SCN Efferents to Peripheral Tissues: Implications for Biological Rhythms

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Abstract The suprachiasmatic nucleus (SCN) is the principal generator of circadian rhythms and is part of an entrainment system that synchronizes the animal with its environment. Here, the authors review the possible communication of timing information from the SCN to peripheral tissues involved in regulating fundamental physiological functions as revealed using a viral, transneuronal tract tracer, the pseudorabies virus (PRV). The sympathetic nervous system innervation of the pineal gland and the sympathetic outflow from brain to white adipose tissue were the first demonstrations of SCN-peripheral tissue connections. The inclusion of the SCN as part of these and other circuits was the result of lengthened postviral injection times compared with those used previously. Subsequently, the SCN has been found to be part of the sympathetic outflow from the brain to brown adipose tissue, thyroid gland, kidney, bladder, spleen, adrenal medulla, and perhaps the adrenal cortex. The SCN also is involved in the parasympathetic nervous system innervation of the thyroid, liver, pancreas, and submandibular gland. Individual SCN neurons appear connected to more than one autonomic circuit involving both sympathetic and parasympathetic innervation of a single tissue, or sympathetic innervation of two different peripheral tissues. Collectively, the results of these PRV studies require an expansion of the traditional roles of the SCN to include the autonomic innervation of peripheral tissues and perhaps the modulation of neuroendocrine systems traditionally thought to be controlled solely by hypothalamic stimulating/inhibiting factors.

Key words tract tracing, pseudorabies virus, pineal gland, adipose tissue, thyroid, sympathetic nervous system, parasympathetic nervous system, adrenal

Most physiological and behavioral responses of organisms do not occur randomly; rather, they occur episodically. Many of these responses coincide with regularly occurring changes in the environment, such as the light-dark cycle. When these responses occur rhythmically in the absence of environmental cues, they are termed *circadian rhythms* (for review, see Rusak and Zucker, 1979). The process by which these physiological and behavioral responses are synchronized with important environmental events, such as the light-dark cycle, is termed *entrainment*. The ability of an animal to entrain to significant environmental events is critical for their survival and ultimately reproductive success. The suprachiasmatic nucleus (SCN) of mammals not only is a critical component of this entrainment system, but it also appears to be a principal generator of most circadian rhythms (for review, see Rusak and Zucker, 1979). The purpose of this mini-review is to discuss how the SCN might communicate timing information to a variety of

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peripheral tissues via neural connections to help regulate such fundamental physiological functions as energy metabolism, food and fluid balance, and cardiovascular, immune, and reproductive responses. Therefore, we especially will focus on the findings of experiments where peripheral tissues have been injected with retrograde transneuronal viral tract tracers that have revealed neural circuits that include the SCN. We will not, however, present an in-depth review of the important studies using traditional retrograde and anterograde tract tracers that, until recently, provided the only neuroanatomical data on SCN efferents (for review, see Watts, 1991; van Esseveldt et al., 2000). Moreover, we will not discuss the exciting research showing that the SCN can act as an "exocrine organ," apparently secreting substances vital for the expression of some (locomotor activity), but not all (endocrine secretions), responses (for review, see LeSauter and Silver, 1998).

THE SCN HAS EFFERENT NEURAL CONNECTIONS TO PERIPHERAL TISSUES

Despite some support for a diffusible signal emanating from the SCN, communication of the brain with peripheral tissues via SCN exocrine secretions seems inefficient in the least. Alternatively, a more rapid and precise mechanism for the SCN to communicate with peripheral tissues is via the autonomic nervous system generally, and the sympathetic nervous system (SNS) specifically. In addition, for some tissues, SCN-to-peripheral tissue communication also involves the parasympathetic nervous system (PSNS; Ueyama et al., 1999). *Neuroanatomical evidence* of the autonomic connections from the SCN to the periphery will be discussed below; however, first we wish to address some of the *functional evidence* for these connections.

STIMULATION OR DESTRUCTION OF THE SCN OR ITS EFFERENT PROJECTIONS AFFECTS PERIPHERAL PHYSIOLOGICAL RHYTHMS

Before the advent of transsynaptic viral tract tracers, it was not technically possible to trace SCN efferents to peripheral tissues within the same animal. Numerous studies, however, have been conducted in which the SCN has been stimulated or destroyed and the resulting changes in the timing of peripheral responses attributed to disruption of SCN clock/output functions. A few examples of these types of experiments are presented below and have been selected because they are supported, in part, by the neuroanatomy defined using transneuronal viral tract tracers (see below for the latter).

