Interactions between endocrine and circadian systems

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Abstract

In most species, endogenous circadian clocks regulate 24-h rhythms of behavior and physiology. Clock disruption has been associated with decreased cognitive performance and increased propensity to develop obesity, diabetes, and cancer. Many hormonal factors show robust diurnal secretion rhythms, some of which are involved in mediating clock output from the brain to peripheral tissues. In this review, we describe the mechanisms of clock–hormone interaction in mammals, the contribution of different tissue oscillators to hormonal regulation, and how changes in circadian timing impinge on endocrine signalling and downstream processes. We further summarize recent findings suggesting that hormonal signals may feed back on circadian regulation and how this crosstalk interferes with physiological and metabolic homeostasis.

Introduction

We live in an environment shaped by various geophysical rhythms. Arguably, one of the most prominent of these rhythms is the succession of day and night. The profound environmental changes brought about by the rotation of the Earth around its axis have promoted the development of endogenous timekeepers that enable an organism to reliably predict the time of day and adjust behavior and physiology accordingly. Not surprisingly, large aspects of our endocrine system are tightly connected to the circadian (from Latin circa diem – about a day) clock. With recent advances in molecular life sciences and medicine, we now realize that this interaction is not only unilateral but also includes endocrine feedback on circadian clock function. This review recapitulates some of the research leading to the picture we have today of the circadian clock system in mammals and provides an overview about the most prominent connection points between circadian and endocrine regulation.

The master circadian pacemaker

In the 1970s, we witnessed a significant breakthrough in the field of chronobiology – the identification of the anatomical entity underlining the mammalian circadian rhythm. It was discovered that information about the external light–dark cycle was passed via the retinohypothalamic tract (RHT) to not only sensory input integrating centers in the thalamus, but also to the hypothalamic suprachiasmatic nucleus (SCN), hinting at the existence of a novel photic input processing hub in the brain (Sousa-Pinto & Castro-Correia 1970, Hendrickson et al. 1972, Moore & Lenn 1972). The SCN is a bilaterally paired structure with high cell body density located adjacent to the third ventricle and directly atop the optic chiasm. It comprises about 50 000 neurons in humans and about 20 000 neurons in rodents. A series of electrical lesion studies provided unequivocal evidence for the
critical role of SCN in the generation of mammalian circadian rhythms. Animals with ablated SCN become behaviorally and physiologically arrhythmic (Moore & Eichler 1972, Stephan & Zucker 1972). Critically, transplanting isolated SCN tissue into SCN-lesioned animals restores circadian rhythmicity (Ralph et al. 1990), and the restored behavioral rhythm of recipients is determined by the donor’s intrinsic period, indicating that the SCN is indeed the master pacemaker generating circadian timing information in animals (Ralph et al. 1990). Brain slice explants of the SCN, but not of other tested brain areas including the cerebral cortex and arcuate nucleus, display robust circadian oscillations in firing rate in vitro, suggesting that the rhythmicity of the SCN is autonomous and self-sustaining (Green & Gillette 1982, Groos & Hendriks 1982).

The molecular clockwork

The *Period* (or *Per*) gene was the first discovered clock gene (Konopka & Benzer 1971), which is conserved from fruit flies to humans. Mutations of *Per* in flies alters the circadian patterns of pupae eclosion and locomotor activity (Konopka & Benzer 1971). Since then, many more clock genes have been identified in different organisms (Zhang & Kay 2010). In the past decades, our knowledge of the molecular clockwork has been significantly expanded. The current model suggests that the central mechanism of the mammalian molecular clock is composed of a set of clock genes intertwined with a delayed interlocking transcriptional–translational feedback loop (TTL), coupled to several auxiliary mechanisms reinforcing robustness and stability (Zhang & Kay 2010). The positive limb of this TTL comprises two basic helix–loop–helix transcription factors, circadian locomotor output cycles kaput (CLOCK), and brain and muscle aryl hydrocarbon receptor nuclear translocator such as BMAL1 or ARNTL. Both form heterodimers via their PER-ARNT-SIM (PAS) domains and activate E-box-element-containing genes by recruiting transcriptional co-activators, chromatin-modifying proteins, and RNA polymerase II. In certain tissues such as the forebrain or the vasculature, CLOCK is functionally replaced by its homolog neuronal PAS domain protein 2 (NPAS2; McNamara et al. 2001, Reick et al. 2001). *Period* (*Per1–3*) and *Cryptochrome* (*Cry1/2*) constitute the negative limb of the TTL. CLOCK–BMAL1 complexes activate the transcription of *Per* and *Cry* genes during the subjective day. PERs and CRYs translocate into the nucleus and form inhibitory complexes. With progress of the circadian cycle, PER/CRY complexes accumulate and so does their inhibitory effect on CLOCK–BMAL1 activity, shutting down *Per* and *Cry* transcription during the night (Lee et al. 2001). The progressive degradation of PER/CRY complexes throughout the night toward the morning releases the inhibition on CLOCK–BMAL1 transcriptional activity and thereby, completes the negative feedback loop of the circadian clock (Fig. 1).

