Timing of Neuropeptide Coupling Determines Synchrony and Entrainment in the Mammalian Circadian Clock

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Abstract

Robust synchronization is a critical feature of several systems including the mammalian circadian clock. The master circadian clock in mammals consists of about 20,000 ‘sloppy’ neuronal oscillators within the hypothalamus that keep robust time by synchronization driven by inter-neuronal coupling. The complete understanding of this synchronization in the mammalian circadian clock and the mechanisms underlying it remain an open question. Experiments and computational studies have shown that coupling individual oscillators can achieve robust synchrony, despite heterogeneity and different network topologies. But, much less is known regarding the mechanisms and circuits involved in achieving this coupling, due to both system complexity and experimental limitations. Here, we computationally study the coupling mediated by the primary coupling neuropeptide, vasoactive intestinal peptide (VIP) and its canonical receptor, VPAC2R, using the transcriptional elements and generic mode of VIP-VPAC2R signaling. We find that synchrony is only possible if VIP (an inducer of Per expression) is released in-phase with activators of Per expression. Moreover, anti-phasic VIP release suppresses coherent rhythms by moving the network into a desynchronous state. Importantly, experimentally observed rhythms in VPAC2R have little effect on network synchronization, but can improve the amplitude of the SCN network rhythms while narrowing the network entrainment range. We further show that these findings are valid across several computational network models. Thus, we identified a general design principle to achieve robust synchronization: An activating coupling agent, such as VIP, must act in-phase with the activity of core-clock promoters. More generally, the phase of coupling is as critical as the strength of coupling from the viewpoint of synchrony and entrainment.

Introduction

Organisms evolved an internal biological timekeeper or ‘clock’ to temporally organize and regulate their physiological processes to best cope with a fundamentally periodic natural environment. In mice, the model system in mammals, this master biological clock consists of 20,000 neurons within the suprachiasmatic nucleus (SCN) in the hypothalamus. Each neuron sustains oscillations in the expression of ‘clock genes’ and firing that are noisy and variable. Inter-neuronal coupling [1,2] lends both precision [3] to the clock via synchronization and robustness to the clock against certain perturbations of the individual neuronal oscillators [4]. Coupling between neurons is mediated by synapses, gap junctions, and neuropeptides, such as vasoactive intestinal peptide (VIP) [5]. Synchronization produced by neuropeptide-based coupling is the central focus of this work.

VIP is one of the most important and well-studied coupling agents responsible for synchrony in the SCN [1,6,7] along with its canonical receptor VPAC2R [8]. In mice, the abolition of VIP-based signaling by knockout of VPAC2R or VIP, or both, leads to weak rhythms or arrhythmicity in behavior, clock protein oscillations, and firing rate in SCN explants [1]. The rhythmicity in a VIP knockout SCN explant can be restored by grafting a normal donor (wild-type) SCN via only paracrine VIP signaling [9]. The downstream targets of VIP binding to VPAC2R acting via G-protein coupled receptor pathways involving the second-messengers cAMP and Ca2+ are activators of the period clock genes (Per1,Per2) [10], and VIP pulses can entrain SCN explants much like periodic light stimuli in mice [11]. VPAC2R has been also shown to be intracellularly expressed in a circadian manner, with the expression peaking in the early morning [12]. The circadian expression/release of VIP is expected, in vivo VIP levels have not been conclusively measured [13–18].

A complete understanding of this critical synchronization phenomenon in the SCN remains an open question. In experimental and computational studies, coupling individual oscillators can achieve this robust synchrony, despite heterogeneity and different network connectivities. But, much less is known regarding the mechanisms and circuits involved in achieving this coupling, both due to system complexity and experimental limitations (as described above for VIP-VPAC2R coupling).

Limiting the scope of possible coupling mechanisms that can achieve synchronization would have significant scientific value.

Ueda et al. [19] and Korenčič et al. [20] identified the interactions between essential clock components (termed ‘core oscillator’) using only conserved transcriptional elements and...
Synchronization among multiple oscillators is a common theme in many biological and engineered systems. Here, we look at its use by the mammalian biological clock to keep accurate time. Through biochemical interactions among a network of inaccurate neuron clocks, a strong precise clock is produced. Although we are gradually learning more about these biochemical interactions, the details still remain largely unclear. Studies, both computational and experimental, have shown that the strength of the rhythmic interaction critically decides if a system can synchronize, i.e., the interactions must be strong enough. In this work, we show that the rhythmic interaction between these neuronal clocks must be timed correctly (in the right phase) in addition to being strong enough to synchronize the network. Activating (repressing) interactions must coincide with other activators (repressors) in each neuron to achieve synchrony. Since this principle imposes certain properties on synchronizing interactions, these properties can be used to identify and understand novel interaction mechanisms. Further, these principles are applicable to interactions between cellular oscillators in other tissues and organisms.

