Published in final edited form as:

Mol Cell Endocrinol. 2012 February 5; 349(1): 3–12. doi:10.1016/j.mce.2011.07.003.

Clocks on top: The role of the circadian clock in the hypothalamic and pituitary regulation of endocrine physiology

Karen J. Tonsfeldt* and Patrick E. Chappell

Department of Biomedical Sciences, College of Veterinary Medicine, Oregon State University, Corvallis, OR 97331

Abstract

Recent strides in circadian biology over the last several decades have allowed researchers new insight into how molecular circadian clocks influence the broader physiology of mammals. Elucidation of transcriptional feedback loops at the heart of endogenous circadian clocks has allowed for a deeper analysis of how timed cellular programs exert effects on multiple endocrine axes. While the full understanding of endogenous clocks is currently incomplete, recent work has re-evaluated prior findings with a new understanding of the involvement of these cellular oscillators, and how they may play a role in constructing rhythmic hormone synthesis, secretion, reception, and metabolism. This review addresses current research into how multiple circadian clocks in the hypothalamus and pituitary receive photic information from oscillators within the hypothalamic suprachiasmatic nucleus (SCN), and how resultant hypophysiotropic and pituitary hormone release is then temporally gated to produce an optimal result at the cognate target tissue. Special emphasis is placed not only on neural communication among the SCN and other hypothalamic nuclei, but also how endogenous clocks within the endocrine hypothalamus and pituitary may modulate local hormone synthesis and secretion in response to SCN cues. Through evaluation of a larger body of research into the impact of circadian biology on endocrinology, we can develop a greater appreciation into the importance of timing in endocrine systems, and how understanding of these endogenous rhythms can aid in constructing appropriate therapeutic treatments for a variety of endocrinopathies.

A significant portion of our current understanding regarding the molecular circadian clock has its roots in studies of the mammalian hypothalamus, and many of the earliest characterized diurnal or circadian physiological rhythms were endocrine in nature. Thus it is not surprising that the circadian clock is intimately and inextricably involved with the hypothalamic-pituitary regulation of multiple endocrine axes. This review, while neither comprehensive nor exhaustive, will attempt to explore current research into the influence of the circadian clock on hypothalamic and pituitary regulation of endocrine function. We will focus on endocrine-regulating outputs from and inputs to the central mammalian clock within the brain, the suprachiasmatic nuclei (SCN), as well as concentrating on more recent work exploring the roles of cell-specific endogenous oscillators within each endocrine axis.

© 2011 Elsevier Ireland Ltd. All rights reserved.

Address for correspondence: Patrick E. Chappell, Department of Biomedical Sciences, College of Veterinary Medicine, Oregon State University, Corvallis, OR 97331, 541-737-5361, patrick.chappell@oregonstate.edu.

*Present address: Neuroscience Graduate Program, Oregon Health & Science University, Portland, OR

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

We will highlight observed circadian rhythms within multiple homeostatic endocrine systems, and how temporal control of these processes confers adaptive advantages.

A hypothalamic center of synchrony: the SCN

The anterior pituitary, or adenohypophysis, is comprised of at least 5 distinct cell types, organized in syncytia within the gland, releasing hormones governing a broad array of homeostatic processes, including somatic growth, metabolism, stress response, thermoregulation, and reproduction and lactation. The posterior pituitary, or neurohypophysis is a ventral extension of neural tissue, releasing the neurohormones oxytocin and arginine vasopressin (AVP) from granular cells within the hypothalamus directly into the general circulation, thus controlling blood pressure and osmolality, and working with adenohypophysial hormones to facilitate parturition and lactation in females. The anterior pituitary, while clearly responsive to feedback signals from target organs, is predominantly sensitive to stimulation (or inhibition) by hypophysiotrophic factors released from the hypothalamus.

The hypothalamus houses several nuclei involved in regulation of endocrine processes, and many of these exhibit circadian rhythms of neurohormone synthesis and release. Central among these regions is the bilateral SCN, which can respond to changes in the ambient light environment by signaling appropriately to multiple endocrine axes. This crucial periventricular region 1) is directly retino-recipient, i.e. receives monosynaptic innervation from the retina via the retinohypothalamic tract (RHT), 2) displays the most robust molecular clock gene cycling in the body, and 3) uses a combination of neuronal and humoral factors to synchronize peripheral clocks to external photic cues (Abrahamson and Moore, 2001). The central role of the SCN in the construction of mammalian circadian rhythms of sleep-wake and locomotor activity patterns has been recognized for decades, and is the subject of several extensive reviews (Abrahamson and Moore, 2001; Gillette and Tischkau, 1999; Herzog and Tosini, 2001; LeSauter and Silver, 1998; Weaver, 1998). We will focus predominantly on how the SCN influences the activity of other hypothalamic nuclei important for the neuroendocrine regulation of physiological processes, as well as how feedback signals from endocrine target tissues may communicate with this central synchronizing oscillator.

As expected from its central role in constructing circadian rhythms, the SCN is a complex, heterogeneous population of neuronal cell types that have been shown to express and release several neuropeptides and biogenic amines. While the majority of SCN neurons synthesize γ -amino-butyric acid (GABA) (Moore and Speh, 1993), which appears to mediate most intra-SCN signaling (Strecker et al., 1997), many cells co-express an array of neuropeptides which also comprise SCN efferent signals. In rats, AVP is expressed in approximately ~37% of SCN neurons, and AVP neurons line the medial halves of the SCN, extending in a crescent to the dorsal and ventral SCN. Vasoactive inhibitory peptide (VIP) comprises approximately ~25% of the cells, and is primarily expressed dorsally and medially in the rostral region of the SCN (Moore et al., 2002). While the exact position of peptide distribution varies by species, the general separation of AVP and VIP described above is maintained among rodents (Morin and Allen, 2006). Gastrin-releasing peptide (GRP) is expressed in the medial SCN, and calretinin is also expressed medially, with some expression dorsally, with these peptides comprising approximately 14% of SCN neurons each. Met-Enkephaline, somatostatin, calbindin, angiotensin II, substance P, and neurotensin are also expressed in the SCN, but at a low (<5%) level of expression, such that these are not considered major contributors of SCN efferent signals (Abrahamson and Moore, 2001; Moore et al., 2002). While the SCN is classically organized by cellular phenotype, mRNA

studies suggest that the regions of AVP and VIP are distinct, but show overlap based on circadian times (Morin and Allen, 2006).

In order for the SCN to act as a synchronizing mechanism within a mammal, its neurons synthesize and secrete many of the above factors with a circadian rhythm, controlled by multiple intracellular oscillators comprised at its root of an elegant transcription-translation feedback loop. Briefly, basic helix-loop-helix (bHLH) PAS domain transcription factors CLOCK and BMAL1 heterodimerize on E-boxes within promoters of the *Period* and *Cryptochrome* genes to stimulate their transcription. Following translation into the PER and CRY proteins, respectively, these factors translocate back to the nucleus to inhibit their own transcription by direct interactions with the CLOCK/BMAL1 heterodimers (Reppert, 2000). An additional regulatory loop exists, controlling antiphasic expression of *Bmal1* (Clock is constitutively expressed in most mammalian tissues), involving the actions of orphan nuclear receptors Rev-erb α and ROR α competing at response elements on the *Bmal1* promoter (Preitner et al., 2003; Sato et al., 2004). In addition to components of the core clock (outlined in more detail in a separate review in this issue), many other transcripts within the SCN are “clock-controlled”, such that abundance of their mRNA (and often protein) also oscillates with a circadian rhythm (Panda et al., 2002). Elucidation of the molecular clock mechanism over the past two decades has led to the somewhat surprising discovery of clock gene expression throughout the body, in many cases capable of oscillating independently of the SCN (Abraham et al., 2005; Fahrenkrug et al., 2008; Granados-Fuentes et al., 2004). Many of these cellular oscillators are present in endocrine glands, and in hormone-responsive target organs, and appear to gate temporally appropriate hormonal secretion and responsiveness to environmental stimuli. Thus, starting from the centrally-located SCN, we will explore neural and humoral signals within the hypothalamus and pituitary, while later articles within this issue will address actions of endogenous and exogenous circadian timing devices in the periphery.

Neuroendocrine efferents from the SCN

Early experiments used chemical or electrolytic lesioning of the SCN to demonstrate the importance of these hypothalamic nuclei to sleep-wake and activity cycles (Arendash and Gallo, 1979; Schwartz and Zimmerman, 1991; Wiegand et al., 1980). Following this, several studies were performed in which fetal SCN grafts were transplanted into SCN-ablated animals (Lehman et al., 1995; Lehman et al., 1987; LeSauter et al., 1996; Matsumoto et al., 1996). Interestingly, even when the grafts were physically prevented from forming synaptic connections with the host brain via a semi-permeable membrane barrier, locomotor rhythmicity was restored in these previously arrhythmic animals, but several hormonal rhythms, particularly luteinizing hormone (LH), cortisol, and melatonin were irreversibly lost (Silver et al., 1996). These data, while demonstrating that some rhythms can be reinstated by humoral factor released from the SCN, also serve to underscore the importance of direct SCN afferents in the control of many endocrine rhythms. Direct connections from the SCN have been described to corticotrophin-releasing hormone- (CRH), thyrotropin-releasing hormone- (TH), and gonadotropin-releasing hormone (GnRH)-containing neurons, as well as other hypothalamic intermediate regions in the regulation of endocrine rhythms (Kalsbeek and Buijs, 2002; Vida et al., 2010). We will investigate the potential contributions of some of the most well-characterized efferent signals from the SCN below.