Additional auxiliary TTLs enhance the stability of the core clock TTL and translate time-of-day information into physiological signals via transcriptional control of clock target genes (Zhang & Kay 2010). Such loops include the nuclear receptors REV–ERBα and REV–ERBβ (NR1D1 and NR1D2) and RORα (NR1F1) which regulate Bmal1 expression via a retinoid orphan receptor responsive elements (Preitner et al. 2002, Ueda et al. 2002, Sato et al. 2004, Akashi & Takumi 2005, Liu et al. 2008), as well as the PAR basic leucine zipper proteins D-box albumin-binding protein and E4 promoter-binding protein (E4BP; NFIL3) (Cowell 2002, Ripperger & Schibler 2006) which feed-back on the expression of *Per* genes via D-box promoter elements (Ripperger et al. 2000).

**Extra-SCN oscillators**

The functional molecular clockwork exists not only in SCN neurons, but (almost) every single cell in the brain and periphery is capable of oscillating in a circadian manner. Molecular clock rhythms have been shown even in cultured cells, such as immortalized fibroblast cells which display robust oscillations of clock gene expression.
after a brief stimulation with high concentrations of serum (Balsalobre et al. 1998). Using single cell imaging techniques, Nagoshi et al. (2004) showed that each fibroblast cell possesses a sustained circadian clock, although at the population level the rhythm dampens quickly as a consequence of a gradual desynchronization between individual cells with different endogenous periods. Application of synchronizing agents such as serum, forskolin, glucocorticoids (GCs), or phorbol esters re-synchronizes individual cells, yielding a transiently phase-coherent population (Nagoshi et al. 2004). These data suggest that the cellular clocks in extra-SCN tissues are actually self-sustained and autonomous in nature, but fail to maintain coherence at the population level, in contrast to the SCN (see below). Similarly, tissue explants from a wide array of peripheral organs including heart, lung, kidney, liver, spleen, pancreas, stomach, cornea, thyroid gland, and adrenal gland show clock gene expression rhythms, but the overall rhythm dampens quickly due to the gradual loss of coherence between individual cells (Yamazaki et al. 2000, Yoo et al. 2004). Similar results have been obtained from tissue explants from various brain regions (Abe et al. 2002, Guilding & Piggins 2007).

SCN communication

In order to achieve a biologically relevant circadian rhythm, it is of utmost importance that individual cells of a specific tissue are synchronized to the external environment. The circadian oscillation of an SCN neuron is coupled to its neighbouring cells in an action-potential-dependent manner (Welsh et al. 1995). This intercellular coupling property bestows the superior robustness and resilience of the SCN circadian rhythm. For example, the SCN explant cultures exhibit robust and persistent circadian oscillations in electrophysiological activity and clock gene expression for an extended period of time, while rhythms in slices from other brain regions and peripheral tissues dampen after a couple of days (Guilding & Piggins 2007, Guilding et al. 2009). SCN explant rhythms are also more resistant to clock gene mutations (Liu et al. 2007) and temperature fluctuations (Abraham et al. 2010, Buhr et al. 2010). One major function of the SCN is to synchronize internal biological processes to external time cues. The SCN receives photic information from both classical photoreceptors – cone and rod cells – as well as melanopsin-containing retinal ganglion cells via the RHT (Hankins et al. 2008). In turn, the SCN innovates other regions of the brain, in particular the hypothalamus. The paraventricular hypothalamic nucleus (PVN) is one of the major loci relaying circadian information from the SCN to the rest of the body (Saeb-Parsy et al. 2000). The PVN is an important integrating center for energy homeostasis, projecting parvocellular neurons to the median eminence to control the release of hormones such as adrenocorticotropic hormone (ACTH) and thyroid-stimulating hormone in the anterior pituitary. The PVN also innervates the sympathetic limb of the autonomous nervous system, thereby allowing the SCN to regulate the sympathetic tone of the body over the course of the day (Buijs et al. 2003). Further projections of the SCN to the dorsomedial hypothalamic nucleus (Luiten et al. 1987), the nucleus accumbens (Phillipson & Griffiths 1985) and the paraventricular thalamic nucleus (Watts & Swanson 1987, Watts et al. 1987) have been described. These connections enable the SCN to exert influence on a plethora of physiological processes such as the reward system, feeding-fasting cycles, cognitive function, locomotor activity, and body temperature (Dibner et al. 2010). In addition to direct neural connections, the SCN secretes diffusible factors, which can function as timing cues. Membrane-encapsulated foetal SCN tissue grafts, which allow only low-molecular-weight particles to diffuse, can restore the rhythmicity of locomotor activity in SCN-lesioned hamsters in the absence of axonal outgrowth (Silver et al. 1996). Transforming growth factor-α (Kramer et al. 2001, Li et al. 2002), prokineticin 2 (PK2; Cheng et al. 2002), and cardiotropin-like cytokine (Kraves & Weitz 2006) have been implicated as SCN-secreted peptides capable of regulating behavioral rhythmicity. Given the physical proximity of the SCN to the third ventricle, these diffusible factors may help propagate the time-of-day information to more remote brain regions via the cerebrospinal ventricular system.