The disruption of pineal melatonin secretion by SCN lesions or by destruction of the SCN-PVN-spinal cord-SNS-pineal circuit will not be reviewed here, as they have been detailed recently (Moore, 1996). Moreover, this polysynaptic pathway from the SCN to the pineal gland has been defined within the same animal, rather than in a series of studies across animals, using viral transneuronal tract tracing methodology (Larsen et al., 1998) and reviewed recently in this journal by Card (2000).

In addition to the role of the SCN in generating the rhythm of pineal melatonin synthesis and release, the SCN plays an integral role in coordinating a variety of other neuroendocrine rhythms and, as with the SCN-pineal studies alluded to above, lesions of the SCN often disrupt the overall secretion and/or timing of these humoral factors. Such disruptions, however, are often most parsimoniously explained by altered synthesis/release of hypothalamic releasing or inhibiting factors. For example, bilateral destruction of the SCN abolishes circadian rhythms in both adrenocorticotrophic hormone (ACTH) and glucocorticoid secretion (Moore and Eichler, 1972; Meyer-Bernstein et al., 1999; Szafarczyk et al., 1983); recent studies, however, implicate the existence of SCN-adrenal cortex connections (see below). SCN lesions also eliminate the diurnal peak in circulating thyroid-stimulating hormone (TSH) (Abe et al., 1979) and decrease the 24-h rhythm of thyroxine (Kalsbeek et al., 2000); again, recent studies suggest SCN-thyroid connections (see below). Collectively, these and other results suggest that the SCN may be important in regulating neuroendocrine responses not only by influencing hypothalamic releasing or inhibiting factors but also via neural connections from the SCN to the peripherally located organs.

THE PSEUDORABIES VIRUS IS A TRANSNEURONAL RETROGRADE TRACT TRACER

Although traditional tract-tracing studies have provided us with valuable information regarding

SCN efferent connections to other neural structures (e.g., Berk and Finkelstein, 1981; Watts et al., 1987), they did not reveal connections to peripheral tissues, because of their limited ability (e.g., *Phaseolus vulgaris leucoagglutinin*; Watts et al., 1987) or inability to travel transsynaptically (e.g., 3H- proline; Swanson and Cowan, 1975). This limitation of the traditional tract tracers has been overcome using neurotropic viruses that travel transneuronally (for review, see Card and Enquist, 1999). Neurotropic viruses (e.g., alpha herpes viruses) specifically invade the nervous system and replicate within synaptically connected populations of neurons and, thus, can define neuronal circuits (reviewed in Card and Enquist, 1999). The most commonly used among the alpha herpes viruses has been the Bartha's K strain of the swine pathogen pseudorabies virus (PRV). PRV infects neurons preferentially leaving surrounding nonneural tissue unaffected; thus, this virus can be used as a transneuronal retrograde tract tracer in the CNS. Specifically, PRV infects neurons after binding to viral attachment protein molecules located on the surface of neuronal membranes. Neurons synapsing on infected cells are exposed to relatively high titers of virus particles that have been exocytosed from the initially infected neuron. The virus is then taken up by second order neurons via synaptic contact and this process continues unhindered, leading to a hierarchical chain of functionally connected infected neurons from peripheral tissues to the brain. PRV acts as a self-amplifying cell marker because it generates more viral progeny in each subsequently infected neuron. The resulting multisynaptic chain of infected neurons can then be visualized relatively easily using standard immunocytochemical techniques. More recently, several genetically engineered, recombinant strains of PRV have been created to examine collateralization (Card and Enquist, 1999). These viruses express unique reporters (e.g., green fluorescing protein, β galactosidase) and thus can be discriminated with antibodies specific to each PRV recombinant (e.g., Sams et al., 1995; Card et al., 1998). Therefore, using these genetically altered viruses, multiple strains of PRV can be injected into the same animal and differential multisynaptic neuronal pathways can be identified by standard dual-labeling immunocytochemistry. Examples of the use of these altered viruses to describe SCN efferent connections to the periphery are given below.