The theory of coupled oscillators describes the role of phase of coupling in determining synchronous and asynchronous states of an oscillator network under the assumption of weak coupling between oscillators (see [33] and references therein). In the circadian realm, Indic et al. [34] show that an altered phase of coupling is necessary to split synchronized SCN oscillations into two anti-phase subgroups and thus explain the splitting behavior in hamsters using a theoretical formulation similar to ours. Ueda et al. [35] use a principle that synchronizing factors released during the day and night must have similar phase response curves to light- and dark-pulses, respectively, to identify potential coupling mechanisms in *Drosophila*. The work of Ueda et al. [35] is consistent with our theory and also suggests a role for these factors in synchrony. Our work, to our knowledge, is the first systematic investigation into the role of the phase (or timing) of neuropeptide coupling in determining properties of the SCN network, including synchrony and entrainment. We show that the phase of coupling is as important as and complements the amplitude of coupling in determining synchrony and entrainment across multiple models. Moreover, we explicitly consider the circadian control of the ligand (VIP) and receptor (VPAC2R) and highlight the asymmetry in the roles played by VIP and VPAC2R.

**Results**

We wish to study the effects of both the timing of VIP release and VPAC2R expression on SCN synchrony and entrainment. Therefore, we first consider the timing of VIP release with constant (constitutive) VPAC2R expression and later incorporate periodic VPAC2R expression at different phases. Intuitively, periodic VIP release is necessary for the model to synchronize as the VIP phase informs neighbors of the phase of the releasing neuron’s clock.

We simulate the network model in Figure 1B (inset) consisting of 100 neurons with a fixed amplitude, but with intrinsic (uncoupled) periods drawn from a normal distribution with a mean of 23.7 h and nominal period spread (standard deviation) of 0.6 h, in accordance with [36]. We initially use an all-to-all network connectivity with no autocrine interactions, but we later evaluate other network connectivities as well. We use certain delays (τ2 and τ3) in the model to control the phase of VIP release and VPAC2R expression, respectively (see Figure 1B (inset)). The phase of a periodic signal is the timing of the peak of the signal. Following chronobiology conventions, the phase over one cycle spans circadian time (CT) 0 to 24 and the peak in *Per* gene expression is the reference phase CT6. The time of day between CT0-12 and CT12-24 are termed *subjective day* and *subjective night*, respectively. The effect of the external light-dark environment is simplified as a sinusoidal VIP signal superimposed on the network in order to qualitatively understand the entrainment properties of the system. Detailed cellular modeling of the complex effect of light on VIP and the endogenous neuronal oscillator is beyond the scope of this work.

We quantify synchrony in the network using different metrics. Period synchrony is required for network synchrony. So, when the entire network oscillates with same period (in practice, if the variance of periods is below a threshold), we test phase synchrony in the network using the Synchronization Index (SI). The SI or Kuramoto order parameter [37] measures the phase alignment of a period synchronized network and takes values between zero and one. The SI is zero, when the phases of neurons are evenly spread between CT0 and CT24, and one, when all neurons are phase aligned. Network rhythm refers to *Per* oscillations averaged over the entire population of neurons. We...
can thus assign an amplitude and period to this network rhythm. It is worth noting that the amplitude or period of the network rhythm is different from mean of the amplitudes or periods of individual oscillators in the network.

Only VIP release in the early subjective day leads to synchrony

We first study the effect of the timing of VIP release alone on network synchrony and entrainment by setting VPAC2R expression to be constant. In addition to the VIP release time, we also varied the spread of intrinsic periods in the network in order to test the robustness of our results. The results are summarized in Figure 1. Time courses of the computational SCN reveal either synchrony (with VIP release at CT4) or desynchrony (at CT16) (Figure 1A). Synchrony is only achieved with VIP release in a small window (henceforth called the region of synchrony (ROS)) within one circadian cycle (Figure 1B). The network always synchronizes for VIP release in the early subjective day (CT0-CT6), the time of peak intrinsic Per production, irrespective of the heterogeneity (period spread) of the network.

Moreover, the system exhibits sharp transitions between regions of synchrony and desynchrony. This reflects a phase transition of the network into a synchronous state, a well-studied phenomenon in classical coupled oscillator theory [38]. This ‘cooperative’
process is nucleated by a small cluster of synchronized neurons that progressively recruit neighbors until the entire network is in synchrony. In particular, synchrony is attained when coupling-induced gene expression and intrinsic gene production in each neuron act synergistically, i.e., coupling and intrinsic gene production are in-phase for VIP release at CT4 (Figure S1A). However, for VIP release at CT16, when the network does not synchronize, the coupling-induced expression and intrinsic production act antagonistically in each neuron (Figure S1A).

The width of the ROS is dependent on the spread of intrinsic (uncoupled) periods of neurons in the network and the strength of coupling between neurons. The ROS is broadened from CT0-CT8 to CT0-CT10 as the period spread in the network is decreased from its nominal value. Increasing the strength of coupling between neurons by over-expressing either VIP or VPAC2R broadens the ROS and can thus compensate for increases in period spread of the network (Figure S1B). While the network achieves perfect phase synchrony at low period spread with an SI of one, at the nominal spread, the phases of individual neurons are not perfectly aligned (SI~0.8) (see Figure 1B). The snapshot of rhythms in individual oscillators in the synchronized network shows that neurons with nominal period spread are arranged in a linear phase pattern (Figure 1C). Within this pattern, some neurons peak earlier (phase advanced) than the network rhythm, while others peak later (phase delayed). Neurons with intrinsic period longer than the mean period are phase advanced and neurons with shorter intrinsic period are phase delayed (Figure 1C). The width of the linear phase ordering is significantly compressed as the period spread of the network is decreased (Figure S1C).