Endocrine rhythms: clock vs behavioral regulation

It has been long been appreciated that the circulating levels of a number of hormones vary over the 24-h cycle (Andrews & Folk 1964). Such a diurnal rhythm of a hormone or metabolite can either be a manifestation of circadian clock control or a direct or indirect response to an environmental rhythm such as the light–dark cycle. Two methodologies have been developed to track down the relative contribution of the endogenous circadian clock to diurnal hormonal rhythms in humans, namely constant routine (CR) and forced desynchrony (FD) protocols. The CR protocol aims to minimize the effects of external time cues.
and behavioral variables by equally distributing such variables across the circadian cycle. Depending on individual experimental goals, it routinely demands constant wakefulness, limited physical activity, equally distributed isocaloric snacks or constant glucose infusion and constant dim light condition (Mills et al. 1978). The FD protocol employs a strategy which schedules a behavioral cycle beyond the entrainable range of the circadian clock (i.e. significantly longer or shorter than 24 h) in a constant dim light environment, resulting in the free running of the endogenous circadian clock. This leads to an even distribution of certain behavioral variables in question across different phase of the circadian cycle (Kleitman 1970).

The clarification of the relative contribution of endogenous and exogenous input to the diurnal rhythm of a physiological system is of particular relevance for understanding the influence of our modern 24/7 lifestyle on the well-being of individuals. Owing to social constraints many rhythmic behaviors such as sleep/wake and food intake/fasting cycles often no longer align with their endogenous pattern controlled by the circadian clock (Scheer et al. 2009, Beccuti & Pannain 2011). Shift workers are an obvious example. Several epidemiological studies indicate that shift workers are predisposed for metabolic disorders and even cancer (Ohayon et al. 2002, Akerstedt 2003). Thus, better knowledge for the mechanistic link between circadian misalignment and hormonal deregulation may help with the development of novel medical regimes to prevent or intervene in the metabolic consequences of shift work.

GCs and melatonin represent two well-studied hormonal systems that are subject to direct and dominant regulation by the circadian clock. The circulating levels of both display robust diurnal patterns (Migeon et al. 1956, Ralph et al. 1971). Using the CR and FD experimental protocols, the secretion rhythms of cortisol (el-Haj Fuleihan et al. 1997, Wehr et al. 2001, Aeschbach et al. 2003, Scheer et al. 2010) and melatonin (Dijk et al. 1999, Wehr et al. 2001, Cain et al. 2010, Gooley et al. 2011) have been shown to be under direct regulation by the circadian clock. Not surprisingly, both hormones also act as major hormonal output pathways that propagate the time signal from the SCN to various other tissues. In the following paragraphs, we will discuss the interaction between the central clock and these two endocrine systems.

