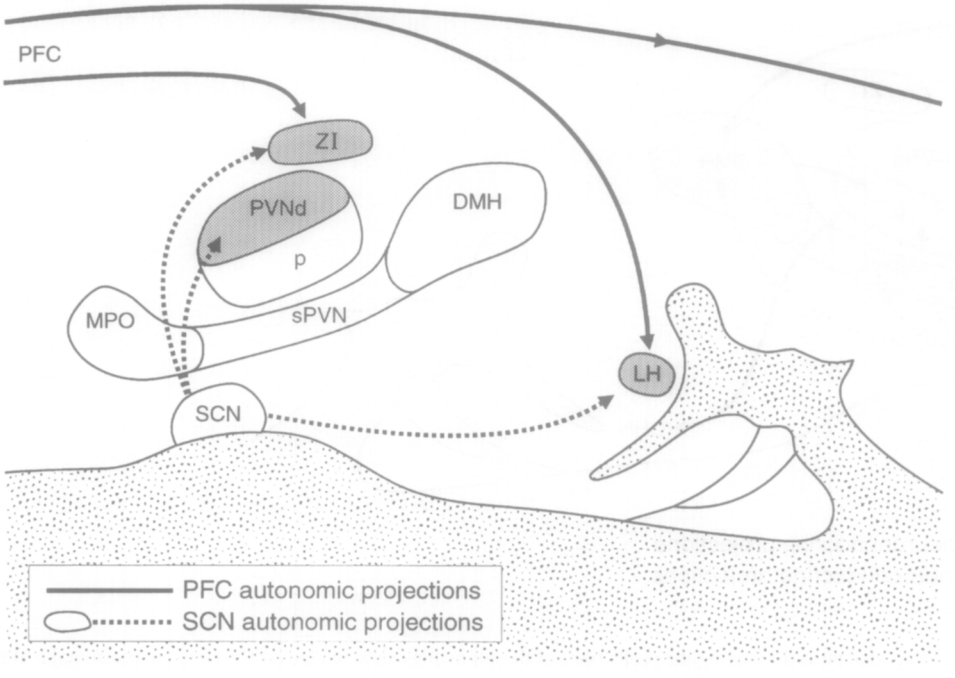

Fig. 5. Sagittal scheme of the hypothalamus illustrating the projections of the suprachiasmatic nucleus (SCN) and prefrontal cortex (PFC) to the autonomic centers in the hypothalamus. Direct SCN and PFC projections are established to neurons in the dorsal cap of the PVN (PVNd), zona incerta (ZI) and lateral hypothalamus (LH). These three regions have direct projections to sympathetic preganglionic neurons in the spinal cord.

volume). This observation supports our view that the PFC, too, by an excitatory input to GABA-ergic neurons in the DMH, reduces the corticosterone stress response (Figs. 4 and 5). Consequently, we would like to propose that emotional/stress information about the environment, for which PFC, septum, amygdala and hippocampus are essential, converges with circadian information in the same areas and possibly the same neurons around the PVN. In this way the anatomical basis is created that enables the coupling of the time-of-the-day message to emotional stress arising from other brain areas. Support for this hypothesis is provided by studies that showed animals responding to a new environment with a time-of-day-dependent increase in ACTH and corticosterone secretion (Buijs et al., 1997). Consequently, the response of the HPA axis to stress (in this case: a new environment) is highly dependent on the signal of the SCN; without the SCN a very enhanced signal in both ACTH and corticosterone is recorded, indicating that the SCN has an overall inhibitory influence on the HPA-axis.

Forebrain interaction with the autonomic nervous system

Recent anatomical observations concurred with the results from electrophysiology that indicated monosynaptic contacts between SCN terminals and PVN neurons. These anatomical data showed close appositions between fibers arising from the SCN and neurons in the PVN retrogradely filled from the spinal cord (Teclemariam-Mesbah et al., 1997; 1999; Buijs et al., 1999). This proved to be the first anatomical demonstration of the site where the SCN may transmit its daily information to the autonomic nervous system.