SCN output signals: Vasopressin

Among the SCN efferent signals, AVP is perhaps the best characterized. It is the only SCN-synthesized peptide for which diurnal rhythms are detectable in cerebrospinal fluid (CSF), putatively either from “spillover” of intrahypothalamic signaling or as a humoral signal (Reppert et al., 1981; Schwartz et al., 1983). The role of vasopressin in generating endocrine

rhythms has been most thoroughly demonstrated in control of corticosterone release in the hypothalamic-pituitary-adrenal (HPA axis). Actions of SCN-derived AVP on neurons of the dorsomedial hypothalamus (DMH) are required to produce daily corticosterone surges (Kalsbeek et al., 1996). Vasopressinergic connections from the SCN to the sub-paraventricular nuclei and the DMH are also believed to be critical for relaying temporal information to corticotrophin-releasing hormone (CRH) neurons in the paraventricular nucleus, which in turn may regulate the rhythmic release of adrenocorticotrophic hormone (ACTH) (Buijs and Van Eden, 2000; Kalsbeek et al., 2010; Kalsbeek et al., 1996; Kalsbeek et al., 2008). Additionally, SCN-derived AVP has been implicated in the regulation of the reproductive axis. Release and synthesis of AVP from the SCN peaks near the time of the preovulatory GnRH surge, a temporally regulated hormonal event which initiates a similar surge in luteinizing hormone (LH) and subsequent ovulation (Funabashi et al., 2000; Kalamatianos et al., 2004; Kalsbeek et al., 1995; Palm et al., 2001a). In oestrogen-treated ovariectomised (OVX) female animals with SCN lesions, intracranially-administered AVP rescues the LH surge (Palm et al., 1999), and can potentiate LH surge release if given during a precise window in the afternoon (Palm et al., 2001a). Even in *Clock* mutant mice, a timed i.c.v. infusion of AVP can stimulate a portion of LH surge release, but only in the presence of elevated oestradiol (Miller et al., 2006). Furthermore, AVP-containing SCN efferents contact neurons in the sexually-dimorphic anteroventral periventricular nuclei (AVPV) that release Kiss1, a newly-characterized and potent stimulator of GnRH release, believed important for the preovulatory GnRH surge (Vida et al., 2010). SCN-derived AVP has also been implicated in generating observed activity rhythms in voles, in which arrhythmic animals show attenuated and arrhythmic release of AVP, even in conjunction with concurrent normal rhythms of other SCN peptides (Jansen et al., 2007). Such a role in rodents, however, has been mostly ruled out by investigations using the Brattleboro rat, a naturally-occurring AVP knockout that bears no gross circadian locomotor abnormalities (Schmale and Richter, 1984). However, in these animals estrous cycle length and corticosterone production appears to be compromised (Boer et al., 1981; Brudieux et al., 1986), confirming that AVP efferents from the SCN play an important role in maintaining endocrine rhythms. Interestingly, SCN transplants from Brattleboro rats into SCN-lesioned hosts are still able to rescue normal locomotor rhythmicity (Boer et al., 1999), suggesting that AVP, while important for select endocrine rhythms, may not play a central role in constructing sleep-wake activity cycles.

SCN output signals: VIP

In contrast to the mostly efferent role of AVP, VIP has been shown to be critical for coupling the multiple cellular oscillators within the SCN, and may play a more central role in maintaining intra-SCN synchrony. Animals harboring a genetically-targeted knockout of VIP or its receptor, VPAC2, exhibit behavioral arrhythmicity (Colwell et al., 2003; Harmar et al., 2002), but its role in influencing endocrine rhythms remains somewhat unexplored, with a predominance of studies, however, investigating regulation of reproduction. There appears to be a direct connection between VIPergic SCN neurons and GnRH neurons (Smith et al., 2000; Van der Beek et al., 1997). Central infusion of VIP alters the timing of the LH surge, and intracranial injections of VIP antisera can also delay and attenuate surge release (Weick and Stobie, 1992). While the overall effects of VIP within the reproductive axis remain ambiguous, approximately 40% of GnRH neurons have been shown to express VIP receptors (Smith et al., 2000), and direct VIP application on to slice preparations have an excitatory effect that is both E2- and time-dependent (Christian and Moenter, 2008). Interestingly, *Vipr2*^{-/-} female mice, while exhibiting significantly altered locomotor rhythms due to SCN-specific disruption, displayed a relatively mild reproductive phenotype, suggesting that SCN-derived VIP signals may not play a critical role in the neuroendocrine regulation of reproduction (Dolatshad et al., 2006). It is possible, then, that the above

temporal changes in GnRH neuronal sensitivity are mediated by oscillators within GnRH neurons, which are explored in greater depth below.

SCN output signals: PK2 and TGF α

Prokineticin 2 (PK2) is a peptide initially characterized in the gut, and like somatostatin (SST) and cholecystokinin (CCK), appears to play a role within the brain, and is putatively an output signal from the SCN. PK2 was only recently identified in the SCN as a secreted factor, and appears involved in coordinating physiological rhythms (Prosser et al., 2007). PK2-containing cells in the SCN have a similar distribution as AVP neurons. Other regions of the hypothalamus express the PK2 receptor (PK2R), especially in the diagonal band of Broca, and preoptic and arcuate nuclei (Zhang et al., 2009). PK2 mRNA, while found throughout the hypothalamus, is highly concentrated within the dorsolateral aspects of the SCN. Infusion of exogenous PK2, while exerting no effect in the subjective day phase, inhibits activity in the subjective night phase, suggesting that this peptide can act to inhibit activity in the light phase, when PK2 levels are elevated, and the decrease of PK2 allows for initiation of activity in the dark (Cheng et al., 2002). The loss of PK2 disrupts locomotor and thermoregulatory mechanisms, but does not seem to affect fertility (Prosser et al., 2007). Interestingly, while PK2KO animals are fertile, PK2 receptor knockout animals are infertile and display abnormal estrous cycles, but the effects on reproduction seem likely mediated via a role of PK2R in GnRH neuronal migration during development (Matsumoto et al., 2006). Indeed, reproductive effects in these animals can be recovered with GnRH administration (Pitteloud et al., 2007). However, in some cases, PK2-deficient animals undergo sufficient GnRH neuronal migration but remain infertile. Because GnRH neurons do not express PK2R, there may be another uncharacterized role of PK2R signaling in reproduction (Martin et al., 2011; Pitteloud et al., 2007). Additionally, the growth factor TGF α has been shown to be released from the SCN, and may also act locally within the brain to inhibit locomotor activity upon exposure to light (Van der Zee et al., 2005). How rhythms of PK2 and TGF α are modulated to produce directionally opposite actions in diurnal vs. nocturnal animals is not yet well understood.

Other players in SCN function: GABA

γ -amino butyric acid (GABA), while synthesized throughout the brain, predominantly in interneuronal populations, is also likely used by the SCN to communicate with other neuroendocrine regulatory regions. There is substantial evidence showing fluctuations in GABAergic tone throughout the day, but whether these function as output signals, or whether GABA may be involved in synchronizing individual SCN neurons, like VIP, is unclear (Aton et al., 2006; Kalsbeek et al., 2006; Liu and Reppert, 2000; Strecker et al., 1997). Directionality of response to GABA is also dictated by the presence or absence of potassium/chloride co-transporters in neuronal cell membranes, such that a cell could be depolarized by GABA instead of hyperpolarized (Gulyas et al., 2001; Rivera et al., 1999). The mRNA for these co-transporters (KCC2 and NKCC1) has been detected in the SCN (Belenky et al., 2010), suggesting that subdivisions of the SCN may be able to respond differentially to GABA to construct more complex neuroendocrine outputs.

In the following subsections of this review, we will continue to focus both on what is currently known about how the SCN influences hypothalamic and pituitary hormone secretion, either via neuronal or humoral pathways, as well as exploring the role of endogenous cell-specific molecular clocks within each population, an investigation that is still in its nascence.

The role of circadian clocks in hypothalamic-pituitary regulation of the stress axis

A few studies have found SCN efferent projections directed at corticotrophin-releasing hormone- (CRH) expressing neurons in the PVN (Buijs et al., 1993), suggesting that circadian output signals may modulate function of the stress axis by acting directly on this hypophysiotropic factor. Indeed, CRH mRNA in the PVN has been shown to be rhythmic, with peak expression occurring near lights on, and nadir expression close to lights off (Girotti et al., 2009). The requirement of these connections, however, for normal HPA axis function are undermined by the observation that rhythmic ACTH secretion patterns persist in SCN-lesioned animals, and also by studies demonstrating that circadian glucocorticoid production can occur independently of ACTH (Oster et al., 2006; Sage et al., 2001). It is again unclear if a CRH synthetic rhythm influences the pituitary, as ACTH release is typically only weakly rhythmic, and POMC expression in corticotropes is relatively constitutive (Girotti et al., 2009). Interestingly, examination of endogenous clock activity within CRH-expressing PVN neurons reveals that *per1*, *per2*, and *bmal1* expression is almost antiphasic to expression patterns of these clock genes in the SCN, whereas clock gene expression rhythms in the anterior pituitary and adrenal cortex are quite robust, and are synchronized with the SCN (Girotti et al., 2009). Rhythms of clock gene expression in all three points of the HPA axis can be modulated by restricted feeding regimens, which additionally can act as a photic-independent zeitgeber to entrain clocks within the SCN (Girotti et al., 2009).

Intriguingly, glucocorticoid feedback from the adrenal cortex appears to have a suppressive effect on the amplitude of circadian rhythms within the hypothalamus and pituitary (Koyanagi et al., 2006; Liu et al., 2006). In adrenalectomized animals, rhythms of CRH mRNA and ACTH release are much more robust than in sham-operated controls (Koyanagi et al., 2006), and AVP expression in the PVN/SON also becomes rhythmic in the absence of glucocorticoids (Liu et al., 2006). It remains unclear whether this effect is mediated by glucocorticoid receptor (GR) binding to promoter elements of clock genes or of downstream clock-controlled genes in these distinct hypothalamic regions. Other lines of evidence, however, suggest that the SCN may exert influence over adrenocortical hormone secretion independently of CRH neurons, namely via efferent projections to brainstem nuclei that modulate E and NE release from the chromaffin medullary tissue, and that it is these innervations that influence glucocorticoid production (Buijs et al., 2003; Buijs et al., 1999). These results suggest that glucocorticoids, in addition to being modulated by adrenal-specific clocks, can feed back at the level of the hypothalamus to influence the HPA axis. It has been demonstrated that dexamethasone, a potent glucocorticoid, can reset clock gene oscillations in multiple peripheral cell types and immortalized cultured cell lines (Balsalobre et al., 2000). The *per1* gene promoter sequence contains multiple glucocorticoid-response elements (GREs), and increases in *per1* gene expression have been reported to be stimulated by glucocorticoids in multiple brain regions that express GR (Reddy et al., 2009). Interestingly, the SCN appears largely devoid of GR, suggesting that any feedback on the central clock may be mediated through intermediary GR-expressing neuronal populations (Segall et al., 2009).