Although PRV has been used extensively as an effective, transneuronal marker, there are some limitations to the technique. For example, PRV may not be a ubiquitous transneuronal marker; some neurons may be resistant to infection because they lack viral receptors or the tissue of interest is only sparsely innervated and/or has fewer viral binding sites (for this and other caveats/pitfalls, see Card, 1998).

THE SCN IS CONNECTED TO PERIPHERAL TISSUES AS REVEALED BY USING VIRAL TRACT TRACING METHODOLOGY

The first evidence that the SCN has neuronal connections to peripheral tissues came from examinations of a likely target tissue, the pineal gland, and a seemingly unlikely target tissue, white adipose tissue (WAT). In terms of the former, a long series of neuroanatomical and functional studies demonstrated a circuit beginning with the SCN and including a sequence of intermediate connections (paraventricular nucleus [PVN] of the hypothalamus, intermediolateral horn of the spinal cord, superior cervical ganglia) ending with the pineal gland that is responsible for the circadian-based synthesis and release of melatonin (MEL) by pinealocytes (for review, see Moore, 1996). This circuitry was confirmed and precisely detailed by injecting PRV into the pineal gland and retrogradely labeling its inputs including the SCN in laboratory rats (Larsen et al., 1998). Implications of the former study and other related studies for SCN control of pineal-generated melatonin signals were reviewed recently in this journal by Card (2000) and, therefore, will not be discussed further here.

The seemingly less likely SCN target is WAT. There was, however, functional evidence for an SCN-SNS-WAT circuit. Thus, coronal microknife cuts located just caudal to the SCN (and thus presumably severing most descending projections, block glucoprivation-induced increases in plasma-free fatty acid concentrations (i.e., block increases in WAT lipolysis; Teixeira et al., 1973). Not surprisingly, similar cuts also block the increases in lipolysis triggered by fasting, forced exercise, cold exposure, and insulin- induced hypoglycemia (Coimbra and Migliorini, 1983). This SCN-SNS-WAT circuit also may help account for the 24-h rhythms of lipolysis/lipogenesis shown by laboratory rats (LeMagnen and Devos, 1970).

The SNS outflow from brain to WAT was revealed by injecting the PRV into the inguinal or epididymal WAT of Siberian hamsters (*Phodopus sungorus*) or laboratory rats and included the SCN among many brain

structures (Bamshad et al., 1998). Because WAT does not have PSNS innervation (for review, see Bartness and Bamshad, 1998), the interpretation of this labeling was that the SCN has efferents that compose part of the sympathetic outflow from brain to WAT (Bamshad et al., 1998). Collectively, the SCN may function to coordinate lipolysis temporally under several conditions. These include the chronic control of daily cycles of lipid mobilization by rhythmically varying the SNS drive on WAT, as well as the acute control of increases in energy demands such as those associated with fasting, cold exposure, and exercise.

Siberian hamsters also increase their lipid mobilization when exposed to short "winter-like" days (Wade and Bartness, 1984) via increases in the SNS drive on WAT, as measured by norepinephrine turnover in this tissue (Youngstrom and Bartness, 1995). SCN lesions block the ability of short day–like exogenously administered MEL signals to trigger short day–like decreases in body fat in pinealectomized Siberian hamsters (Bartness et al., 1991). This finding, together with the localization of functional MEL receptors for photoperiodic responses (MEL_{1a} receptors) in the SCN of this species (Reppert et al., 1994), suggested a possible mechanism underlying the short day–induced decreases in body fat by Siberian hamsters. Perhaps MEL_{1a} receptors are located on SCN neurons that are part of the sympathetic outflow to WAT (Bamshad et al., 1998). By labeling the sympathetic outflow from brain to WAT using PRV combined with the labeling of brain MEL_{1a} receptors using in situ hybridization, we recently found PRV-labeled neurons that also expressed MEL_{1a} receptor mRNA in several brain regions, including the SCN (Song and Bartness, 2001). Thus, the increased duration of MEL secretion in short days may increase MEL_{1a} receptor stimulation that, in turn, increases the sympathetic drive on WAT, thereby increasing lipolysis and decreasing adiposity in this species (Song et al., 2001).