Since melatonin secretion from the pineal gland is strongly under the influence of the SCN and shows a very pronounced day/night rhythm, we determined, by means of transneuronal virus tracing, the pathway between the SCN and the pineal. These anatomical studies showed that, seen from the site of the biological clock, the PVN is indeed the first relay station, after which the neurons in the intermedio-lateral column (IML) in the spinal cord transmit SCN information onwards, to the superior cervical ganglion, and then to the pineal (Larsen et al., 1998; Teclemariam-Mesbah et al., 1999). These and other anatomical studies demonstrated the monosynaptic interaction between the SCN and the autonomic parts of the PVN and thus provided the anatomical basis for the interaction of the biological clock with the autonomic nervous system. An as yet not very well understood phenomenon is that, depending on the conditions, the adrenal responds to the same amount of ACTH with a liberation of different quantities of corticosterone (Dallman et al., 1987). For example, the adrenal has a circadian variation in its sensitivity to ACTH; just before the onset of the activity period, the adrenal cortex is the most sensitive to ACTH. Consequently, we suspected that the SCN might influence the adrenal gland also via the autonomic nervous system.

In order to demonstrate such a putative connection between the SCN and the adrenal cortex we investigated, by means of transneuronal tracing, the CNS neurons in command of the adrenal (Buijs et al., 1999); first order neurons were found in the IML. These neurons were shown to receive a synaptic input from PVN neurons; next, second order neurons were shown in the autonomic part of the PVN. Interestingly, these neurons were shown to receive an input from the SCN. Especially the PVN dendrites received a strong SCN input, which corroborated our earlier observations with pineal and spinal cord retrograde tracing (Teclemariam-Mesbah et al., 1997; 1999); furthermore, the third order neurons found in the SCN were, among other things, vasopressin- and VIP-containing neurons. Interestingly, in addition to autonomic PVN neurons, prefrontal cortex neurons are labeled after tracer injection in the adrenal as well; the time of appearance of these neurons is in between the moments of appearance in the hypothalamus of the 2nd and 3rd order neurons (Fig. 6). Because the distance to the spinal cord is further from the PFC than from the hypothalamus, it is likely that these neurons are also 2nd order (see chapter by Van Eden and Buijs, this volume). This puts the PFC in the same position as the PVN and some brain stem nuclei, namely that it is able to affect the sympathetic neurons controlling the adrenal directly.

Third order neurons in control of the adrenal appear in the MPO, BNST, central amygdala and

subfornical organ, which is an interesting observation because these areas are known to be involved in the control of the HPA axis as well. The virus tracing data consequently indicate that these structures also have the capacity to influence not only the neuroendocrine neurons of the PVN, but also the sympathetic autonomic neurons of the PVN or brain, and consequently also affect the neural control of the adrenal. Interestingly, in spite of its dense input to the PVN, the DMH contains just a few 3rd order neurons, suggesting that the DMH might largely interact with the neuroendocrine PVN and that its interaction with the autonomic PVN will thus be quite limited.

The hypothalamic projections from the medial amygdala and ventromedial PFC are aimed especially at the DMH and subPVN, and may consequently serve to control, indirectly, the neuroendocrine part of the PVN (Fig. 4). Interestingly, with respect to their projections, large parts of the dorsomedial PFC show a close resemblance to the projections of the autonomic part of the PVN and give input to (para)sympathetic in and output regions of the brain stem such as the NTS and nucleus ambiguus. Furthermore, similar to the PVN, the dorsomedial part of the PFC also provides input to the sympathetic motor neurons in the spinal cord, located in the IML (Fig. 6).