The role of circadian clocks in the hypothalamic-pituitary regulation of thyroid function

Examination of secretory patterns of thyroid-stimulating hormone (TSH), thyroxine (T4), and triiodothyronine (T3) reveals several diurnal patterns, although it is unclear if these rhythms exist in the absence of light cues (Kalsbeek et al., 2000). Thyroid hormone release

patterns are also sexually dimorphic, with more robust rhythms of T3 present in females, and lower amplitudes in males (Kalsbeek et al., 2000). The SCN may exert indirect effects on the timing of thyroid hormone release, as SCN lesions resulted in altered T3 and T4 release (Kalsbeek et al., 2000). In these SCN-lesioned animals, a blunting of TSH rhythmic secretion was observed, suggesting that part of the effects of the clock on thyroid function may be mediated at the level of the pituitary (Kalsbeek et al., 2000). Although thyrotropin-releasing hormone has been found within the SCN itself, retrograde parvovirus labeling of thyroid follicular cells reveals initial trace in TRH-expressing cells of the PVN, with the SCN found to be labeled one day later (Kalsbeek et al., 2000). These retrograde tracing studies suggest that similar to SCN effects on glucocorticoid production, this central pacemaker may signal to the thyroid gland via sympathetic nervous system innervation. A separate influence over thyroid function, as well as the function of other endocrine axes, may be mediated predominantly via melatonin, an indoleamine secreted by the pineal gland. All cells within the pars tuberalis (PT) of many mammals have been shown to express TSH β (Rudolf et al., 1993), and mRNA rhythms of this glycoprotein accordingly are modulated by melatonin, peaking just after lights-off (Arai and Kameda, 2004). As circulating melatonin rises in the dark phase, it rapidly inhibits expression of both TSH β and its heteromeric α -glycoprotein subunit (α -GSU) (Aizawa et al., 2007). The regulation of TSH by melatonin may be complex, as the protein availability and mRNA expression levels oscillate out of phase in LD, with higher protein concentrated within thyrotropes even as expression levels fall (Aizawa et al., 2007). Melatonin appears to induce a decrease in TSH secretion at night, allowing the hormone to accumulate in thyrotropes, but delaying secretion until the light-induced inhibition of melatonin the following day (Aizawa et al., 2007). The respective effects of SCN afferents and endogenous clock gene expression in the pineal gland are reviewed separately in this issue, and will not be covered in depth here.

The role of circadian clock in hypothalamic-pituitary regulation of the somatolactotrophs

Growth hormone (GH) and somatostatin (SST) rhythms act in opposition to each other, presumptively regulating feeding rhythms. GH, secreted by specialized somatotroph cells in the anterior pituitary, exhibit daily rhythms of release during the subjective night that can be influenced by the sleep-wake cycle but appear to also persist under constant conditions (Vaccarino et al., 1995). Similar to the finding that TRH is expressed within the SCN, SST), a potent inhibitor of growth hormone secretion, has also been localized to the SCN, and exhibits expression rhythms with peaks during the subjective day that persist even in DD (Nishiwaki et al., 1995). Also, direct intracranial infusion of GH-releasing hormone (GHRH) into the SCN acted to advance rhythms of activity in constant darkness, but only when administered in the subjective day, raising the hypothesis that feeding cues may entrain SCN function via one or both of these somatotrophic factors (Vaccarino et al., 1995).

Prolactin (PRL), synthesized by anterior pituitary lactotrophs, and under inhibitory control by tuberoinfundibular (TIDA) dopamine release from the median eminence, also displays circadian-timed surges, but only in females in the proestrous phase, or OVX, estrogen-replaced females, as gonad removal eliminates this rhythm in females (Arey et al., 1989; Furudate, 1991; Urbanski and Ojeda, 1986). The PRL surge seems to require a functioning SCN, as SCN-lesioned females exhibit neither PRL rhythms nor synthetic rhythms of dopamine (DA) release from the TIDA tract (Kawakami and Arita, 1981; Kawakami et al., 1980). similar to what is observed with differential control of rhythmic synthesis in two AVP-producing populations, only TIDA neurons in females exhibit rhythms of DA synthesis; these patterns are not found in nigrostriatal or mesolimbic DA populations, and are only observed post-pubertally (Mai et al., 1994). SCN-derived AVP has been suggested as a PRL-modulating factor, likely acting to inhibit the activity of TIDA neurons. In addition

to the observed decreases in AVP tone just prior to the PRL surge, 3rd ventricle infusion of AVP during the surge inhibits PRL release (Palm et al., 2001b). It would appear, however, that AVP-mediated inhibition of TIDA activity is also supplemented by a PRL-stimulatory factor originating in the SCN, since SCN lesions (including AVP efferents) prevent induction of the PRL surge, even in the presence of estrogen (Pan and Gala, 1985).

In addition to control via AVP, endogenous clocks within TIDA neurons may be involved in modulating sensitivity to afferent inhibition from other neuronal sources, as some studies have demonstrated a time-dependent increase in TIDA neuronal sensitivity to cholinergic inhibition (Shieh and Pan, 1998; Shieh and Pan, 1995). Interestingly, this circadian rhythm of sensitivity persists in OVX females, suggesting that E2 is not required for an increased responsiveness to inhibitory afferent signals, but may instead be a requisite for transducing the signal of the as-yet-uncharacterized PRL-stimulating factor released (putatively rhythmically) by the SCN (Shieh and Pan, 1995). Core clock genes are expressed in TIDA neurons, and appear to play a role in normal patterns of TIDA dopamine release (Sellix et al., 2006; Sellix and Freeman, 2003). Antisense knockdown of *Per1*, *Per2*, and *Clock* in TIDA neurons decreased DA release in a dose-dependent fashion (Sellix et al., 2006). It is unclear if these endogenous clock rhythms are required to transduce either stimulatory or inhibitory afferent signals from the SCN, as these experiments have not yet been attempted in SCN-lesioned females.

In the pituitary lactotroph cells, endogenous clock oscillations may play a role in PRL synthesis itself, as E-boxes within PRL promoter regions were found to be bound by various clock components using chromatin immunoprecipitation (ChIP) techniques (Bose and Boockfor, 2010). Lactotroph clocks may then work in conjunction with rhythmic hypothalamic TIDA clocks and signals from the SCN to gate production of PRL to coincide with a decrease in dopaminergic tone. It is currently unclear how E2 may act as a permissive agent for these intracellular processes, although possibilities regarding E2-mediated effects on patterning of clock-controlled genes are explored below in the following section on clocks in the reproductive axis.

The role of circadian clocks in hypothalamic-pituitary regulation of the reproductive axis

Similar to what has been observed with the proestrus PRL surge, there is a growing body of evidence implicating circadian mechanisms in the generation of preovulatory gonadotropin surges. Like PRL, LH surge release is temporally regulated to precede E2-mediated sexual receptivity in females. The LH surge clearly reveals a circadian release pattern in the presence of E2, as constitutively elevated E2 administration via silastic capsule implants in OVX female rats and mice results in the occurrence of LH surges on multiple consecutive days, peaking toward the end of the light phase (Christian et al., 2005; Legan and Karsch, 1975). This pattern of GnRH surge secretion and neuronal activation is synchronized with activity rhythms even in the grass rat, a diurnal rodent, in which Fos expression in GnRH neurons occurs 12h out of phase compared to nocturnal animals. Interestingly, these antiphasic rhythms persist in the absence of exogenous light cues, implicating endogenous circadian mechanisms (Mahoney et al., 2004). The SCN likely plays a crucial role in generating timed signals to GnRH neurons to increase neuronal activity, and thus secretion of the peptide to stimulate LH release from pituitary gonadotrope cells. SCN lesions prevent E2-primed LH surge generation (Schwartz and Zimmerman, 1991), and this hormonal release is not rescued by transplantation of SCN grafts into SCN-lesioned females (Silver et al., 1996), suggesting that the SCN makes crucial neuronal connections that result in an increase in GnRH activity and secretion. Additionally, phenobarbital injections in hamsters, which delay the proestrus LH surge, suppress expression of the core clock gene *Period1* in

the SCN, suggesting further that disruption of endogenous clocks in this hypothalamic region affects GnRH surge secretion (Legan et al., 2009). While some earlier studies suggested that SCN efferents may synapse directly on GnRH perikarya in the mPOA, (Van der Beek et al., 1997) it is currently accepted that the anteroventral periventricular nuclei (AVPV) is a required hypothalamic region for GnRH surge generation (Chappell and Levine, 2000; Gu and Simerly, 1997; Petersen et al., 2003; Watson et al., 1995). The AVPV represents a site of neurons expressing kisspeptin (*Kiss1*), releasing the peptide that has been shown to be a potent stimulatory signal for GnRH secretion (Gottsch et al., 2004; Han et al., 2005; Irwig et al., 2004; Messenger et al., 2005). GnRH neurons express the cognate receptor for Kiss1, Kiss1R (Clarkson et al., 2008; Gottsch et al., 2006; Kauffman et al., 2007; Seminara, 2005), and *Kiss1* expression has been shown recently to be up-regulated by E2 in this region only, whereas its expression is inhibited by E2 further caudally in the arcuate nuclei (ARC) (Adachi et al., 2007; Estrada et al., 2006; Herbison, 2008; Jacobi et al., 2007; Smith, 2008a, b).

In addition to likely being stimulated by afferent signals originating in the SCN, it was recently demonstrated that a subpopulation of GnRH neurons express functional endogenous clocks, both *in vivo* and in the immortalized GnRH-secreting GT1-7 cell line (Chappell et al., 2003; Gillespie et al., 2003; Hickok and Tischkau, 2010; Olcese et al., 2003; Zhao and Kriegsfeld, 2009). Normal expression of clock gene patterning may be required for typically observed GnRH secretion, as overexpression of the mutant CLOCK-Δ19 protein in GT1-7 cells disrupts ultradian pulse patterns of GnRH release (Chappell et al., 2003). In support of this, *Clock* mutant mice expressing this dominant negative *Clock* gene are subfertile, and exhibit dramatically lengthened estrous cycles (Chappell et al., 2003; Miller et al., 2004). The extent of core clock disruption on reproductive patency, however, may be mitigated by other redundant mechanisms, since these mice are still capable of breeding, and this affect appears to be strain-dependent (Kennaway et al., 2004). Knockouts of the core clock gene *Bmal1* are infertile, although both of the aforementioned fertility deficits can be traced to problems with clock gene mutation/deletion in the periphery, affecting steroid hormone production, gametogenesis, and parturition and implantation abnormalities in females (Boden et al., 2010; Ratajczak et al., 2009).