SCN–adrenal interaction

The adrenal gland is an endocrine organ composed of two anatomically distinct structures – the cortex and medulla. The cortical part produces multiple corticosteroid hormones, while the medulla produces epinephrine and norepinephrine. The adrenal cortex is further organized into three functionally distinct subregions: the outermost zona glomerulosa producing mineralocorticoids, the middle zona fasciculata producing GCs (mainly cortisol in humans, corticosterone in rodents) and the innermost androgen-producing zona reticulata. A diurnal rhythm of the excretion of urinary ketosteroids was reported in the mid 20th century (Pincus et al. 1954). In the 1970s, along with the identification of the SCN as the master circadian pacemaker, the circadian secretion of corticosteroids was established as a robust hormonal output of the SCN clock (Moore & Eichler 1972, Liu et al. 2008). Only during the last decade, however, has the anatomical and molecular basis underlying the circadian production of corticosteroids been unveiled. Cholesterol is the precursor for the biosynthesis of steroid hormones. LDL – bound cholesterol – is imported into adrenocortical cells via LDL receptors. Cholesterol is then transported into mitochondria via steroidalogenic acute regulatory protein (STAR). This import constitutes the rate-limiting step of steroidogenesis (Miller & Bose 2011). Inside the mitochondria, the side chain of cholesterol is first removed by cytochrome P450scC to become pregnenolone, which is then subjected to a series of enzyme-regulated reactions to become GC (Miller & Bose 2011). GC secretion is highly stress responsive. Together with epinephrine, GCs boost energy production and prepare the body for foraging and fight-or-flight situations. GCs exert effects on a wide array of physiological systems. In times of high energy demand, GCs help maintain blood glucose levels by promoting gluconeogenesis in liver and lipolysis in adipose tissues (Kwon & Hermayer 2013). GCs also play an important role in modulating immune (Silverman & Sternberg 2012) and cognitive functions (Sandi 2011). The majority of the effects of GCs are mediated by its ubiquitously expressed cognate nuclear receptors, glucocorticoid receptors (GRs) (Silverman & Sternberg 2012). Interestingly, despite the widespread expression pattern of GR within the brain, the SCN is devoid of GR (Okamura 2007).

The secretion of GC is the end product of hypothalamic–pituitary–adrenal (HPA) axis activation. Pituitary-released ACTH activates adrenocortical steroidogenesis through the melanocortin 2 receptor (MC2R), via a cAMP–PKA-dependent pathway which transcriptionally stimulates steroidogenic genes such as STAR and CYP11A1 (Miller & Bose 2011).

Blood levels of GCs display a robust circadian rhythm, overlaid by less regular ultradian pulses with a period of
The circadian rise of GCs is phase-locked to the time of awakening, peaking at few hours before the onset of the active phase, i.e. the early morning for diurnal animals such as humans and the evening for nocturnal animals such as mice (Moore & Eichler 1972, Gomez-Abellan et al. 2012). This GC rise promotes arousal and boosts performance during the early active phase. Importantly, GC rhythms persist under constant environmental conditions, suggesting that they are driven by the endogenous circadian clock. Surgical ablation of the SCN completely abolishes the circadian rhythm of GC in blood, indicating that the SCN is the origin of GC rhythmicity (Moore & Eichler 1972, Stephan & Zucker 1972). HPA axis activity upstream of the adrenal is also rhythmic (Watts et al. 2004, Henley et al. 2009), which led to the hypothesis that circadian regulation of GC release may be an indirect response to SCN-induced corticotrophin-releasing hormone (CRH) expression. However, this view has been challenged by several observations. First, the timing of CRH expression in the hypothalamus of pro-opiomelanocortin (POMC; precursor peptide of ACTH) in the anterior pituitary and the plasma GC surge are not organized in the expected sequential manner (Watts et al. 2004, Girotti et al. 2009). Also, implantation of ACTH pellets can restore the rhythmicity of GC in hypophysectomized rats, while denervation of the adrenal gland abolishes the daily GC rhythm, suggesting that ACTH rhythmicity per se is dispensable for the blood GC rhythm (Ottenweller et al. 1978, Ottenweller & Meier 1982). Conversely, stimulation of adrenal sympathetic nerves results in potentiated GC responses which can be abolished by hypophysectomy (Edwards & Jones 1993), suggesting a permissive function of pituitary-derived ACTH and a more direct role of sympathetic innervation in the regulation of the circadian GC rhythm. Indeed, it has been shown in viral tracing experiments that the adrenal is connected to the SCN via the spinal cord and the PVN (Buijs et al. 1999). In a more recent study, it has been shown that light signals are transmitted to the adrenal cortex via the SCN, inducing an up-regulation of PER1 expression and secretion of GC independent of ACTH (Ishida et al. 2005).