In addition to this SCN-SNS-WAT circuitry, there is another SCN-SNS-adipose tissue circuit. Brown adipose tissue (BAT), unlike WAT, functions to generate heat primarily using lipid fuels and has long been known to be richly innervated by the SNS (for review, see Himms-Hagen, 1991; Bartness et al., 2001). Until the advent of the PRV tract-tracing technology, the origins of the sympathetic outflow from brain to BAT only were inferred from lesion and stimulation experiments (for review, see Himms-Hagen, 1991; Bartness et al., 2001). Application of PRV to interscapular BAT (IBAT) in Siberian hamsters and laboratory rats, however, revealed the CNS origins of the sympathetic outflow to BAT, and these included the SCN among other structures (Bamshad et al., 1999). This neuroanatomical link between the SCN and BAT is supported by earlier functional studies showing an increase in IBAT thermogenesis after electrical stimulation of the retinohypothalamic tract that innervates the SCN (Amir, 1989), or after glutamate injections directly into the SCN (Amir et al., 1989). It also appears that the most dorsomedial aspects of the SCN are the most highly infected after virus injections into BAT or WAT from Siberian hamsters and laboratory rats (Bamshad et al., 1998; Bamshad et al., 1999). Moreover, it was the arginine vasopressin containing neurons in the SCN that were most frequently infected after BAT PRV injections (Bamshad et al., 1997). Collectively, this SCN-SNS-BAT circuitry may be involved in the nycthemeral cycles of body temperature (Himms-Hagen, 1991) and also may be involved in the circadian timing of torpor bouts in Siberian hamsters, as SCN lesions block the torpor-associated rhythmic daily decreases in body temperature (Ruby et al., 1989). Finally, although the exact role of the SCN in BAT thermogenesis is not known at this time, it should be noted that SCN lesions do not block circadian rhythms of body temperature in squirrel monkeys (Albers et al., 1984) and laboratory rats (Satinoff and Prosser, 1988) (cf. Syrian hamsters; Osborne and Refinetti, 1995). These findings suggest a more complicated control of body temperature rhythms than sole control by the SCN and/or by BAT.

The likely reason we and others (see below) were able to see the involvement of rostral forebrain structures like the SCN in the PRV tract-tracing studies discussed above was that a longer postinjection time was used (e.g., 6 days; Bamshad et al., 1998; Bamshad et al., 1999) than in the original virus studies of others (i.e., 4 days). Thus, SCN neurons were not infected in previous studies after PRV injections into the kidney (Schramm et al., 1993), pancreas (Loewy and Haxhiu, 1993; Loewy et al., 1994), or adrenal medulla (Strack et al., 1989). With 6- to 7-day postinjection times, however, SCN-kidney (Sly et al., 1999) and SCN-pancreas (Ueyama et al., 1999) connections have been identified.

Recently, PRV injections into the adrenal cortex of laboratory rats resulted in the infection of SCN neurons (Buijs et al., 1999). Although it seems improbable that only the adrenal cortex, and not also the underlying adrenal medulla, was injected with the viral tract tracer, the authors' interpretation of these results was

that the SCN is connected to the adrenal cortex. Moreover, they suggest that this presumed SCN–adrenal cortex circuit may underlie the ability of light to inhibit rapidly corticosterone secretion that cannot be explained by decreases in ACTH secretion (Buijs et al., 1999). Note that, as suggested above, it seems likely that the injected virus also spread to the adrenal medulla and that SCN-adrenal medulla connections via the SNS do exist (Ueyama et al., 1999; **(ADD AUTHOR'S INITIALS)**Bamshad, Demas, and Bartness, unpublished observations).

Components of the thyroid axis (e.g., thyroxine [T4], triiodothyronine [T3], thyrotropin, thyrotropin-stimulating hormone [TSH]) show 24-h rhythms, especially TSH (e.g., Jordan et al., 1980; Kalsbeek et al., 2000). These cyclical fluctuations of thyroid hormones and TSH suggest a circadian basis and involvement of the SCN, although they have not been studied under constant conditions to our knowledge. SCN lesions decrease overall circulating concentrations of TSH (e.g., Abe et al., 1979; Kalsbeek et al., 2000), as well as altering their 24-h rhythms (Abe et al., 1979; Kalsbeek et al., 2000). Moreover, SCN lesions have more marked effects on **(OR SHOULD THESE READ T3 AND T4 AS ABOVE?)**T_3 and T_4 than on TSH concentrations (Kalsbeek et al., 2000). Tests of the effects of SCN lesions on the circadian rhythms of thyroid hormones or TSH release, as apposed to their 24-h rhythms, have not been done, however. Nevertheless, PRV injections into the thyroid label SCN neurons, suggesting possible involvement in the rhythmic release of thyroid hormones and perhaps TSH (Kalsbeek et al., 2000). As with other neuroendocrine systems involving hypothalamic stimulating/inhibiting hormones, SCN efferents could contribute to the control of TSH-containing neurons in the PVN (e.g., Aizawa and Greer, 1981) to regulate thyroid hormone secretions (Watts et al., 1987). The relative contributions of SCN efferents in controlling PVN TSH-containing neurons and in controlling the innervation of the thyroid itself, however, remain to be determined.