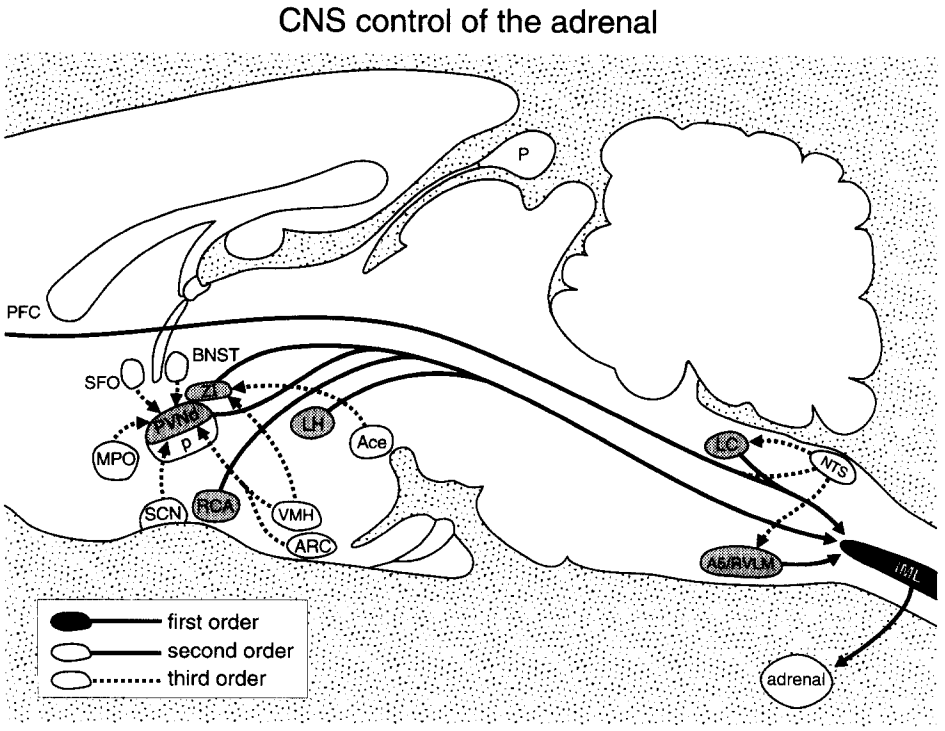

Fig. 6. Sagittal scheme of the rat brain illustrating the presence of first, second and third order neurons in control of the adrenal. After pseudorabies virus injection in the adrenal, first order neurons appear in the intermediolateral column of the spinal cord (IML) where the sympathetic preganglionic neurons are labelled. The next day second order neurons are labelled in the brain stem: the A5/rostroventrolateral medulla (A5/RVLM) and locus coeruleus (LC). In the hypothalamus: the lateral hypothalamus (LH), the retrochiasmatic area (RCA), the autonomic paraventricular nucleus (dorsal and ventral part) (PVNd) and the zona incerta (ZI). Only in the prefrontal cortex are second order neurons detected. The next day third order neurons are detected in the brain stem and nucleus tractus solitarius (NTS), hypothalamus, suprachiasmatic nucleus (SCN), arcuate nucleus (ARC), ventro- and dorsomedial hypothalamus (VMH), medial preoptic area (MPO), subfornical organ (SFO), bed nucleus of the stria terminalis (BNST) and central nucleus of the amygdala (Ace).

Moreover, the dorsomedial PFC not only projects to the 1st order sympathetic or parasympathetic motor neurons, but also to the zona incerta (ZI), where a large group of 2nd order autonomic neurons is located. This pattern of spatial separation of autonomic and neuroendocrine projections that can be discerned in the PFC, can also be observed in a similar way in the amygdala; the medial part of the amygdala contains the majority of the neuroendocrine projections and the central part the autonomic projections (unpubl. observations). This spatial separation of projections within the same anatomical structure does not necessarily mean that these functions are also functionally separated; intranuclear or intraregional interaction has already been documented extensively for the PFC and the amygdala. Of all the cortical and neocortical areas, the autonomic projections seem to be a specific property of the PFC and central amygdala; even third order neurons are not observed in other cortical or neocortical areas. The close anatomical correlation with SCN projections in and around the PVN warrants a comparison with the SCN with respect to the way the SCN affects physiology or homeostatic mechanisms.