Well before the discovery of clock genes or peripheral clocks, it was shown that adrenal glands when isolated and cultured in vitro display a robust circadian rhythm of metabolism and steroid secretion (Andrews & Folk 1964). In line with this, we now know that about 5% of the whole genome – including all canonical clock genes – show rhythmic expression in the mouse adrenal gland (Oster et al. 2006a). By transplanting adrenal glands from arrhythmic PER2/CRY1 double mutant mice to WT adrenalectomized mice, and vice versa, we have provided evidence that a local adrenocortical clock imposes a circadian gating mechanism altering ACTH sensitivity during the course of the day (Oster et al. 2006b). This observation was further supported in a study that used a knock down of BMAL1 in the adrenal cortex (Son et al. 2008). Taken together, this illustrates that while the SCN is indispensable for the circadian rhythm of GC secretion, the adrenal clock provides an additional level of control to modulate the proficiency of GC production across the circadian cycle and further clocks along the HPA axis may be involved (Fig. 2).

**SCN–pineal interaction**

Unlike mice and humans, many non-mammalian vertebrates can perceive photic information by extra-retinal photoreceptors (Menaker et al. 1997, Foster & Soni 1998), e.g. in the pineal. The pineal gland is an ancient organ that exists in most vertebrates (Menaker et al. 1997). In mammals, it is buried deep beneath the skull and lies within the furrow of the two hemispheres. In consequence, its photoreceptive function is lost. However, in most cases its physiology is still strongly influenced by light. A major function of the pineal is its secretion of the hormone melatonin derived from the amino acid tryptophan (Barrett & Bolborea 2012). In mammals, melatonin exerts its effects via binding to its two widely expressed cognate receptors – MT1 and MT2. The melatonin receptors belong to the Gαi/q-protein-coupled receptor superfamily (Barrett & Bolborea 2012). Owing to the widespread expression of melatonin receptors, melatonin has been reported to modulate several physiological systems such as immune function (Srinivasan et al. 2011), metabolism (Nduhirabandi et al. 2012), and higher brain functions (Srinivasan et al. 2012). In birds and reptiles, the pineal-melatonin system is an essential part of the circadian clockwork (Gaston & Menaker 1968, Tosini & Menaker 1998). In contrast, no overt circadian disruption is observed in pinealectomized mammals (Quay 1970, 1972), but melatonin may play an important regulatory role in distributing the time signal of the SCN (see below).

The daily pattern of melatonin secretion profile has a robust profile – being low during the day; rising and peaking during the night. In contrast to the GC rhythm which is anti-phasic in nocturnal and diurnal animals, high melatonin is always confined to the dark phase. SCN lesions abolish melatonin rhythms (Klein & Moore 1979, Reppert et al. 1981). The SCN connects to the
pineal gland via a multi-synaptic autonomic pathway which sequentially involves the PVN and then the pre-ganglionic neurons of the intermediolateral cell column of the spinal cord and finally the noradrenergic sympathetic neurons of the superior cervical ganglion (Drijfhout et al. 1996, Moore 1996, Larsen et al. 1998, Teclemariam-Mesbah et al. 1999; Fig. 3). The SCN releases GABA to inhibit the sympathetic input to the pineal gland during the daytime while this inhibition is released during the night (Kalsbeek et al. 2000). In addition, the SCN sends a constant glutamatergic stimulatory input to the pineal gland which is overwhelmed by the inhibitory mechanism during the night (Perreau-Lenz et al. 2004).

The role of clock genes in regulating pineal gland rhythmicity has received little attention, mainly due to the fact that many of the mouse genetic models used to study the function of the molecular clock are maintained on genetic backgrounds carrying mutations in two key enzymes of melatonin synthesis, arylalkylamine N-acetyl-transferase (AANAT) and hydroxyindole-O-methyl-transferase (HIOMT), resulting in melatonin deficiency (Goto et al. 1989, Roseboom et al. 1998, Vivien-Roels et al. 1998). ClockΔ19 mutants (Vitaterna et al. 1994) were...
back-crossed into a melatonin-proficient strain, showing that the Clock-Δ19 mutation leads to phase delays and dampening of the melatonin rhythm in constant darkness conditions while GC rhythms were completely abolished (Kennaway et al. 2003, 2006). More recently, it has been demonstrated that the melatonin biosynthesis pathway can genetically suppress the circadian perturbations of Clock-Δ19 mutation (Shimomura et al. 2010), suggesting a role of melatonin in contributing to the robustness of the SCN clock (see below).