What role, then, may endogenous clocks play in GnRH and LH surge generation? Evaluation of mice harboring a deletion of clock factors specifically targeted in GnRH neurons is underway in our laboratory, and preliminary results indicate the presence of reproductive abnormalities. Additionally, GnRH neurons may utilize endogenous clocks to time sensitivity to afferent stimulation, most likely by Kiss1. We have very recently demonstrated *in vitro* that GT1-7 cells exhibit rhythms of *Kiss1R* mRNA expression, and that this rhythm is potentiated by E2 exposure (unpublished observations). Confirming the involvement of a functional circadian clock in this phenomenon, GT1-7 subclones overexpressing CLOCK-Δ19 exhibit blunted E2-induced oscillations in *Kiss1R* expression, as well as altered responsiveness to both E2 and Kiss1. It remains unclear how steroid hormones may act to modulate rhythmic expression in neuroendocrine cell types, but E2 modulation of rhythmic gene expression is another example alongside the apparent glucocorticoid-mediated repression of AVP rhythmic expression in the PVN noted above. Future studies will require detailed promoter analyses to determine the nature of potential interactions among clock transcription factors and nuclear receptors, which could represent another novel regulatory mechanism by which the circadian clock times cellular processes.

The expression and release of Kiss1 is likely also influenced by a combination of endogenous clocks in kisspeptin neurons (K. Tonsfeldt, unpublished observations) and reception of signals from the SCN. Recent studies in rodents, which have distinct AVPV and ARC Kiss1 populations, show that expression of *Kiss1* in the AVPV is higher in the

afternoon of proestrus than in the morning, coincident with the GnRH surge (Robertson et al., 2009), and that Kiss1 neurons can be stimulated by SCN-derived AVP (Williams et al., 2011). Importantly, these effects are only found in E2-primed females, again implicating this sex steroid as a permissive factor for oscillating gene expression. Since kisspeptin neurons in the AVPV are enriched with ER α , the mechanisms by which E2 exert these effects will need to be explored further.

While clock genes are expressed with robust rhythms in the anterior pituitary, including gonadotropes, few studies have been performed on these cells specifically to draw conclusion about endogenous oscillations in these cells. The expression of clock genes in the L β T2 gonadotrope cell line can be stimulated by GnRH treatment, apparently using EGR-1 and MAPK-dependent mechanisms (Resuehr et al., 2009), and there is evidence that GnRH receptor expression is influenced by direct activation by CLOCK and BMAL1 (Resuehr et al., 2007). It is yet unclear exactly how GnRH affects rhythmic gene expression patterns in the pituitary, but one potential hypothesis posits that pulsatile GnRH primes gonadotropes to stimulate LH and FSH synthesis, although this remains largely unexplored.

The role of circadian clocks in the neurohypophysis

Circadian rhythms of AVP are readily measured in the CSF of multiple species, but these rhythms of AVP abundance appear to originate in the SCN, whereas AVP released into the circulation by the posterior pituitary from cells in the PVN/SON appear to be responsive predominantly to changes in blood volume and serum osmolality. In primates, oxytocin (OT) has been found to be rhythmic in CSF, even under constant lighting conditions (Amico et al., 1989; Artman et al., 1982; Reppert et al., 1984), but these results have been harder to replicate in rodent models, where both light exposure, prior handling, and activity associated with feeding can modulate OT secretion (Devarajan and Rusak, 2004; Mens et al., 1982; Windle et al., 1992). Interestingly, in the primate, early studies found that the CSF rhythm of OT concentration was not affected by SCN lesions, suggesting that at least in this species, OT rhythms may be mediated by an independent oscillator in the PVN/SON (Reppert et al., 1984). Recent studies in rodent models have demonstrated the presence of Per1 gene expression in a subset of neurons in the PVN, which co-localizes with neurons expressing both AVP and OT (Dzirbikova et al., 2011; Tavakoli-Nezhad et al., 2007). Interestingly, these same studies were unable to detect rhythms of AVP or OT expression in the PVN/SON, even while confirming robust rhythms of AVP in the SCN. Together, these results suggest that magnocellular neurohypophyseal cells may possess endogenous clocks, but that under most basal conditions, these clocks are not coupled to secretion of their respective neurohormones. While complex results regarding rhythms of OT and AVP secretion have been observed (Saeb-Parsy and Dyball, 2004), a recent study in OT knockouts suggests that the mere presence of this peptide appears to play a significant role in the timing of parturition, as OTKO mice delivered pups at random times throughout the subjective day, in contrast to wild-type littermates which deliver predictably within a window dictated by the LD cycle (Roizen et al., 2007).

Endocrine feedback signals to hypothalamic clocks: Example- the reproductive axis

Estrogen

The actions of estrogen on the SCN have been thoroughly examined, although results are still equivocal. Evidence for estrogenic modulation of the circadian clock has existed for some time: “Scalloping”, or phase advances of locomotor activity, was described in cycling hamsters on the day of estrous in the late 1970s (Morin et al., 1977). It was also demonstrated that exogenous estrogen treatment could shorten the period of ovariectomized

hamsters, an effect absent in gonadectomized males (Zucker et al., 1980). While scalloping and activity increase have been attributed primarily to an increase in locomotor activity by estrogen in the mPOA (Ogawa et al., 2003), other circadian processes have demonstrated estrogenic modulation. The dispute regarding the role of estrogen on the SCN stems primarily from conflicting evidence for estrogen receptors alpha (ER α) and beta (ER β) in the SCN. Studies prior to the discovery of ER β pointed to little or no estrogen receptor expression in the SCN (Simerly, 1993; Simerly et al., 1990). However, recent studies have demonstrated both ER α and ER β immunoreactivity and mRNA in the SCN of mice, humans and rats (Kruijver and Swaab, 2002; Vida et al., 2008). In general, these studies suggest that ER β expression is more robust than ER α in the SCN. However, there is some question to the quality of the ER β -IR data due to weak commercial antibodies (Vida et al., 2008). The expression of ER α and ER β is sexually dimorphic and appears more robustly in females in the dorsolateral region of the SCN (Vida et al., 2008). Interestingly, it has been demonstrated in breast carcinoma cells that ER α expression can be directly modulated by the clock gene *Per2* via physical interactions between clock components and the nuclear steroid hormone receptor (Gery et al., 2007), suggesting that the expression pattern or transcriptional capability of ER α may fluctuate throughout the day. To complicate potential scenarios of estrogenic regulation, ER α expression can be induced by E2 exposure *in vitro* (Saceda et al., 1988), which has been shown to concurrently diminish the number of ER-immunoreactive cells (Vida et al., 2008), and ER β activation can alter ER α expression (Lindberg et al., 2002), suggesting that ER expression may be elusive in the SCN due to fluctuations over the estrous and circadian cycles, as well as by effects of the estrogen receptors themselves. Alternatively, estrogen's actions on the SCN may be mediated through ER α -positive efferents that project to the SCN from the bed nucleus of the stria terminalis (BNST), preoptic area (POA), or amygdala (De La Iglesia et al., 1999).

The actions of estrogen on the SCN have been described from a molecular to a physiological level. Estrogen treatment of ovariectomized rats enhances the normal phase-advance response by increasing levels of p-CREB and Fos, suggesting that estrogen may modulate the ability of organisms to respond to environmental light changes (Abizaid et al., 2004). Estrogen treatment can also increase the expression of gap junction channels connexin-43 and connexin-32 in the SCN, which are critical to SCN coupling (Shinohara et al., 2000). The ability of estrogen to modify individual components of the molecular circadian clock was first demonstrated by Nakamura et al. (Nakamura et al., 2001), in which they demonstrated that estrogen could increase *Cry2* mRNA expression in the female rat SCN. This study was followed up by the observation that estrogen treatment advanced the rhythm of *Per2* in the SCN, but not in the cortex (Nakamura et al., 2001). Furthermore, estrogen treatment has been shown to modify the spontaneous firing rate, which is a classical diurnal rhythm in individual SCN neurons. In high concentrations (100 μ M), E2 has been shown to depolarize and increase the spontaneous firing rate (SFR) of SCN neurons in slices from pre-pubertal male rats, indicating that E2 can exert a rapid, excitatory effect directly on SCN neurons (Fatehi and Fatehi-Hassanabad, 2008). Taken altogether, there is significant evidence that estrogen can alter SCN function on both a molecular and behavioral level.

Androgen

There has been less investigation into the role of androgen receptors (AR) and androgenic effects in the SCN. Initial observations of castrated hamsters and mice showed decreased activity levels but longer active periods, which could be recovered with exogenous testosterone propionate (TP) treatment (Daan et al., 1975; Iwahana et al., 2008). AR expression in the SCN is more abundant in males and occurs predominantly in GRP-containing cells in the ventrolateral core of the SCN (Iwahana et al., 2008; Karatsoreos et al., 2007). AR expression does not vary over the circadian day, but is induced by

testosterone levels (Karatsoreos et al., 2007). Analogous to what was observed in females, castration reduces the Fos response during a phase advance, an effect that is reversible by treatment with the non-aromatizable dihydrotestosterone (DHT) (Karatsoreos et al., 2007). Interestingly, DHT treatment is not able to recover the rhythmic abnormalities seen after castration, suggesting that TP must be aromatized to estrogen to produce the locomotor effects. However, the presence of AR in the SCN and the non-locomotor actions of TP on period length suggest that the SCN can be modulated by the actions of androgens.

Other feedback hormones

Similar to the sex steroids estrogen and testosterone, glucocorticoid feedback from the adrenal cortex may also play a role in influencing clock function within the hypothalamus and pituitary. While research into GR-mediated feedback is limited, this steroid hormone appears to act at extra-SCN sites within the hypothalamus, often to repress circadian oscillations of gene expression, as covered above in the section describing the stress axis.

Control of homeostatic endocrine processes is influenced at multiple loci for adaptive advantage

Taken together, it is readily apparent that circadian clocks in the hypothalamus and anterior and posterior pituitaries play a crucial role in the timing of hormone synthesis and release, and allow for the temporal control of multiple endocrine processes to coincide with optimal organ function during times which confer maximum adaptive advantage. While the current literature draws a complex picture, it is becoming clearer that circadian control of endocrine axes likely involves multiple cell- and tissue-specific oscillators, coordinated in timing appropriate signal release, reception, and second messenger signal coupling for optimal function, all synchronized by factors from the SCN to ensure that proper functioning is temporally confined to adapt to the natural terrestrial light environment.

>The review explores circadian influences on hypothalamic and pituitary endocrine function.

>Literature regarding endocrine influences on the mammalian clock, the suprachiasmatic nucleus, tissue-level endogenous oscillators, and several endocrine rhythms are extensively covered.

> The reproductive axis is explored as an example of convergence of endocrine and circadian factors.

> The review concludes that circadian regulation is critical to most endocrine functions, but will take many more years of research to further delineate the mechanisms behind these complex processes.