Control of water balance by the kidney and of water excretion by the bladder also appears under the influence of the SCN as revealed by PRV tract-tracing experiments. Thus, although no SCN-infected neurons were found in an earlier study after PRV injections into the kidney (Schramm et al., 1993), with longer post-PRV injection intervals (6–7 days), an SCN-to-kidney circuit was identified (Sly et al., 1999). In addition, an SCN-bladder circuit also was revealed after such extended postinjection survival times (Sly et al., 1999).

The involvement of the SCN in PSNS innervation of peripheral tissues has not been discussed above, largely because several of these ultimate SCN targets do not appear to have PSNS innervation (e.g., WAT, BAT, pineal gland). SCN-PSNS-peripheral tissue circuits have been defined, however, using the PRV. In addition to the SCN-SNS-thyroid circuit discussed above, a PSNS counterpart exists (Kalsbeek et al., 2000). There also is SCN-PSNS innervation of the pancreas and submandibular gland (Ueyama et al., 1999), as well as the liver (la Fleur et al., 2000). A previous report of no SCN-PSNS-pancreas connections more likely was due to the shorter (4 day) postinjection interval in the earlier study (Jansen et al., 1997) than in the more recent one (5–6 days; Ueyama et al., 1999). Moreover, individual SCN neurons appear connected to more than one autonomic circuit. Thus, by using isogenic PRV mutants to enable separate identification of virus-infected neurons connected to two different peripheral targets, single SCN neurons have been shown to be part of the SNS outflow from the brain to the adrenal medulla as well as part of the PSNS outflow from the brain to the submandibular gland (Ueyama et al., 1999). In addition, other individual SCN neurons appear to be part of the SNS outflow from the brain to two different sympathetic targets—the stellate ganglion (the site of the cell bodies of postsympathetic ganglionic neurons innervating the heart) and the adrenal medulla (Ueyama et al., 1999). The participation of neurons in multiple autonomic pathways has been suggested as the neuroanatomical origins coordinating the "fight-or-flight" response (Jansen et al., 1995) championed by Walter Cannon (1927).

Finally, a recent preliminary report (Drazen et al., 2000) suggested that the SCN has efferents ultimately innervating the spleen via the SNS. Sympathetic innervation of the spleen seems reasonable given that SNS innervation of lymphoid tissue exists for the spleen, thymus, lymph nodes, and bone marrow using traditional tract-tracing and immunohistochemical techniques (Bellinger et al., 2001). Importantly, the SNS plays a *functional* role in mediating a wide range of immune responses such as lymphocyte trafficking and proliferation, as well as modulating cytokine production (Elenkov et al., 2000). In terms of possible SCN-mediation of immune function, there is daytime inhibition of the production of tumor necrosis factor-α, but not other immune factors such as interleukin-1 beta or interleukin-6 (DeRijk et al., 1997). These cyclical changes in immune products, however,

could be secondary to SCN-mediated changes in cortisol secretion (DeRijk et al., 1997). To our knowledge, there has been no test of circadian variations in immune function where SCN lesions were made and animals were housed under constant conditions.

DOES THE SCN REGULATE CLOCK GENE EXPRESSION IN PERIPHERAL TISSUES?