PER1 deficiency has been shown to enhance Aanat transcription, enzymatic activity and hence melatonin secretion (Chen & Baler 2000, Christ et al. 2010). In CRY1/2 double-deficient mice on a melatonin-proficient genetic background not only is the melatonin rhythm blunted under light–dark conditions, but also photic suppression of melatonin is abolished (Yamanaka et al. 2010). Together, these data suggest that clock genes impinge on pineal melatonin rhythmicity. However, owing to the lack of suitable genetic models to study the tissue-specific function of clock genes in melatonin-proficient strains, the physiological role of the molecular clock in the pineal itself remains largely unclear.

Hormonal feedback to the circadian clock

The stabilizing role of melatonin in SCN regulation mentioned above suggests that hormonal rhythms – we have discussed circadian regulation of GC and melatonin secretion – are not merely an output of the central clock. They can also feedback to the various levels of the circadian system and thereby intervene the circadian rhythm of physiology and behavior of animals (Fig. 4). In the following section, we will use these and some other hormones as examples to illustrate the crosstalk within the clock–hormones circuitry in mammals.
Cortisol

Exposure to jetlag or sleep perturbations (such as sleep restriction or shift work) results in a transient mismatch between the internal circadian time and the external light–dark cycle. Symptoms of jetlag include decreased alertness, motor coordination and cognitive performance, sleep disturbances, gastrointestinal disruption, and loss of appetite (Waterhouse et al. 2005). Sleep restriction and daytime sleep – hallmarks of a night shift work schedule – are associated with increased BMI and risk of metabolic syndrome, and alterations in circulating endocrine parameters such as insulin, glucose, and GCs (Wu et al. 2008, Rehman et al. 2010, Baron et al. 2011). Cortisol rhythms are also affected by jet travel, even when only three or fewer time zones are crossed (Doane et al. 2010), as well as by relatively subtle advances in sleep timing (Dijk et al. 2012).

GC steroids secreted from the adrenal gland are integral regulators of energy metabolism as well as the response to immune challenge and stress. GC disruption is associated with a variety of disorders. Cushing’s disease is characterized by excess cortisol, with symptoms including hypertension, hyperglycemia, sleep disorders, depression, and weight gain (Carroll & Findling 2010). Addison’s disease, characterized by a lack of cortisol, is accompanied by symptoms of weight loss, elevated sensitivity to stress, hypotension, mood disorders, and hypoglycemia (Mitchell & Pearce 2012).

GCs have been shown to directly affect circadian clock gene expression in a number of tissues, such as white adipose tissue, liver, and kidney (Gomez-Abellan et al. 2012, Pezu¨k et al. 2012). Adrenalectomy shortens re-entrainment in the SCN, lung, and kidney following phase shifts, suggesting that GCs may serve to stabilize the phase of peripheral clocks against external noise (Pezük et al. 2012). In the case of jetlag-induced circadian desynchrony, it was shown that manipulation of the GC rhythm could speed up or slow down activity adaptation to the new light–dark schedule, depending on the intervention time (Kiessling et al. 2010). This study...
highlights the exciting therapeutic potential of GCs in the treatment of jetlag and other desynchrony disorders.

**Melatonin**

The best-studied physiological effect of melatonin is its modulatory function on sleep/wake cycle regulation in humans. Application of exogenous melatonin has been shown to decrease the latency to sleep, increase total sleep time, and promote sleep maintenance (Sack et al. 1997, Sharkey et al. 2001). In contrast, blocking the nocturnal release of melatonin by suppressing the sympathetic innervation to the pineal results in increased total wake time (Van Den Heuvel et al. 1997). Moreover, exogenous melatonin can influence sleep macro architecture (Dijk et al. 1995, 1997). Because of its sleep-promoting effect, melatonin treatment is frequently used to ameliorate the symptoms of jetlag or to improve sleep quality during the daytime in night-shift workers (Aeschbach et al. 2009).