References

- Abizaid A, Horvath B, Keefe DL, Leranath C, Horvath TL. Direct visual and circadian pathways target neuroendocrine cells in primates. *Eur. J. Neurosci.* 2004; 20:2767–2776. [PubMed: 15548220]
- Abraham U, Prior JL, Granados-Fuentes D, Piwnica-Worms DR, Herzog ED. Independent circadian oscillations of Period1 in specific brain areas in vivo and in vitro. *J. Neurosci.* 2005; 25:8620–8626. [PubMed: 16177029]
- Abrahamson EE, Moore RY. Suprachiasmatic nucleus in the mouse: retinal innervation, intrinsic organization and efferent projections. *Brain Res.* 2001; 916:172–191. [PubMed: 11597605]
- Adachi S, Yamada S, Takatsu Y, Matsui H, Kinoshita M, Takase K, Sugiura H, Ohtaki T, Matsumoto H, Uenoyama Y, Tsukamura H, Inoue K, Maeda K. Involvement of anteroventral periventricular

- metastin/kisspeptin neurons in estrogen positive feedback action on luteinizing hormone release in female rats. *J. Reprod. Dev.* 2007; 53:367–378. [PubMed: 17213691]
- Aizawa S, Hoshino S, Sakata I, Adachi A, Yashima S, Hattori A, Sakai T. Diurnal change of thyroid-stimulating hormone mRNA expression in the rat pars tuberalis. *J. Neuroendocrinol.* 2007; 19:839–846. [PubMed: 17927662]
- Amico JA, Levin SC, Cameron JL. Circadian rhythm of oxytocin in the cerebrospinal fluid of rhesus and cynomolgus monkeys: effects of castration and adrenalectomy and presence of a caudal-rostral gradient. *Neuroendocrinology.* 1989; 50:624–632. [PubMed: 2515462]
- Arai Y, Kameda Y. Diurnal rhythms of common alpha-subunit mRNA expression in the pars tuberalis of hamsters and chickens. *Cell Tissue Res.* 2004; 317:279–288. [PubMed: 15300491]
- Arendash GW, Gallo RV. Effect of lesions in the suprachiasmatic nucleus-retrochiasmatic area on the inhibition of pulsatile LH release induced by electrical stimulation of the midbrain dorsal raphe nucleus. *Neuroendocrinology.* 1979; 28:349–357. [PubMed: 379679]
- Arey BJ, Averill RL, Freeman ME. A sex-specific endogenous stimulatory rhythm regulating prolactin secretion. *Endocrinology.* 1989; 124:119–123. [PubMed: 2909362]
- Artman HG, Reppert SM, Perlow MJ, Swaminathan S, Oddie TH, Fisher DA. Characterization of the daily oxytocin rhythm in primate cerebrospinal fluid. *J. Neurosci.* 1982; 2:598–603. [PubMed: 7077368]
- Aton SJ, Huettnier JE, Straume M, Herzog ED. GABA and Gi/o differentially control circadian rhythms and synchrony in clock neurons. *Proc. Natl. Acad. Sci. U.S.A.* 2006; 103:19188–19193. [PubMed: 17138670]
- Balsalobre A, Brown SA, Marcacci L, Tronche F, Kellendonk C, Reichardt HM, Schutz G, Schibler U. Resetting of circadian time in peripheral tissues by glucocorticoid signaling. *Science.* 2000; 289:2344–2347. [PubMed: 11009419]
- Belenky MA, Sollars PJ, Mount DB, Alper SL, Yarom Y, Pickard GE. Cell-type specific distribution of chloride transporters in the rat suprachiasmatic nucleus. *Neuroscience.* 2010; 165:1519–1537. [PubMed: 19932740]
- Boden MJ, Varcoe TJ, Voultsios A, Kennaway DJ. Reproductive biology of female Bmal1 null mice. *Reproduction.* 2010; 139:1077–1090. [PubMed: 20200203]
- Boer GJ, van Esseveldt KE, van der Geest BA, Duindam H, Rietveld WJ. Vasopressin-deficient suprachiasmatic nucleus grafts re-instate circadian rhythmicity in suprachiasmatic nucleus-lesioned arrhythmic rats. *Neuroscience.* 1999; 89:375–385. [PubMed: 10077320]
- Boer K, Boer GJ, Swaab DF. Reproduction in Brattleboro rats with diabetes insipidus. *J. Reprod. Fertil.* 1981; 61:273–280. [PubMed: 7205775]
- Bose S, Boockfor FR. Episodes of prolactin gene expression in GH3 cells are dependent on selective promoter binding of multiple circadian elements. *Endocrinology.* 2010; 151:2287–2296. [PubMed: 20215567]
- Brudieux R, Krifi MN, Laulin JP. Release of aldosterone and corticosterone from the adrenal cortex of the Brattleboro rat in response to administration of ACTH. *J Endocrinol.* 1986; 111:375–381. [PubMed: 3027224]
- Buijs RM, Van Eden CG. The integration of stress by the hypothalamus, amygdala and prefrontal cortex: balance between the autonomic nervous system and the neuroendocrine system. *Prog. Brain. Res.* 2000; 126:117–132. [PubMed: 11105644]
- Buijs RM, van Eden CG, Goncharuk VD, Kalsbeek A. The biological clock tunes the organs of the body: timing by hormones and the autonomic nervous system. *J. Endocrinol.* 2003; 177:17–26. [PubMed: 12697033]
- Buijs RM, Markman M, Nunes-Cardoso B, Hou YX, Shinn S. Projections of the suprachiasmatic nucleus to stress-related areas in the rat hypothalamus: a light and electron microscopic study. *J. Comp. Neurol.* 1993; 335:42–54. [PubMed: 7691904]
- Buijs RM, Wortel J, Van Heerikhuize JJ, Feenstra MG, Ter Horst GJ, Romijn HJ, Kalsbeek A. Anatomical and functional demonstration of a multisynaptic suprachiasmatic nucleus adrenal (cortex) pathway. *Eur. J. Neurosci.* 1999; 11:1535–1544. [PubMed: 10215906]
- Chappell PE, Levine JE. Stimulation of gonadotropin-releasing hormone surges by estrogen. I. Role of hypothalamic progesterone receptors. *Endocrinology.* 2000; 141:1477–1485. [PubMed: 10746653]

- Chappell PE, White RS, Mellon PL. Circadian gene expression regulates pulsatile gonadotropin-releasing hormone (GnRH) secretory patterns in the hypothalamic GnRH-secreting GT1-7 cell line. *J. Neurosci.* 2003; 23:11202–11213. [PubMed: 14657179]
- Cheng MY, Bullock CM, Li C, Lee AG, Bermak JC, Belluzzi J, Weaver DR, Leslie FM, Zhou QY. Prokineticin 2 transmits the behavioural circadian rhythm of the suprachiasmatic nucleus. *Nature.* 2002; 417:405–410. [PubMed: 12024206]
- Christian CA, Moenter SM. Vasoactive intestinal polypeptide can excite gonadotropin-releasing hormone neurons in a manner dependent on estradiol and gated by time of day. *Endocrinology.* 2008; 149:3130–3136. [PubMed: 18326000]
- Christian CA, Mobley JL, Moenter SM. Diurnal and estradiol-dependent changes in gonadotropin-releasing hormone neuron firing activity. *Proc. Natl. Acad. Sci. U. S. A.* 2005; 102:15682–15687. [PubMed: 16230634]
- Clarkson J, d'Anglemont de Tassigny X, Moreno AS, Colledge WH, Herbison AE. Kisspeptin-GPR54 signaling is essential for preovulatory gonadotropin-releasing hormone neuron activation and the luteinizing hormone surge. *J. Neurosci.* 2008; 28:8691–8697. [PubMed: 18753370]
- Colwell CS, Michel S, Itri J, Rodriguez W, Tam J, Lelievre V, Hu Z, Liu X, Waschek JA. Disrupted circadian rhythms in VIP- and PHI-deficient mice. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 2003; 285:R939–R949. [PubMed: 12855416]
- Daan S, Damassa D, Pittendrigh CS, Smith ER. An effect of castration and testosterone replacement on a circadian pacemaker in mice (*Mus musculus*). *Proc. Natl. Acad. Sci. U. S. A.* 1975; 72:3744–3747. [PubMed: 1059163]
- De La Iglesia HO, Blaustein JD, Bittman EL. Oestrogen receptor-alpha-immunoreactive neurones project to the suprachiasmatic nucleus of the female Syrian hamster. *J. Neuroendocrinol.* 1999; 11:481–490. [PubMed: 10444305]
- Devarajan K, Rusak B. Oxytocin levels in the plasma and cerebrospinal fluid of male rats: effects of circadian phase, light and stress. *Neurosci. Lett.* 2004; 367:144–147. [PubMed: 15331139]
- Dolatshad H, Campbell EA, O'Hara L, Maywood ES, Hastings MH, Johnson MH. Developmental and reproductive performance in circadian mutant mice. *Hum. Reprod.* 2006; 21:68–79. [PubMed: 16210390]
- Dzirbikova Z, Kiss A, Okuliarova M, Kopkan L, Cervenka L, Zeman M. Expressions of per1 Clock Gene and Genes of Signaling Peptides Vasopressin, Vasoactive Intestinal Peptide, and Oxytocin in the Suprachiasmatic and Paraventricular Nuclei of Hypertensive TGR[mREN2]27 Rats. *Cell Mol. Neurobiol.* 2011
- Estrada KM, Clay CM, Pompolo S, Smith JT, Clarke IJ. Elevated KiSS-1 expression in the arcuate nucleus prior to the cyclic preovulatory gonadotrophin-releasing hormone/luteinising hormone surge in the ewe suggests a stimulatory role for kisspeptin in oestrogen-positive feedback. *J. Neuroendocrinol.* 2006; 18:806–809. [PubMed: 16965299]
- Fahrenkrug J, Hannibal J, Georg B. Diurnal rhythmicity of the canonical clock genes *Per1*, *Per2* and *Bmal1* in the rat adrenal gland is unaltered after hypophysectomy. *J. Neuroendocrinol.* 2008; 20:323–329. [PubMed: 18208549]
- Fatehi M, Fatehi-Hassanabad Z. Effects of 17 β -estradiol on neuronal cell excitability and neurotransmission in the suprachiasmatic nucleus of rat. *Neuropsychopharmacology.* 2008; 33:1354–1364. [PubMed: 17687263]
- Funabashi T, Shinohara K, Mitsushima D, Kimura F. Gonadotropin-releasing hormone exhibits circadian rhythm in phase with arginine-vasopressin in co-cultures of the female rat preoptic area and suprachiasmatic nucleus. *J. Neuroendocrinol.* 2000; 12:521–528. [PubMed: 10844580]
- Furudate S. Effects of estradiol on the prolactin surges and the feedback mechanisms of gonadotropins in pseudopregnant rats. *Jikken Dobutsu.* 1991; 40:203–208. [PubMed: 1904364]
- Gery S, Virk RK, Chumakov K, Yu A, Koeffler HP. The clock gene *Per2* links the circadian system to the estrogen receptor. *Oncogene.* 2007; 26:7916–7920. [PubMed: 17599055]
- Gillespie JM, Chan BP, Roy D, Cai F, Belsham DD. Expression of circadian rhythm genes in gonadotropin-releasing hormone-secreting GT1-7 neurons. *Endocrinology.* 2003; 144:5285–5292. [PubMed: 12960008]