As discussed above with respect to the role of the SCN in adrenal control, several physiological studies have demonstrated a daily variation in the sensitivity of the adrenal to ACTH. Since light selectively stimulates SCN neurons to become activated, and SCN neurons in general have a low electrical activity during the dark period, we investigated blood corticosterone levels after subjecting animals to nocturnal light exposure. Light immediately decreased blood corticosterone levels without affecting ACTH levels, but it did so only at the beginning of the dark period (see Fig. 3). As we could not see this effect in SCN-lesioned animals, we took this as evidence for a multisynaptic pathway between the SCN and the adrenal cortex (Buijs et al., 1997, 1999); furthermore we did not observe significant changes in blood adrenalin and noradrenalin, indicating that the physiological effect is indeed due to the neuronal effect on the adrenal cortex. Interestingly, in humans, who, contrary to rats, are day-active individuals, we observed the opposite effect of light: an increase in cortisol after light exposure only in the early morning (Scheer et al., 1999a). Consequently, the combined data show that the SCN is not only capable of orchestrating the secretion of ACTH in a timely manner, but also of setting the sensitivity of the adrenal for the oncoming ACTH via its direct and indirect projections to the PVN the SCN!

Since we also observed immediate effects of light on the heart rate of healthy human subjects and opposite changes on heart rate in rats (Scheer et al., 1999b), we hypothesize that this SCN/ PVN pathway transmits the circadian activity/rest setting to all organs of the body. Since virus tracing from many different organs produces, at least via the sympathetic branch, more or less the same pattern in the hypothalamus, PFC and amygdala, we are confident that the PVN, SCN, PFC and amygdala influence not only the adrenal cortex but also many other organs in the body.

We would like to propose that the interaction of the SCN with the autonomic nervous system and the neuroendocrine system that we demonstrated, not only goes for the SCN, but also for the PFC and the amygdala. These two brain regions also seem to have access both to the neuroendocrine and to the autonomic system and it is suggested that they also take care of setting the sensitivity of the peripheral organs to different stimuli. There is a wealth of literature that demonstrates that in depression or other psychiatric disorders where dysfunction of PFC or amygdala is known to be present, there are pronounced disturbances of hormonal as well as autonomic responses, especially after emotional stimuli.

The changed sensitivity of the adrenal to ACTH and the insensitivity to cortisol feedback as found during depression and schizophrenia may support this idea (Lammens et al., 1995); the PFC and/or amygdala may influence the secretion of cortisol from the adrenal directly, without using the HPA axis. Lesion studies indicating an enhanced secretion of corticosterone after ablating the PFC indicate that the PFC plays an inhibitory role in corticosterone secretion; data indicating enhanced cortisol secretion in depression without simultaneous elevation of ACTH support this idea. The observation that the PFC is less active in depression and certainly less active in schizophrenic patients might be the basis for the observed endocrine and

autonomic changes in these patients. Furthermore, observations of a correlated decrease in melatonin secretion in depressive or schizophrenic patients (Monteleone et al., 1997; Nathan et al., 1999) only emphasize that a change in autonomic regulation might be the basis of enhanced cortisol secretion in depressive illness. The fact that this enhanced cortisol secretion is insensitive to dexamethasone feedback would suggest that these autonomic projecting neurons have no sensitivity or a changed sensitivity to glucocorticoids in depression.

Conclusion

The forebrain control of the neuroendocrine and autonomic PVN shows many similarities between the participating structures. The SCN, ventral hippocampus, amygdala, septum and prefrontal cortex all have access to the neuroendocrine PVN via, mainly, indirect pathways.

The SCN has direct access to the autonomic PVN and thus sets the sensitivity of the organs to the hormones by a neuronal pathway. Consequently the biological clock, the SCN, sets the endocrine/autonomic balance, depending on the day/night, sleep/wake rhythm.

The PFC and central amygdala have direct access to sympathetic and/or parasympathetic motor nuclei in brainstem and spinal cord. In this way the amygdala and PFC resemble the PVN functionally; within their anatomical boundaries they control (via the PVN) the adenohypophysis, or, directly, the autonomic nervous system.

The amygdala and PFC will set the endocrine/autonomic balance, depending on the emotional status. The information about the different set points, time-of-day, emotion and food balance, converges in the medial part of the hypothalamus where the major players for regulating the homeostatic balance are concentrated. We would like to propose that this interaction forms part of the explanation why emotional disturbances have such profound effects on the homeostatic balance of the individual and vice versa.

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