Beyond its effect on sleep, melatonin has been shown to directly signal to the SCN. In contrast to GRs (see above), high densities of MT1 and MT2 receptors in the SCN have been demonstrated (Gillette & McArthur 1996). In rodents, timed daily administration of high concentrations of exogenous melatonin can entrain the free-running endogenous rhythm under constant darkness conditions (Armstrong et al. 1986, Redman & Armstrong 1988). Similarly, timed application of melatonin can entrain blind human subjects (Arendt & Broadway 1987, Sack et al. 2000). In vitro, melatonin application to cultured SCN explants affects amplitude and phase of the circadian rhythm of neuronal firing (Liu et al. 1997, Shimomura et al. 2010). The acute inhibitory effect of melatonin on neuronal activity seems to be mediated by MT1 receptor (Liu et al. 1997), while the phase-resetting effect relies on MT2 receptor signalling (Hunt et al. 2001). It is worthy of mention that melatonin is also capable of modulating the production of adrenal GCs. In humans and monkeys, acute melatonin administration suppresses the production of cortisol (Torres-Farfan et al. 2003, Campino et al. 2011). More recently, it has been demonstrated using foetal rats that timed melatonin application can entrain adrenal gland rhythms (Torres-Farfan et al. 2011). Thus, together melatonin and GC rhythms appear to stabilize circadian phase and precision for different physiological systems.

**Ghrelin and insulin**

The timing of food intake is an important entrainment signal for peripheral clocks, best characterized in, but not limited to, the liver and adipose clocks (Stephan 2002). Anticipatory behavior just before scheduled feeding (food anticipatory activity (FAA)) is seen in animals with restricted access to food. This is characterized by increased activity and changes to body temperature, GC rhythms and hepatic P450 enzymatic function (Krieger et al. 1977, Hirao et al. 2006), which function to prepare the body for the anticipated food intake. When food access is confined to the normal rest period, these processes can uncouple peripheral oscillators from the central clock that stays locked to the light regimen. Ghrelin is secreted in anticipation of feeding, regardless of the light–dark cycle, from gastric oxyntic gland cells which possess a functional clock (LeSauter et al. 2009). Ghrelin stimulates appetite via its actions on the hypothalamic orexigenic peptides, neuropeptide Y and orexin, and on mesolimbic reward centres (Abizaid et al. 2006, Toshinai et al. 2006). In shift workers, the post-prandial ghrelin slump is attenuated, perhaps contributing to overeating (Schiavo-Cardozo et al. 2012). Ghrelin administration increases FAA; however, studies on rodents lacking functional ghrelin signalling are contradictory. Mice lacking ghrelin receptors are reported to have dampened FAA (LeSauter et al. 2009), whilst mice lacking preproghrelin show intact FAA responses during restricted feeding (RF; Szentiirmai et al. 2010). Ghrelin can feed back onto the circadian clock by directly affecting clock gene expression in the SCN (Yannielli et al. 2007). In vivo studies show that ghrelin treatment increases food intake, but only shifts behavioral rhythms under fasted conditions (Yannielli et al. 2007).

Insulin represents another potential food-inducible clock synchronizer. Insulin secretion from pancreatic beta cells is clock-gated, and disruption of the positive arm of the clock – CLOCK or BMAL1 – results in hypoinsulinemia (Marcheva et al. 2010, Sadacca et al. 2011), while disruption of the clock’s negative regulators – PERs and CRYs – is associated with hyperinsulinemia (Zhao et al. 2012, Barclay et al. 2013). Insulin sensitivity is reduced in shift workers, and accompanied by increased beta cell activity, suggesting a pre-diabetic state (Esquirol et al. 2012). But insulin can also feed back to the clock. Tahara et al. (2011) used daytime RF in mice to demonstrate insulin-dependent alterations of clock gene rhythms in the liver, and a similar response was seen in primarily cultured rat hepatocytes (Yamajuku et al. 2012). It would be remiss to discuss the effects of insulin on the clock without discussing the effects of glucose as a direct function of insulin signalling. Glucose can directly affect circadian gene expression in fibroblasts and the SCN (Hirota et al. 2002, Iwanaga et al. 2005). In the absence of...
insulin signalling, for example in diabetic rats, circadian clock phase is shifted in the heart, suggesting that high glucose levels can directly impinge on clock regulation (Young et al. 2002). Under RF conditions, sucrose (but not lipid) induces phase shifts and FAA (Stephan & Davidson 1998).

The concept of food-inducible factors acting as powerful entertainers of the clock system is ratified by a number of studies which employ RF to rescue clock gene rhythms as well as physiological rhythms under desynchronised conditions. In a rat model of night work, restricting food intake to the normal activity phase restores glucose rhythms and prevents weight gain (Salgado-Delgado et al. 2010). In a study on a mouse model of shift work, restoring normal food intake rhythms concurrently restores clock gene rhythmicity in the liver, as well as triglyceride, glycerol and GC rhythms, and gluconeogenesis (Barclay et al. 2012). While these data suggest a direct link between peripheral clock regulation and energy homeostasis, the phase relationship between clock gene expression and the transcriptional activity of metabolism-associated genes is variable, suggesting an interplay between local and systemic factors (Reznick et al. 2013).