- Gillette MU, Tischkau SA. Suprachiasmatic nucleus: the brain's circadian clock. *Recent Prog. Horm. Res.* 1999; 54:33–58. discussion 58–9. [PubMed: 10548871]
- Girotti M, Weinberg MS, Spencer RL. Diurnal expression of functional and clock-related genes throughout the rat HPA axis: system-wide shifts in response to a restricted feeding schedule. *Am. J. Physiol. Endocrinol. Metab.* 2009; 296:E888–E897. [PubMed: 19190255]
- Gottsch ML, Clifton DK, Steiner RA. Kisspeptin-GPR54 signaling in the neuroendocrine reproductive axis. *Mol. Cell Endocrinol.* 2006; 254:254–255. 91–96.
- Gottsch ML, Cunningham MJ, Smith JT, Popa SM, Acohido BV, Crowley WF, Seminara S, Clifton DK, Steiner RA. A role for kisspeptins in the regulation of gonadotropin secretion in the mouse. *Endocrinology.* 2004; 145:4073–4077. [PubMed: 15217982]
- Granados-Fuentes D, Prolo LM, Abraham U, Herzog ED. The suprachiasmatic nucleus entrains, but does not sustain, circadian rhythmicity in the olfactory bulb. *J. Neurosci.* 2004; 24:615–619. [PubMed: 14736846]
- Gu GB, Simerly RB. Projections of the sexually dimorphic anteroventral periventricular nucleus in the female rat. *J. Comp. Neurol.* 1997; 384:142–164. [PubMed: 9214545]
- Gulyas AI, Sik A, Payne JA, Kaila K, Freund TF. The KCI cotransporter, KCC2, is highly expressed in the vicinity of excitatory synapses in the rat hippocampus. *Eur. J. Neurosci.* 2001; 13:2205–2217. [PubMed: 11454023]
- Han SK, Gottsch ML, Lee KJ, Popa SM, Smith JT, Jakawich SK, Clifton DK, Steiner RA, Herbison AE. Activation of gonadotropin-releasing hormone neurons by kisspeptin as a neuroendocrine switch for the onset of puberty. *J. Neurosci.* 2005; 25:11349–11356. [PubMed: 16339030]
- Harmar AJ, Marston HM, Shen S, Spratt C, West KM, Sheward WJ, Morrison CF, Dorin JR, Piggins HD, Reubi JC, Kelly JS, Maywood ES, Hastings MH. The VPAC(2) receptor is essential for circadian function in the mouse suprachiasmatic nuclei. *Cell.* 2002; 109:497–508. [PubMed: 12086606]
- Herbison AE. Estrogen positive feedback to gonadotropin-releasing hormone (GnRH) neurons in the rodent: the case for the rostral periventricular area of the third ventricle (RP3V). *Brain Res. Rev.* 2008; 57:277–287. [PubMed: 17604108]
- Herzog ED, Tosini G. The mammalian circadian clock shop. *Semin Cell Dev Biol.* 2001; 12:295–303. [PubMed: 11463214]
- Hickok JR, Tischkau SA. In vivo circadian rhythms in gonadotropin-releasing hormone neurons. *Neuroendocrinology.* 2010; 91:110–120. [PubMed: 19786732]
- Irwig MS, Fraley GS, Smith JT, Acohido BV, Popa SM, Cunningham MJ, Gottsch ML, Clifton DK, Steiner RA. Kisspeptin activation of gonadotropin releasing hormone neurons and regulation of KiSS-1 mRNA in the male rat. *Neuroendocrinology.* 2004; 80:264–272. [PubMed: 15665556]
- Iwahana E, Karatsoreos I, Shibata S, Silver R. Gonadectomy reveals sex differences in circadian rhythms and suprachiasmatic nucleus androgen receptors in mice. *Horm. Behav.* 2008; 53:422–430. [PubMed: 18164002]
- Jacobi JS, Martin C, Nava G, Jezierski MC, Clapp C, Martinez de la Escalera G. 17-Beta-estradiol directly regulates the expression of adrenergic receptors and kisspeptin/GPR54 system in GT1-7 GnRH neurons. *Neuroendocrinology.* 2007; 86:260–269. [PubMed: 17728535]
- Jansen K, Van der Zee EA, Gerkema MP. Vasopressin immunoreactivity, but not vasoactive intestinal polypeptide, correlates with expression of circadian rhythmicity in the suprachiasmatic nucleus of voles. *Neuropeptides.* 2007; 41:207–216. [PubMed: 17553561]
- Kalamatianos T, Kallo I, Goubillon ML, Coen CW. Cellular expression of V1 α vasopressin receptor mRNA in the female rat preoptic area: effects of oestrogen. *J. Neuroendocrinol.* 2004; 16:525–533. [PubMed: 15189327]
- Kalsbeek A, Buijs RM. Output pathways of the mammalian suprachiasmatic nucleus: coding circadian time by transmitter selection and specific targeting. *Cell Tissue Res.* 2002; 309:109–118. [PubMed: 12111541]
- Kalsbeek A, van der Vliet J, Buijs RM. Decrease of endogenous vasopressin release necessary for expression of the circadian rise in plasma corticosterone: a reverse microdialysis study. *J Neuroendocrinol.* 1996; 8:299–307. [PubMed: 8861286]

- Kalsbeek A, Buijs RM, Engelmann M, Wotjak CT, Landgraf R. In vivo measurement of a diurnal variation in vasopressin release in the rat suprachiasmatic nucleus. *Brain Res.* 1995; 682:75–82. [PubMed: 7552330]
- Kalsbeek A, Fliers E, Franke AN, Wortel J, Buijs RM. Functional connections between the suprachiasmatic nucleus and the thyroid gland as revealed by lesioning and viral tracing techniques in the rat. *Endocrinology.* 2000; 141:3832–3841. [PubMed: 11014240]
- Kalsbeek A, Fliers E, Hofman MA, Swaab DF, Buijs RM. Vasopressin and the output of the hypothalamic biological clock. *J. Neuroendocrinol.* 2010; 22:362–372. [PubMed: 20088910]
- Kalsbeek A, Verhagen LA, Schallij I, Foppen E, Saboureaux M, Bothorel B, Buijs RM, Pevet P. Opposite actions of hypothalamic vasopressin on circadian corticosterone rhythm in nocturnal versus diurnal species. *Eur. J. Neurosci.* 2008; 27:818–827. [PubMed: 18279365]
- Kalsbeek A, Palm IF, La Fleur SE, Scheer FA, Perreau-Lenz S, Ruiter M, Kreier F, Cailotto C, Buijs RM. SCN outputs and the hypothalamic balance of life. *J. Biol. Rhythms.* 2006; 21:458–469. [PubMed: 17107936]
- Karatsoreos IN, Wang A, Sasanian J, Silver R. A role for androgens in regulating circadian behavior and the suprachiasmatic nucleus. *Endocrinology.* 2007; 148:5487–5495. [PubMed: 17702841]
- Kauffman AS, Clifton DK, Steiner RA. Emerging ideas about kisspeptin- GPR54 signaling in the neuroendocrine regulation of reproduction. *Trends Neurosci.* 2007; 30:504–511. [PubMed: 17904653]
- Kawakami M, Arita J. Neural structures essential for the control of prolactin surges in the female rat. *Exp. Brain Res.* 1981 Suppl 3:274–293. [PubMed: 6972876]
- Kawakami M, Arita J, Yoshioka E. Loss of estrogen-induced daily surges of prolactin and gonadotropins by suprachiasmatic nucleus lesions in ovariectomized rats. *Endocrinology.* 1980; 106:1087–1092. [PubMed: 6987050]
- Kennaway DJ, Boden MJ, Voultsios A. Reproductive performance in female Clock Delta19 mutant mice. *Reprod. Fertil. Dev.* 2004; 16:801–810. [PubMed: 15740704]
- Koyanagi S, Okazawa S, Kuramoto Y, Ushijima K, Shimeno H, Soeda S, Okamura H, Ohdo S. Chronic treatment with prednisolone represses the circadian oscillation of clock gene expression in mouse peripheral tissues. *Molecular Endocrinology.* 2006; 20:573–583. [PubMed: 16269518]
- Kruijver FP, Swaab DF. Sex hormone receptors are present in the human suprachiasmatic nucleus. *Neuroendocrinology.* 2002; 75:296–305. [PubMed: 12006783]
- Legan SJ, Karsch FJ. A daily signal for the LH surge in the rat. *Endocrinology.* 1975; 96:57–62. [PubMed: 1167356]
- Legan SJ, Donoghue KM, Franklin KM, Duncan MJ. Phenobarbital blockade of the preovulatory luteinizing hormone surge: association with phase-advanced circadian clock and altered suprachiasmatic nucleus Period1 gene expression. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 2009; 296:R1620–R1630. [PubMed: 19297538]
- Lehman MN, Silver R, Gladstone WR, Kahn RM, Gibson M, Bittman EL. Circadian rhythmicity restored by neural transplant. Immunocytochemical characterization of the graft and its integration with the host brain. *J. Neurosci.* 1987; 7:1626–1638. [PubMed: 3598638]
- Lehman MN, LeSauter J, Kim C, Berriman SJ, Tresco PA, Silver R. How do fetal grafts of the suprachiasmatic nucleus communicate with the host brain? *Cell Transplant.* 1995; 4:75–81. [PubMed: 7728336]
- LeSauter J, Silver R. Output signals of the SCN. *Chronobiol. Int.* 1998; 15:535–550. [PubMed: 9787940]
- LeSauter J, Lehman MN, Silver R. Restoration of circadian rhythmicity by transplants of SCN "micropunches". *J. Biol. Rhythms.* 1996; 11:163–171. [PubMed: 8744243]
- Lindberg MK, Weihua Z, Andersson N, Moverare S, Gao H, Vidal O, Erlandsson M, Windahl S, Andersson G, Lubahn DB, Carlsten H, Dahlman-Wright K, Gustafsson JA, Ohlsson C. Estrogen receptor specificity for the effects of estrogen in ovariectomized mice. *J. Endocrinol.* 2002; 174:167–178. [PubMed: 12176656]
- Liu C, Reppert SM. GABA synchronizes clock cells within the suprachiasmatic circadian clock. *Neuron.* 2000; 25:123–128. [PubMed: 10707977]