Outside the ROS, while the neurons continue to oscillate (Figure 1A), the phases of the neurons are dispersed (Figure 1C). Although the neurons continue to interact, their ordering strongly resembles an uncoupled network of neurons as measured by the SI (Figure 1B). Despite the strong coupling signal between neurons in the network, the coupling maintains the system in a ‘uncoupled’-like state and prevents synchrony. An almost perfect positive correlation between individual neuron periods in the desynchronized network and their intrinsic periods further supports this view (not shown). Thus, the coupling might be viewed as forcing desynchrony in the neuronal network.

The period of the Pe gene network rhythms is close to the average intrinsic period of individual neurons in the network (Figures S1D and S1F). The period of the network rhythm is modulated by the phase of VIP release within the ROS. The amplitude of the network rhythms is boosted by synchrony within the ROS as measured by both absolute amplitude and amplitude relative to the mean Pe expression (Figures S1E and S1G). While individual neuron amplitudes without VIP-VPAC2R signaling is ~9, coupling reduces individual neuron amplitudes irrespective of the VIP release phase. Individual neurons in the coupled network have smaller amplitudes outside the ROS as compared to within the ROS. In other words, there is a significantly higher amplitude reduction (penalty) in desynchrony than in synchrony.

The network entrains to sufficiently-strong external light-dark cycles (Zeitgeber) both within the ROS (at CT4) and outside the ROS (at CT16) (Figure S2A). Thus, the phase of VIP release does not affect the ability of the network to entrain to a Zeitgeber. However, the network shows different entrainment characteristics with VIP release at CT4 and at CT16. A synchronized SCN network (VIP release at CT4) behaves as a ‘strong’ oscillator that shows a narrow entrainment range and a phase angle of entrainment highly sensitive to the Zeitgeber period (see [39,40] for definition of ‘strong’ and ‘weak’ oscillators). On the other hand, an SCN under desynchrony (VIP release at CT16) behaves as a ‘weak’ oscillator that exhibits a wide entrainment range and a phase angle of entrainment less sensitive to Zeitgeber period.

Circadian VPAC2R expression in the early subjective day boosts circadian rhythm amplitude and narrows the network entrainment range

We now further introduce circadian VPAC2R expression into the model. We study both the effect of the timing of VPAC2R expression and the strength of circadian modulation of VPAC2R when VIP is released at either CT4 or CT16. VPAC2R expression is under Pe TTFL control and its strength is varied, while keeping the mean VPAC2R expression a constant, as shown in Figure 2A. Surprisingly, the ability of the network to synchronize is unaffected by the peak phase of VPAC2R expression (Figure 2B). As before, the SCN network can synchronize only with VIP release in the early subjective day (CT4), but not in the subjective night (CT16).

The phase of VPAC2R expression affects, nevertheless, the amplitude of both individual oscillators in the network and the network rhythm (Figure 2C). VPAC2R peak expression in the early subjective day (coinciding with the ROS for VIP release) significantly improves the amplitude of individual oscillators and the network rhythm relative to constant VPAC2R expression. This boost in the network amplitude is caused by an increase in individual rhythm amplitude, since the synchronization index remains unchanged for different VPAC2R phases. VPAC2R expression in the subjective night suppresses the network rhythm and individual amplitudes. We also observed modulation of the network period by the phase of VPAC2R expression (Figure S3A).

Next, we vary the strength of VPAC2R modulation between 0, meaning constant VPAC2R expression, and 1, meaning maximal circadian VPAC2R expression with no basal component (see Figure 2A). The amplitude of the network rhythm initially increases and then starts to decrease with increased strength of VPAC2R modulation for VPAC2R expression peak at CT4 (Figure 2D). Recall that VPAC2R expression at CT4 enhances network rhythm amplitude by boosting individual amplitudes (Figure 2C). On the other hand, with VPAC2R expression at CT16, where VPAC2R modulation reduces network amplitudes, increasing the strength of VPAC2R expression reduces amplitudes even further. However, while increasing strength of VPAC2R expression at CT16 reduces amplitudes, it results in period synchrony over a larger range of VPAC2R expression strengths than with VPAC2R expression at CT4. Similarly, the network period increases and decreases with changes in the strength of VPAC2R expression with VPAC2R peak at CT4 and CT16, respectively (Figure S3B).

The entrainment properties of the network are also modulated by the phase of VPAC2R expression irrespective of the phase of VIP release (Figure S2B). Peak VPAC2R expression in the subjective night (at CT16) leads to an wider entrainment range relative to constitutive VPAC2R expression (Figure S2A) in both a synchronized (VIP release at CT4) and desynchronized (VIP release at CT16) SCN network. In other words, VPAC2R expression at CT16 makes the network behave as a ‘weaker’ oscillator than with constitutive VPAC2R expression [39]. On the other hand, while peak VPAC2R expression in the early subjective day (CT4) results in a much narrower entrainment range or a ‘stronger’ oscillator, peak VPAC2R expression at an intermediate phase (CT10) leads to an intermediate network entrainment range. Thus, VPAC2R expression phase can smoothly tune the entrainment range of the SCN network and the network’s qualitative behavior as a ‘strong’ or ‘weak’ oscillator.
The effect of the phase of VIP release and VPAC2R expression is independent of model and network properties

Finally, we test the robustness of our previous results on the network topology in the proposed model and the choice of model. We studied first a randomized network topology, since in the SCN only 10% of SCN cells express VIP [41] and only 30% of VIP cells express VPAC2R [42] making the network connectivity very heterogeneous. Replacing the all-to-all network connection with a random network topology or a local ring topology has little effect on the qualitative conclusions on synchrony and the timing of VIP release (Figures 3A and 3B). The change in topology and percentage connectivity of each neuron only alters the width of the ROS. Our conclusions regarding forced desynchrony, when the activating coupling agent is not in-phase with promoter of Per production, also remain unchanged.