**Leptin and adiponectin**

It is widely established that clock disruption results in metabolic perturbations, and ultimately obesity (reviewed in Froy (2010)). Conversely, high fat diet (HFD) can dampen clock gene rhythmicity in the liver and fat, and well as affecting behavioral rhythms (Kohsaka et al. 2007). HFD results in loss of diurnal feeding patterns in rodents, and subsequent alteration to GC, insulin, and glucose rhythms (Kohsaka et al. 2007). A study by Kaneko et al. (2009) showed altered clock gene expression in the brainstem of mice fed with a HFD, as well as in genetically obese mice such as ob/ob (lacking the leptin gene) and KK-A(y) mice (a spontaneous diabetic mouse model). However, arguably the most dramatic effects of HFD and obesity are the effects seen on circulating adipokines such as leptin and adiponectin.

Leptin is secreted from white adipose tissue in response to glucose stimulation, and signals via appetite centres in the hypothalamus to promote satiety and prevent excess energy consumption. Circulating leptin shows a diurnal rhythm, peaking in the night in humans. In the models of obesity, leptin resistance can occur and in the absence of leptin’s anorexigenic effects, this is accompanied by overeating (reviewed in Gautron & Elmquist (2011)). In humans, acute HFD feeding results in lower 24-h leptin (Havel et al. 1999), whereas hyperleptinemia and changes in leptin rhythmicity are observed in obese subjects in accordance with increased fat mass (Considine et al. 1996, Rosenbaum et al. 1996, van Dielen et al. 2002). Despite having no direct effect on locomotor activity, leptin can induce PER expression in the SCN of female mice and potentiate the phase-shifting effects of light in these animals (Mendoza et al. 2011). Ex vivo, leptin stimulation can reset the phase of the SCN clock (Prosser & Bergeron 2003).

Adiponectin possesses insulin-sensitizing and anti-inflammatory properties (reviewed in Harwood (2012)). Circulating adiponectin levels inversely correlate with obesity and leptin levels, and weight loss results in increased adiponectin (Hu et al. 1996, Yang et al. 2001, Matsubara et al. 2002). Adiponectin secretion shows both ultradian and circadian rhythms, with a nadir in the early hours of the morning in healthy adults (Gavrila et al. 2003, Scheet et al. 2010). In rodents, adiponectin peaks in the end of the light phase (inactive phase) and its rhythm is shifted under HFD (Ríos-Lugo et al. 2010). Bullen and colleagues showed decreased adiponectin levels relative to fat mass following HFD in rodents (Barnea et al. 2006, Bullen et al. 2007). To assess the effect of adiponectin on the circadian clock, Hashinaga and colleagues used Kk-Ta mice, a polygenic model of metabolic syndrome with hypoadiponectinemia. These mice have a shorter activity period under constant conditions and dampened circadian locomotor rhythms with increased light-phase activity relative to controls. Clock gene rhythms are phase-advanced in the liver and skeletal muscle in these mice. The introduction of the human adiponectin transgene into the liver of these mice restores locomotor rhythmicity, as well as hepatic clock gene phase (Hashinaga et al. 2013). These studies strongly indicate that leptin, adiponectin, and maybe other adipokines may have direct effects on molecular clock function.

**Summary and outlook**

In summary, many components of the endocrine system show strong circadian rhythmicity in both rodents and humans. Some of these hormones, such as melatonin and cortisol, are involved in disseminating the SCN timing signal to other parts of the body. Endocrine rhythms respond to factors that compromise the clock function, such as HFD, obesity, jetlag, and sleep disruption. In turn, the endocrine system feeds back on central and peripheral clocks to adapt circadian rhythms to altered physiological state. Given the profound effects endocrine and circadian
systems have on general well-being and the development of various disorders, this mutual interaction might provide new targets for pharmacological interventions at the systemic level. Recent studies have shown that resetting of clock function (Hirota et al. 2010) and with the recent discovery of drugs directly impinging on clock function (Hirota et al. 2010, Solt et al. 2012) it may be possible to rescue endocrine regulation under desynchrony conditions such as shift work.

Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of their reports.

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