- Liu RY, Unmehopa UA, Zhou JN, Swaab DF. Glucocorticoids suppress vasopressin gene expression in human suprachiasmatic nucleus. *J. Steroid Biochem. Mol. Biol.* 2006; 98:248–253. [PubMed: 16483767]
- Mahoney MM, Sisk C, Ross HE, Smale L. Circadian regulation of gonadotropin-releasing hormone neurons and the preovulatory surge in luteinizing hormone in the diurnal rodent, *Arvicanthis niloticus*, and in a nocturnal rodent, *Rattus norvegicus*. *Biol. Reprod.* 2004; 70:1049–1054. [PubMed: 14668212]
- Mai LM, Shieh KR, Pan JT. Circadian changes of serum prolactin levels and tuberoinfundibular dopaminergic neuron activities in ovariectomized rats treated with or without estrogen: the role of the suprachiasmatic nuclei. *Neuroendocrinology.* 1994; 60:520–526. [PubMed: 7845542]
- Martin C, Balasubramanian R, Dwyer AA, Au MG, Sidis Y, Kaiser UB, Seminara SB, Pitteloud N, Zhou QY, Crowley WF Jr. The Role of the Prokineticin 2 Pathway in Human Reproduction: Evidence from the Study of Human and Murine Gene Mutations. *Endocr. Rev.* 2011
- Matsumoto S, Basil J, Jetton AE, Lehman MN, Bittman EL. Regulation of the phase and period of circadian rhythms restored by suprachiasmatic transplants. *J. Biol. Rhythms.* 1996; 11:145–162. [PubMed: 8744242]
- Matsumoto S, Yamazaki C, Masumoto KH, Nagano M, Naito M, Soga T, Hiyama H, Matsumoto M, Takasaki J, Kamohara M, Matsuo A, Ishii H, Kobori M, Katoh M, Matsushime H, Furuichi K, Shigeyoshi Y. Abnormal development of the olfactory bulb and reproductive system in mice lacking prokineticin receptor PKR2. *Proc. Natl. Acad. Sci U. S. A.* 2006; 103:4140–4145. [PubMed: 16537498]
- Mens WB, Andringa-Bakker EA, Van Wimersma Greidanus TB. Changes in cerebrospinal fluid levels of vasopressin and oxytocin of the rat during various light-dark regimes. *Neurosci. Lett.* 1982; 34:51–56. [PubMed: 7162697]
- Messenger S, Chatzidaki EE, Ma D, Hendrick AG, Zahn D, Dixon J, Thresher RR, Malinge I, Lomet D, Carlton MB, Colledge WH, Caraty A, Aparicio SA. Kisspeptin directly stimulates gonadotropin-releasing hormone release via G protein-coupled receptor 54. *Proc. Natl. Acad. Sci. U. S. A.* 2005; 102:1761–1766. [PubMed: 15665093]
- Miller BH, Olson SL, Turek FW, Levine JE, Horton TH, Takahashi JS. Circadian clock mutation disrupts estrous cyclicity and maintenance of pregnancy. *Curr. Biol.* 2004; 14:1367–1373. [PubMed: 15296754]
- Miller BH, Olson SL, Levine JE, Turek FW, Horton TH, Takahashi JS. Vasopressin regulation of the proestrous luteinizing hormone surge in wild-type and Clock mutant mice. *Biol. Reprod.* 2006; 75:778–784. [PubMed: 16870944]
- Moore RY, Speh JC. GABA is the principal neurotransmitter of the circadian system. *Neurosci. Lett.* 1993; 150:112–116. [PubMed: 8097023]
- Moore RY, Speh JC, Leak RK. Suprachiasmatic nucleus organization. *Cell Tissue Res.* 2002; 309:89–98. [PubMed: 12111539]
- Morin LP, Allen CN. The circadian visual system, 2005. *Brain Res. Rev.* 2006; 51:1–60. [PubMed: 16337005]
- Morin LP, Fitzgerald KM, Zucker I. Estradiol shortens the period of hamster circadian rhythms. *Science.* 1977; 196:305–307. [PubMed: 557840]
- Nakamura TJ, Shinohara K, Funabashi L, Kimura F. Effect of estrogen on the expression of Cry1 and Cry2 mRNAs in the suprachiasmatic nucleus of female rats. *Neurosci. Res.* 2001; 41:251–255. [PubMed: 11672838]
- Nishiwaki L, Okamura H, Kanemasa K, Inatomi T, Ibata Y, Fukuhara C, Inouye ST. Differences of somatostatin mRNA in the rat suprachiasmatic nucleus under light-dark and constant dark conditions: an analysis by in situ hybridization. *Neurosci. Lett.* 1995; 197:231–234. [PubMed: 8552306]
- Ogawa S, Chan J, Gustafsson JA, Korach KS, Pfaff DW. Estrogen increases locomotor activity in mice through estrogen receptor alpha: specificity for the type of activity. *Endocrinology.* 2003; 144:230–239. [PubMed: 12488349]

- Olcese J, Domagalski R, Bednorz A, Weaver DR, Urbanski HF, Reuss S, Middendorff R. Expression and regulation of mPer1 in immortalized GnRH neurons. *Neuroreport*. 2003; 14:613–618. [PubMed: 12657897]
- Oster H, Damerow S, Kiessling S, Jakubcakova V, Abraham D, Tian J, Hoffmann MW, Eichele G. The circadian rhythm of glucocorticoids is regulated by a gating mechanism residing in the adrenal cortical clock. *Cell Metab*. 2006; 4:163–173. [PubMed: 16890544]
- Palm IF, Van Der Beek EM, Wiegant VM, Buijs RM, Kalsbeek A. Vasopressin induces a luteinizing hormone surge in ovariectomized, estradiol-treated rats with lesions of the suprachiasmatic nucleus. *Neuroscience*. 1999; 93:659–666. [PubMed: 10465449]
- Palm IF, van der Beek EM, Wiegant VM, Buijs RM, Kalsbeek A. The stimulatory effect of vasopressin on the luteinizing hormone surge in ovariectomized, estradiol-treated rats is time-dependent. *Brain Res*. 2001a; 901:109–116. [PubMed: 11368957]
- Palm IF, van der Beek EM, Swarts HJ, van der Vliet J, Wiegant VM, Buijs RM, Kalsbeek A. Control of the estradiol-induced prolactin surge by the suprachiasmatic nucleus. *Endocrinology*. 2001b; 142:2296–2302. [PubMed: 11356675]
- Pan JT, Gala RR. Central nervous system regions involved in the estrogen-induced afternoon prolactin surge. I. Lesion studies. *Endocrinology*. 1985; 117:382–387. [PubMed: 4006863]
- Panda S, Antoch MP, Miller BH, Su AI, Schook AB, Straume M, Schultz PG, Kay SA, Takahashi JS, Hogenesch JB. Coordinated transcription of key pathways in the mouse by the circadian clock. *Cell*. 2002; 109:307–320. [PubMed: 12015981]
- Petersen SL, Ottem EN, Carpenter CD. Direct and indirect regulation of gonadotropin-releasing hormone neurons by estradiol. *Biol. Reprod*. 2003; 69:1771–1778. [PubMed: 12890720]
- Pitteloud N, Zhang C, Pignatelli D, Li JD, Raivio L, Cole LW, Plummer L, Jacobson-Dickman EE, Mellon PL, Zhou QY, Crowley WF Jr. Loss-of-function mutation in the prokineticin 2 gene causes Kallmann syndrome and normosmic idiopathic hypogonadotropic hypogonadism. *Proc. Natl. Acad. Sci. U.S.A.* 2007; 104:17447–17452. [PubMed: 17959774]
- Preitner N, Brown S, Ripperger J, Le-Minh N, Damiola F, Schibler U. Orphan nuclear receptors, molecular clockwork, and the entrainment of peripheral oscillators. *Novartis Found. Symp*. 2003; 253:89–99. discussion 99–109. [PubMed: 14712916]
- Prosser HM, Bradley A, Chesham JE, Ebling FJ, Hastings MH, Maywood ES. Prokineticin receptor 2 (Prokr2) is essential for the regulation of circadian behavior by the suprachiasmatic nuclei. *Proc. Natl. Acad. Sci. U. S. A.* 2007; 104:648–653. [PubMed: 17202262]
- Ratajczak CK, Boehle KL, Muglia LJ. Impaired steroidogenesis and implantation failure in *Bmal1* $-/-$ mice. *Endocrinology*. 2009; 150:1879–1885. [PubMed: 19056819]
- Reddy TE, Pauli F, Sprouse RO, Neff NF, Newberry KM, Garabedian MJ, Myers RM. Genomic determination of the glucocorticoid response reveals unexpected mechanisms of gene regulation. *Genome Res*. 2009; 19:2163–2171. [PubMed: 19801529]
- Reppert SM. Cellular and molecular basis of circadian timing in mammals. *Semin Perinatol*. 2000; 24:243–246. [PubMed: 10975430]
- Reppert SM, Artman HG, Swaminathan S, Fisher DA. Vasopressin exhibits a rhythmic daily pattern in cerebrospinal fluid but not in blood. *Science*. 1981; 213:1256–1257. [PubMed: 7268432]
- Reppert SM, Perlow MJ, Artman HG, Ungerleider LG, Fisher DA, Klein DC. The circadian rhythm of oxytocin in primate cerebrospinal fluid: effects of destruction of the suprachiasmatic nuclei. *Brain Res*. 1984; 307:384–387. [PubMed: 6467006]
- Resuehr D, Wildemann U, Sikes H, Olcese J. E-box regulation of gonadotropin-releasing hormone (GnRH) receptor expression in immortalized gonadotrope cells. *Mol. Cell Endocrinol*. 2007; 278:36–43. [PubMed: 17928134]
- Resuehr HE, Resuehr D, Olcese J. Induction of mPer1 expression by GnRH in pituitary gonadotrope cells involves EGR-1. *Mol. Cell Endocrinol*. 2009; 311:120–125. [PubMed: 19616057]
- Rivera C, Voipio J, Payne JA, Ruusuvuori E, Lahtinen H, Lamsa K, Pirvola U, Saarma M, Kaila K. The K⁺/Cl⁻ co-transporter KCC2 renders GABA hyperpolarizing during neuronal maturation. *Nature*. 1999; 397:251–255. [PubMed: 9930699]