The qualitative observation that VIP release only in the early subjective day leads to synchrony and a lack of effect of the peak phase of VPAC2R expression on network synchrony appears to be robust to the choice of model as well. In the Goodwin-like model of Gonze et al. [26] defined in (7), the ROS for constitutive VPAC2R expression is restricted to a small window between CT2-4 (Figure 3C). As before, the poor synchrony in the subjective night is driven by desynchrony in the neurons as evidenced by robust individual neuron oscillation amplitudes, but very small network rhythm amplitudes (Figure S4A). As before, with oscillatory VPAC2R expression, the network remains in a state of synchrony (VIP release at CT4) or desynchrony (VIP release CT14) irrespective of the peak phase of VPAC2R expression (Figure 3D). However, as before, the phase of VPAC2R expression modulates the network amplitude and period (Figure S4B).

One feature shared by both our DDE model and the Goodwin-like Gonze model is that VIP-VPAC2R coupling acts independently of the endogenous Per gene transcription according to an ‘OR’-logic gate (discussed in the Models section). While this is consistent with experimental data on the Per promoter [43], several prior models [26,28,29,31] assume a dependent ‘AND’-logic gate for the coupling-driven and endogenous gene transcription. We verified using a variant of our DDE model (5) that our

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Figure 2. Simulations with circadian VPAC2R expression. (A) Variation of VPAC2R expression profile for increasing fraction of expression under circadian control (green < blue < red). The shape of VPAC2R expression is the same as the Per expression (black); (B) Synchrony measured with SI for different peak phases of VPAC2R expression at two different VIP release phases: CT4 within the ROS and CT16 outside the ROS. The SI for constant VPAC2R expression with VIP release at CT4 is also shown for comparison; (C) The network rhythm amplitude with VIP release at CT4 and CT16 following the same color scheme as (B). The open circles are the mean of individual neuron amplitudes in the network with VIP release at CT4. The dashed line is the network amplitude for VIP release at CT4 with constant VPAC2R expression for reference. (D) Variation in network amplitude with different strengths of circadian VPAC2R receptor expression for VPAC2R expression at CT 4 and CT16 and VIP release at CT4 (corresponding to yellow plot in panel (C). The shaded region is the strength of circadian VPAC2R expression used in plots (B) and (C). (In all panels, circles are used when period synchrony is achieved and squares when incomplete synchrony is reached).
results were insensitive to the precise mechanism of coupling-driven and endogenous Per gene transcription (Figure S4C).

**Discussion**

Our first key result is that VIP-based coupling must have a peak phase in the early subjective day to ensure synchronization in the SCN network. In showing this, we assumed that some combination of VIP production and release is under circadian control resulting in periodic VIP-based coupling (ignoring effects of the light environment). The experimental evidence regarding this assumption is mixed and sometimes inconsistent in mice and rats, two closely-related model species. In rats, overwhelming evidence suggests that both VIP mRNA expression and protein levels do not oscillate in constant darkness (DD condition) and oscillate only under light-dark (LD) conditions [15,17,44,45]. However, VIP released into the extracellular medium in cultured rat SCN is circadian [18]. In mice, Dardente et al. [13] and Laemle et al. [16] show that VIP mRNA and protein levels in the SCN are circadian even under DD conditions. Although the circadian nature of VIP release is debated, we can study its effects via computational modeling.

We identified an important design principle for coupling in the SCN: for activating coupling agents, the inducing activity of the coupling agent must be in-phase with the endogenous activity of the target gene’s promoters. This result is aided by our choice of a minimalist delay-based model of the SCN, and the generality of this principle across other choices of SCN model and network connectivity further strengthens our assertion. In fact, this principle is a generalization of prior work on the phasing of transcriptional regulation in single oscillators [19,20] to a network of oscillators. Ueda et al. [19] and Korenci et al. [20] both show that activating transcription factors should be in-phase, and suppressing transcription factors must be anti-phase with the activating transcription factors to achieve large amplitude. Clearly, neurons with improved amplitude from optimal inter-neuronal coupling can better influence and align wayward neurons back into a stable relationship. We also expect therefore that coupling agents that suppress core clock transcription or enhance degradation to follow the anti-phase with intrinsic gene production design principle, although we do not have a biological example for this.