As discussed above, considerable evidence is accumulating to support the notion of an important role of the SCN in the regulation of peripheral tissue function via autonomic output circuits. The precise means by which this regulation occurs, however, remain unknown. One intriguing idea is that SCN efferents control peripheral physiology by altering the functioning of *peripheral* clock genes. In the last few years, several mammalian genes have been identified that appear to be "master genes" controlling circadian rhythmicity in mammals. Two of the most extensively studied genes include the *clock* gene and the mammalian analogs to the *per* (*period*) gene first discovered in *Drosophila* (Konopka, 1979). The *clock*, *rPer1*, and *rPer2* genes appear to be involved in regulating circadian locomotor rhythms in mammals (Tei et al., 1997) and are found in peripheral tissues as well as the brain (e.g., Sun et al., 1997). This raises the possibility that SCN efferents may modulate *clock* and/or *per* gene expression directly, and the subsequent changes in gene expression lead to changes in overt rhythms in both physiology and behavior. At least one study has provided support for this notion. A robust rhythm in *rPer2* expression is seen in both the SCN and peripheral tissues (e.g., spleen, heart, lung, liver) in rats (Sakamoto et al., 1998). Lesions of the SCN abolish the rhythms in *rPer2* expression, suggesting that the rhythm in *rPer2* expression is regulated by the SCN (Sakamoto et al., 1998). Such lesions, however, also abolish *rPer2* rhythms in peripheral mononuclear leukocytes that are not innervated, suggesting that the SCN lesions led to the abolishment of a humoral factor (Oishi et al., 1998). Although the idea of direct regulation of *clock* gene expression by SCN outputs is intriguing, more research is required to test it.

CONCLUDING THOUGHTS

Over the past few years, the use of the transsynaptic tract tracer PRV has allowed neuroscientists to begin to map the neural connections between the SCN and peripheral tissues in several physiological systems. The results of these studies have begun to shed new light on the important role of the SCN in the regulation of physiological and neuroendocrine rhythms. Importantly, such studies have begun to redefine the functions of the SCN not simply as a "master clock" regulating locomotor and other behavioral rhythms but also as playing an integral role in the coordination and integration of a wide range of physiological and neuroendocrine functions. For example, the regulation of energy balance involves energy intake and energy expenditure, as well as the modulation of lipid and carbohydrate metabolism, and requires extensive synchronization of behavioral (e.g., foraging and feeding), neural (i.e., SNS, PSNS), and humoral (e.g., insulin, glucagon, epinephrine, glucocorticoids) factors, most or all of which are entrained to environmental influences (e.g., photocycle, photoperiod, ambient temperature). In addition, communication of biological rhythm information from the SCN to peripheral tissues via autonomic neural outflows suggests a possible additional influence on the rhythmic secretions of several hormones long known to exhibit 24-h rhythms but thought to be exclusively under the control of hypothalamic releasing factors (e.g., glucocorticoids, thyroid hormones). Thus, the synthesis and secretion of these and other hormones also might be modulated via innervation of SCN efferent projections that comprise the SNS and/or PSNS outflows from the brain.

Because there are considerable gaps in our knowledge of the control of peripheral physiology by SCN efferents to peripheral targets, as noted throughout this review, additional research is required. For example, except for the SCN-SNS-pineal circuit, the exact connectivity between the SCN and the peripheral targets discussed above is unknown. Although time course studies can indicate the progression of the virus through the nervous system, they do not necessarily indicate the sequence of connections among the sites. For example, injections of PRV into WAT or BAT show a progression across days from the spinal cord to the brainstem, then the midbrain and finally the forebrain. A simple interpretation of this finding would be that forebrain sites (e.g., SCN) are connected to midbrain sites, midbrain sites are connected to brainstem sites, and brainstem sites are connected to spinal cord sites. We know, however, that there are monosynaptic forebrain and brainstem connections to the intermediolateral horn of the spinal cord. Thus, the

order within which sites become infected after peripheral PRV injections does not necessarily provide connectivity information. With PRV-infected forebrain neurons seen 4 days after peripheral injections (e.g., in the PVN), why does it take approximately 2 more days to see infections in SCN neurons? The answer to this and other questions await the results of future neuroanatomical and functional studies.

Collectively, the use of the viral transsynaptic tract tracer, PRV, has revealed connections of the SCN to a variety of peripheral tissues. The existence of these SCN–to–peripheral tissue circuits forces us to expand our view of the role of the SCN in the control of the secretion of several hormones (e.g., thyroid, glucocorticoids) to include the autonomic innervation of these tissues and to add other peripheral tissues to the growing list of SCN efferent targets.

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