- Robertson JL, Clifton DK, de la Iglesia HO, Steiner RA, Kauffman AS. Circadian regulation of Kiss1 neurons: implications for timing the preovulatory gonadotropin-releasing hormone/luteinizing hormone surge. *Endocrinology*. 2009; 150:3664–3671. [PubMed: 19443569]
- Roizen J, Luedke CE, Herzog ED, Muglia LJ. Oxytocin in the circadian timing of birth. *PLoS One*. 2007; 2:e922. [PubMed: 17895964]
- Rudolf T, Filler T, Wittkowski W. Pars tuberalis specific cells within the pars distalis of the adenohypophysis. An ontogenetic study. *Ann. Anat.* 1993; 175:171–176. [PubMed: 8489037]
- Saceda M, Lippman ME, Chambon P, Lindsey RL, Ponglikitmongkol M, Puente M, Martin MB. Regulation of the estrogen receptor in MCF-7 cells by estradiol. *Molecular Endocrinology*. 1988; 2:1157–1162. [PubMed: 3216858]
- Saeb-Parsy K, Dyball RE. Responses of cells in the rat supraoptic nucleus in vivo to stimulation of afferent pathways are different at different times of the light/dark cycle. *J. Neuroendocrinol.* 2004; 16:131–137. [PubMed: 14763999]
- Sage D, Maurel D, Bosler O. Involvement of the suprachiasmatic nucleus in diurnal ACTH and corticosterone responsiveness to stress. *Am. J. Physiol. Endocrinol. Metab.* 2001; 280:E260–E269. [PubMed: 11158929]
- Sato TK, Panda S, Miraglia LJ, Reyes TM, Rudic RD, McNamara P, Naik KA, FitzGerald GA, Kay SA, Hogenesch JB. A functional genomics strategy reveals *Rorα* as a component of the mammalian circadian clock. *Neuron*. 2004; 43:527–537. [PubMed: 15312651]
- Schmale H, Richter D. Single base deletion in the vasopressin gene is the cause of diabetes insipidus in Brattleboro rats. *Nature*. 1984; 308:705–709. [PubMed: 6717565]
- Schwartz WJ, Zimmerman P. Lesions of the suprachiasmatic nucleus disrupt circadian locomotor rhythms in the mouse. *Physiol. Behav.* 1991; 49:1283–1287. [PubMed: 1896511]
- Schwartz WJ, Coleman RJ, Reppert SM. A daily vasopressin rhythm in rat cerebrospinal fluid. *Brain Res.* 1983; 263:105–112. [PubMed: 6839163]
- Segall LA, Milet A, Tronche F, Amir S. Brain glucocorticoid receptors are necessary for the rhythmic expression of the clock protein, *PERIOD2*, in the central extended amygdala in mice. *Neurosci. Lett.* 2009; 457:58–60. [PubMed: 19429162]
- Sellix MT, Freeman ME. Circadian rhythms of neuroendocrine dopaminergic neuronal activity in ovariectomized rats. *Neuroendocrinology*. 2003; 77:59–70. [PubMed: 12624542]
- Sellix MT, Egli M, Poletini MO, McKee DT, Bosworth MD, Fitch CA, Freeman ME. Anatomical and functional characterization of clock gene expression in neuroendocrine dopaminergic neurons. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 2006; 290:R1309–R1323. [PubMed: 16373438]
- Seminara SB. Metastin and its G protein-coupled receptor, GPR54: critical pathway modulating GnRH secretion. *Front Neuroendocrinol.* 2005; 26:131–138. [PubMed: 16309735]
- Shieh K, Pan J. Ontogeny of the diurnal rhythm of tuberoinfundibular dopaminergic neuronal activity in peripubertal female rats: possible involvement of cholinergic and opioidergic systems. *Neuroendocrinology*. 1998; 68:395–402. [PubMed: 9873203]
- Shieh KR, Pan JT. An endogenous cholinergic rhythm may be involved in the circadian changes of tuberoinfundibular dopaminergic neuron activity in ovariectomized rats treated with or without estrogen. *Endocrinology*. 1995; 136:2383–2388. [PubMed: 7750459]
- Shinohara K, Funabashi T, Mitushima D, Kimura F. Effects of estrogen on the expression of connexin32 and connexin43 mRNAs in the suprachiasmatic nucleus of female rats. *Neurosci. Lett.* 2000; 286:107–110. [PubMed: 10825648]
- Silver R, LeSauter J, Tresco PA, Lehman MN. A diffusible coupling signal from the transplanted suprachiasmatic nucleus controlling circadian locomotor rhythms. *Nature*. 1996; 382:810–813. [PubMed: 8752274]
- Simerly RB. Distribution and regulation of steroid hormone receptor gene expression in the central nervous system. *Adv. Neurol.* 1993; 59:207–226. [PubMed: 8380515]
- Simerly RB, Chang C, Muramatsu M, Swanson LW. Distribution of androgen and estrogen receptor mRNA-containing cells in the rat brain: an in situ hybridization study. *J. Comp. Neurol.* 1990; 294:76–95. [PubMed: 2324335]
- Smith JT. Sex steroid control of hypothalamic Kiss1 expression in sheep and rodents: Comparative aspects. *Peptides*. 2008a

- Smith JT. Kisspeptin signalling in the brain: steroid regulation in the rodent and ewe. *Brain Res. Rev.* 2008b; 57:288–298. [PubMed: 17509691]
- Smith MJ, Jiennes L, Wise PM. Localization of the VIP2 receptor protein on GnRH neurons in the female rat. *Endocrinology.* 2000; 141:4317–4320. [PubMed: 11089568]
- Strecker GJ, Wuarin JP, Dudek FE. GABAA-mediated local synaptic pathways connect neurons in the rat suprachiasmatic nucleus. *J. Neurophysiol.* 1997; 78:2217–2220. [PubMed: 9325388]
- Tavakoli-Nezhad M, Tao-Cheng JH, Weaver DR, Schwartz WJ. PER1-like immunoreactivity in oxytocin cells of the hamster hypothalamo-neurohypophyseal system. *J. Biol. Rhythms.* 2007; 22:81–84. [PubMed: 17229927]
- Urbanski HF, Ojeda SR. Development and amplification of mid-afternoon surges of prolactin secretion in ovariectomized immature rats. *J. Endocrinol.* 1986; 110:361–366. [PubMed: 3746168]
- Vaccarino FJ, Sovran P, Baird JP, Ralph MR. Growth hormone-releasing hormone mediates feeding-specific feedback to the suprachiasmatic circadian clock. *Peptides.* 1995; 16:595–598. [PubMed: 7479289]
- Van der Beek EM, Horvath TL, Wiegant VM, Van den Hurk R, Buijs RM. Evidence for a direct neuronal pathway from the suprachiasmatic nucleus to the gonadotropin-releasing hormone system: combined tracing and light and electron microscopic immunocytochemical studies. *J. Comp. Neurol.* 1997; 384:569–579. [PubMed: 9259490]
- Van der Zee EA, Roman V, Ten Brinke O, Meerlo P. TGF α and AVP in the mouse suprachiasmatic nucleus: anatomical relationship and daily profiles. *Brain Res.* 2005; 1054:159–166. [PubMed: 16051199]
- Vida B, Hrabovszky E, Kalamatianos L, Coen CW, Liposits Z, Kallo I. Oestrogen receptor alpha and beta immunoreactive cells in the suprachiasmatic nucleus of mice: distribution, sex differences and regulation by gonadal hormones. *J. Neuroendocrinol.* 2008; 20:1270–1277. [PubMed: 18752649]
- Vida B, Deli L, Hrabovszky E, Kalamatianos T, Caraty A, Coen CW, Liposits Z, Kallo I. Evidence for suprachiasmatic vasopressin neurones innervating kisspeptin neurones in the rostral periventricular area of the mouse brain: regulation by oestrogen. *J Neuroendocrinol.* 2010; 22:1032–1039. [PubMed: 20584108]
- Watson RE Jr, Langub MC Jr, Engle MG, Maley BE. Estrogen-receptive neurons in the anteroventral periventricular nucleus are synaptic targets of the suprachiasmatic nucleus and peri-suprachiasmatic region. *Brain Res.* 1995; 689:254–264. [PubMed: 7583329]
- Weaver DR. The suprachiasmatic nucleus: a 25-year retrospective. *J Biol Rhythms.* 1998; 13:100–112. [PubMed: 9554572]
- Weick RF, Stobie KM. Vasoactive intestinal peptide inhibits the steroid-induced LH surge in the ovariectomized rat. *J Endocrinol.* 1992; 133:433–437. [PubMed: 1613444]
- Wiegand SJ, Terasawa E, Bridson WE, Goy RW. Effects of discrete lesions of preoptic and suprachiasmatic structures in the female rat. Alterations in the feedback regulation of gonadotropin secretion. *Neuroendocrinology.* 1980; 31:147–157. [PubMed: 6771669]
- Williams WP 3rd, Jarjisian SG, Mikkelsen JD, Kriegsfeld LJ. Circadian control of kisspeptin and a gated GnRH response mediate the preovulatory luteinizing hormone surge. *Endocrinology.* 2011; 152:595–606. [PubMed: 21190958]
- Windle RJ, Forsling ML, Guzek JW. Daily rhythms in the hormone content of the neurohypophysial system and release of oxytocin and vasopressin in the male rat: effect of constant light. *J. Endocrinol.* 1992; 133:283–290. [PubMed: 1613430]
- Zhang C, Truong KK, Zhou QY. Efferent projections of prokineticin 2 expressing neurons in the mouse suprachiasmatic nucleus. *PLoS One.* 2009; 4:e7151. [PubMed: 19784373]
- Zhao S, Kriegsfeld LJ. Daily changes in GT1-7 cell sensitivity to GnRH secretagogues that trigger ovulation. *Neuroendocrinology.* 2009; 89:448–457. [PubMed: 19141986]
- Zucker I, Fitzgerald KM, Morin LP. Sex differentiation of t-e circadian system in the golden hamster. *Am. J. Physiol.* 1980; 238:R97–R101. [PubMed: 7356053]