Antiphase VIP action results in desynchrony among robustly oscillating neurons resembling adult SCN in Cry1, 2−/− mice.
Moreover, Cry1, 2−/− SCN explants from neonates are synchronous and lose synchrony only during development. Our theory on timing of coupling suggests an explanation for this effect, since the phase of VIP action has been measured to change by several hours with age [44]. Recent work on another coupling factor, GABA, has shown that it works against synchrony under steady-state conditions [47], but acts to resynchronize the SCN under anti-phase configurations [48]. These observations are consistent with the hypothesis that GABA signaling endogenously occurs at the ‘incorrect phase’ (out-of-phase with repressors, since GABA is an inhibitory neuropeptide). Thus, GABA normally occurs at the ‘incorrect phase’ (out-of-phase with repressors, since GABA is an inhibitory neuropeptide). Thus, GABA normally reduces synchrony among neurons, and only when neurons of the dorsal and ventral SCN are forced out-of-phase under extreme photoperiods [48], GABA behaves as a synchronizing neuropeptide. This role reversal of GABA with the phase configuration of the SCN is similar to the change in behavior of VIP from a synchronizer to a desynchronizer with overexpression of the neuropeptide [49] and supports our view that the phase and strength are complementary features of coupling.

Entrainment of the network to an external Zeitgeber revealed differences in its behavior under synchronizing and desynchronizing VIP action. A synchronized network behaves as a ‘strong’ oscillator, while a desynchronized network behaves as a ‘weak’ oscillator. Comparing a coupled SCN network and uncoupled lung tissue, Abraham et al. [39] show by measuring entrainment ranges that the strength of coupling determines the ‘strong’ or ‘weak’ nature of oscillators. Our results reveal that even for the same strength of coupling, the phase of coupling determines the entrainment behavior of the SCN oscillator. This complementarity between coupling strength and phase also applies to recent observations that weakened coupling by means of vasopressin receptor knockouts speeds up jet-lag shifts in mice [50]. Simulations of 8 h jet-lag advances and 8 h delays showed faster re-entrainment with anti-phase VIP action (i.e., in a desynchronized SCN) (Figure S2C). These results are also consistent with the recent finding that desynchronizing the network with VIP prior to a jet-lag protocol leads to faster re-entrainment after the jet-lag shift [49].

Another general feature of this coupling is the asymmetric effect of circadian control of the neuropeptide and its receptor on synchrony in the network. Synchrony in a network requires exchange of phase information between the constituent oscillators. Circadian release of neuropeptide informs the network of the phase of the releasing neuron aiding consensus in-phase and ultimately synchrony. However, the phase of the receptor merely modulates the effect of the rest of the network on the neuron. Thus, circadian neuropeptide release is globally informative, while circadian receptor expression is only locally informative.

Next, we assemble available literature data on the timing of different aspects of VIP-VPAC2R signaling. Laemle et al. [16] show VIP immunoreactivity within SCN neurons peaking in the early subjective day and late subjective night entirely consistent with our prediction. Dardente et al. [13] estimate peak VIP mRNA expression at around CT18 allowing VIP release to occur in the early subjective day after delays in translation and extracellular release. While Shinohara et al. [18] show VIP release in multiple rat SCN explants in a range between CT16-CT24, attribution of CTs is made difficult by significantly shortened period (approx. 21 h) of VIP oscillations. Moreover, these measurements come from neonatal SCNs and there is some evidence that VIP mRNA phases may change by up to 12 hours during development [44]. The available data on VIP timing are thus mostly consistent with our prediction of VIP release in the early subjective day.

Information on second-messengers and transcription factors in the G-protein coupled receptor-mediated VIP-VPAC2R signaling to the core circadian clock [7] can also be used to test our predictions indirectly. Brancaccio et al. [51] and Enoki et al. [52] both show using real-time reporters that intracellular Ca2+ oscillates and peaks at CT6-7. Moreover, cAMP, the other important second-messenger, also peaks in the early to mid subjective day (CT6) as reported by O’Neill et al. [53]. Finally, VIP-VPAC2R signaling converges on the core circadian clock through the transcription factor CREB. CREB-mediated activation of Per gene expression occurs in the mid-subjective day (CT16) [54]. Although this evidence reinforces our claim that coupling must occur within a certain circadian time window in the subjective day, the particular CT of action is likely to be determined by other factors including entrainment, period control and SCN heterogeneity.

The most direct measurements of VPAC2R expression under DD conditions come from An et al. [12] showing peak bioluminescent staining of SCN slices at subjective dawn (CT0). Since we expect synchrony with robust amplitude with VIP in-phase with VPAC2R in the early subjective day, the study reinforces both our claims regarding phase of VIP release and VPAC2R expression. Moreover, VPAC2R mRNA expression is found to either peak around dawn (ZT0) [15] under LD conditions, or be circadian, but biphasic, in DD and LD conditions [55]. However, recent work on post-transcriptional control [56] has shown that having circadian mRNA expression is no guarantee of circadian protein levels and vice versa. Circadian VPAC2R expression did not have a significant effect on synchrony in our model, but could tune the amplitude and period of the network. We also observed that the phase of circadian VPAC2R alters the entrainment behavior of the network by modulating the network entrainment range. That VPAC2R expression in the early subjective day narrows the entrainment range is expected, since under similar VPAC2R expression phase we observe increased network amplitude. As noted in [39], changes in network amplitude and period indeed affect the entrainment properties of the network oscillator. Although we identify several critical network properties modulated by circadian VPAC2R expression, we cannot resolve the specific role of the circadian VPAC2R expression that is observed [12].

Evidence in the literature cited above support our claims, but our predictions could be more directly tested. An experiment that significantly alters the phase of VIP release in an SCN explant would be expected to desynchronize the individual rhythms and damp out network rhythms. It would, for example, be interesting to test the effects of putting the Vip gene under the control of the Bmal1 promoter. This would, in principle, put VIP production in anti-phase to its normal physiological release time. A more easily-accessible phenotype of alterations in the phase of VIP-VPAC2R coupling is the period of an explant or behavioral rhythms. The period of network rhythms is affected by both phase of VIP release and VPAC2R expression in our model (Figures S1C, S2E and S3A). Moreover, the phase angle of entrainment would also change with the change in period. These experiments could follow the approach of previous studies on VIP and VPAC2R signaling [6,42].
under different physiological and environmental conditions, such as with GABA and VIP observed in [48]. We did not, however, take into account the heterogeneity in the expression of these different neuropeptides and their receptors. VIP is mainly expressed in the core region, where oscillators appear to be weak or damped, while AVP is expressed predominantly in the shell with robust oscillations [57]. VIP is expressed in 10% of SCN neurons [41], up to 90% of neurons express the VPAC2R [12], thereby allowing these neurons to respond to VIP signaling: While VPAC2R is expressed in only 30% of VIP cells, it is expressed in 50% of AVP cells [42]. So the core VIP neurons seem to signal a different class of shell AVP neurons. Thus, there is a complex interaction of coupling with a core clock mechanism, they are have not accounted for. Since our results are based on the interaction of coupling with a core clock mechanism, they are qualitatively independent of these specific neuronal and network properties and could be applied to other functionally-similar neuropeptides in other organisms, such as pigment dispersing factor (PDF) in Drosophila [7,39].

Models

We use a minimalist computational model of each neuronal oscillator and VIP-VPAC2R-based coupling with the fewest assumptions in order to identify design principles in the SCN. This minimal model course-grains more detailed molecular models of the SCN [4,20,23–31] by focusing on the underlying qualitative features of circadian neurons relevant to VIP-VPAC2R-mediated coupling. Oscillations in each SCN neuron originate from a transcriptional-translational feedback loop (TTFL) [60], where core clock proteins suppress their own transcription after a time delay due to processes such as translation, post-translational modification and nuclear transport. A periodic signal is defined by its amplitude, period and phase; in practice, the amplitude is the distance between the maximum and minimum, period is time elapsed between successive maxima, and the phase is the timing of a marker, such as the peak, within one period. In such periodic signals, there is a definite relationship between time and phase, and thus, a phase difference is equivalent to the time elapsed between events. This permits phase differences between biochemical processes to be simply described using time delays, using a formulation called delay-differential equation (DDE) models. From a technical viewpoint, a DDE formulation allows the representation of components with different phases using the fewest model parameters. Using this idea, the interaction of 6 core genes in the circadian liver clock was modeled by Korenčič et al. [20].

The TTFL in each neuron is reduced to a single clock gene Per that is transcribed and then inhibits its own transcription after a delay. In other words, the TTFL is a negative feedback loop with a delay. This lumped gene is conceived as a combination of two core clock genes, period 1 (Per1) and period 2 (Per2). These two clock genes are upregulated by VIP [10], and VIP-based coupling causes additional production of the lumped Per. The availability of bioluminescence time series from mouse SCN explants Per::LUC transgene and PER2::LUC fusion protein made this model choice convenient [61].

The Per mRNA \( p \) in each neuron is produced at a constant rate, but suppressed after a delay \( \tau_1 \) (see first term in rhs) and linearly degraded:

\[
\frac{dp}{dt} = \frac{1}{(c+p_1)^2} - d \ p,
\]

with the notation \( p_1 = p(t - \tau_1) \). The conditions and choice of parameters \( c,d,\tau_1 \) that lead to oscillations with a period of about 24 h have been discussed in [20, Supplementary Information].

While VIP coupling increases Per expression [10], the mechanistic effect on Per gene regulation in (1) is unclear. On the one hand, coupling-induced expression and intrinsic mRNA production might function like an ‘OR’ logic gate, where one of these two modes of gene expression is sufficient to produce Per mRNA [43]. On the other, coupling-induced transcription in vivo (outside the promoter constructs of [43]) might function through enhancers that require both modes of expression behaving like an ‘AND’ logic gate. In this work, we use the ‘OR’ logic as it is supported by more evidence. Nevertheless, we also tested the ‘AND’ logic formulation where coupling-driven transcription is also modulated by endogenous gene activation (see Figure S4C).

As described earlier, the biophysical mechanism of Per expression by VIP-VPAC2R binding is largely unknown. Exogenous VIP addition appears to produce a transcriptional response much faster than the 24 h time-scale of circadian oscillations (Erik Herzog, personal communication). We thus assume that the VIP-VPAC2R complex immediately and proportionally upregulates Per expression:

\[
\frac{dp}{dt} = \frac{1}{(c+p_1)^2} - d \ p + \frac{z}{[\text{VIP} - \text{VPAC2R complex}].}
\]

The experimental and theoretical formulations of receptor-ligand binding is discussed in detail in [62]. The simplest model for a ligand \( L \) binding receptor \( R \) to form a complex \( C \), \( R + L \rightarrow C \), is

\[
[C] = R_T \frac{L}{K_D + L},
\]

where \( R_T \) is the total receptor number per cell and \( K_D \) is the dissociation constant for the binding. In biological terms, this model (3) assumes fast binding kinetics and the binding being receptor-limited. It is unknown in the SCN whether VIP or VPAC2R is limiting, and hence, we might also consider the alternate scenario of limiting VIP, which we leave for the future. The coupling upregulates Per gene expression in proportion to the complex concentration \([C]\) (2).

We consider VIP and VPAC2R to be under core clock control, since the features of VIP release and VPAC2R expression are not well known and beyond the scope of this work. We assume that both VIP and VPAC2R have the same shape as Per gene expression. We have checked that the qualitative results presented here are unaffected by this assumption using waveform shaping functions.

The timing of VIP release and VPAC2R expression relative to Per expression are varied using time delays \( \tau_2 \) and \( \tau_3 \), respectively (see Figure 1B (inset)). The SCN neurons release VIP into the common extracellular medium, and thereafter VIP binds to the nearby VPAC2R. The SCN neurons influenced by VIP release from a particular neuron determine the connectivity (outgoing links) of the releasing neuron. We leave open the possibility that a neuron might signal itself (autocrine) in addition to other neurons (paracrine).

The \((j,i)\)–th element of the connectivity matrix \( A \) is the contribution of VIP release from neuron \( i \) to binding at neuron \( j \). All neurons are fixed to release the same amount of VIP in order to equalize their influence on the network, i.e., columns of \( A \) add
to unity. We then explore the impact of different topologies on the network by our choice of A.

The total effect of coupling on a neuron is determined by both the total incident VIP and its VPAC2R expression (3). The varying phases of VIP and VPAC2R are then combined using (2) and (3) into

$$\frac{dp^i}{dt} = \frac{1}{(c+p'^{i}_1)^2} - d \ p^i + R_T \left( 1 - \lambda + \lambda \ \frac{p^{i}_1}{P} \right) \frac{\sum_j a_{ij} p^{i}_2}{k_D + \sum_j a_{ij} p^{i}_2}, \quad i = 1, \ldots, N.$$  \hspace{1cm} (4)

In order to compare the effects of circadian VPAC2R expression against constitutive VPAC2R expression, we normalize the VPAC2R expression with a constant mean. The weighting can be against constitutive VPAC2R expression, we normalize the total incident VIP

$$\frac{VPAC2R \ expression}{R_T(p^{i}_1)} = \frac{\sum_j a_{ij} p^{i}_2}{K_D + \sum_j a_{ij} p^{i}_2}, \quad i = 1, \ldots, N.$$  \hspace{1cm} (5)

In order to compare the effects of circadian VPAC2R expression against constitutive VPAC2R expression, we normalize the VPAC2R expression with a constant mean. The weighting can be against constitutive VPAC2R expression, we normalize the total incident VIP

For the AND logic gate formulation (4) as:

$$\frac{dp^i}{dt} = \frac{1}{(c+p'^{i}_1)^2} - d \ p^i + R_T \left( 1 - \lambda + \lambda \ \frac{p^{i}_1}{P} \right) \frac{\sum_j a_{ij} p^{i}_2}{k_D + \sum_j a_{ij} p^{i}_2},$$  \hspace{1cm} (4)

$$L(t) = L_{LD} \left[ 1 + \sin \left( \frac{2\pi t}{T_{LD}} \right) \right].$$  \hspace{1cm} (6)

A closely-related coupled oscillator model is the Kuramoto phase model with delayed coupling [63]. The phase-reduced model with coupling defined in [63] shows a similar bistability between asynchronous and synchronous states, and stability of these states can be controlled using the delay (or phase) of coupling between oscillators [64,65].

We simulate the network model (4) consisting of N = 100 neurons using the dde23 solver in MATLAB [66]. Each neuron’s TTFL is parameterized as \( \tau_T = 9.5, \epsilon = 0.35 \) and \( d = 0.5 \) that results in robust oscillations with a 23.7 h period and amplitude of 8.7.

The phase-reduced model with delayed coupling [63] shows a similar bistability between asynchronous and synchronous states, and stability of these states. We refer to this weighting as the ‘strength of circadian VPAC2R expression’. The model is thus

$$\frac{dp^i}{dt} = \frac{1}{(c+p'^{i}_1)^2} - d \ p^i + R_T \left( 1 - \lambda + \lambda \ \frac{p^{i}_1}{P} \right) \frac{\sum_j a_{ij} p^{i}_2}{k_D + \sum_j a_{ij} p^{i}_2}, \quad i = 1, \ldots, N.$$  \hspace{1cm} (4)

In order to study the entrainment properties of the SCN model, we made a simplifying assumption that external light input contributes to the baseline VIP levels in the extracellular medium of the SCN. Since we focus on synchrony in this work, a detailed exploration of various effects of light on SCN neurons is beyond the scope of this work. We incorporate the effect of light L(t) into (4) as:

$$\frac{dp^i}{dt} = \frac{1}{(c+p'^{i}_1)^2} - d \ p^i + R_T \left( 1 - \lambda + \lambda \ \frac{p^{i}_1}{P} \right) \frac{\sum_j a_{ij} p^{i}_2 + L(t)}{k_D + \sum_j a_{ij} p^{i}_2},$$  \hspace{1cm} (6)

The Goodwin-based neuron model

We test the robustness of our results (obtained from the DDE formulation (4)) to the choice of model by running simulations using an alternate circadian core model – the one of Gonzé et al. [26]. In the modified Gonzé et al. model, the circadian oscillator in neuron i with clock gene mRNA \( X_i \), clock protein \( Y_i \) and transcriptional inhibitor \( Z_i \) in a network with connectivity A is governed by:

$$\frac{dX^i}{dt} = \frac{r \ \left( Z^{i}_{\text{avg}} \right)^\mu - k \ X^i}{K + X^i} - k \ X^i + X^i$$  \hspace{1cm} (7)

$$\frac{dY^i}{dt} = v X^i - k \ \frac{Y^i}{K + Y^i}$$  \hspace{1cm} (7)

$$\frac{dZ^i}{dt} = v Y^i - k \ \frac{Z^i}{K + Z^i}$$  \hspace{1cm} (7)

with parameters \( r = 0.7, k = 0.35, \mu = 4, K = 1, K_i = 2, R_T = 0.075 \). Equation (7) corresponds to the original DDE system in (4) with phase control of both VIP and VPAC2R oscillations by \( \tau_2 \) and \( \tau_3 \) respectively.
Supporting Information

Figure S1  Additional plots to constant receptor simulations in Figure 1. (A) Comparison on a phase portrait of the rate of intrinsic Per production and coupling-induced Per gene expression at two circadian times 12 h apart. With VIP release at CT4, intrinsic and coupling-based production are strongly correlated ($\rho = 0.91$), while at CT16, they are strongly anti-correlated ($\rho = -0.82$); (B) Comparison of synchrony measured using SI in a network with high period spread for nominal coupling strength and increased coupling strength by either over-expression of VIP or VPAC2R (higher than nominal in Figure 1B); (C) Per gene expression in the coupled network as neuron phases plotted against the intrinsic period for low period spreads. Compare against the nominal spread plot in Figure 1C; (D) and (F) Comparison of periods of the network rhythm and intrinsic (uncoupled) neuron periods for nominal and low period spreads, respectively; (E) and (G) Amplitude and relative amplitude comparison between mean of individual coupled neurons and intrinsic period spreads. The individual neuron amplitude is 8.7 without any VIP signaling. (In all panels, circles are used when period spread is reached.)

(TIF)

Figure S2  Entrainment of the network to a VIP-based Zeitgeber described by (6). (A) The phase angle of entrainment of the network with constitutive VPAC2R expression at two phases of VIP release (synchrony at CT4 and desynchrony at CT16) for different Zeitgeber periods ($T_{LD}$) from 19 h to 26 h. The network phase is the timing of the peak of the network rhythm and the Zeitgeber phase is the timing of the peak of the sinusoidal Zeitgeber (see (6)). Period detuning is the difference between the intrinsic period of the network and Zeitgeber period. No phase synchrony within the network are shown. (B) The phase angle of entrainment versus period detuning like in (A) with oscillatory VPAC2R expression peaks at CT4, 10 and 16; (C) The double-plotted actogram for a simulated jet-lag experiment for 8 h advance (left) and 8 h delay (right) for VIP release causing synchrony and desynchrony and constitutive VPAC2R expression. The network phase and Zeitgeber phase (triangles) are plotted on each day after the jet-lag shift on day 0. On each row, the phases on day $n$ and $n+1$ are shown (double-plotted) for easy visualization.

(TIF)

Figure S3  Additional plots to circadian receptor expression simulations in Figure 2. (A) Modulation of the network period by the timing of VPAC2R expression with VIP release fixed at CT4; (B) Change in the extent of modulation of the network period by the strength of circadian receptor expression with VIP release at CT4.

(TIF)

Figure S4  Additional plots to simulations in Figure 3. (A) Comparison of network and mean individual neuron amplitudes for the Gonze et al. model [26] for the core oscillator (see (7)) for different phases for VIP release; (B) Modulation of the network amplitude (left) and period (right) by the timing of VPAC2R expression with VIP release at CT4 in the Gonze et al. model; (C) Effect of phase of VIP release in the DDE model in this work for multiplicative (‘AND’ logic gate) activation of Per transcription by VIP-VPAC2R coupling as defined in (5). As in Figure 1, the synchronization index, the network time-courses at two different VIP release phases, comparison of network and individual neuron amplitudes and the phase ordering of neurons within the network are shown.

(TIF)

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Author Contributions

Conceived and designed the experiments: BA HH. Performed the experiments: BA. Analyzed the data: BA EDH HH. Wrote the paper: BA.

References

1. Aton SJ, Colwell CS, Harmar AJ, Waschek J, Herzog ED (2005) Vasoactive intestinal polypeptide mediates circadian rhythmicity and synchrony in mammalian clock neurons. Nat Neurosci 8: 476–483.

2. Maywood ES, Reddy AB, Wong GK, O’Neill JS, O’Brien JA, et al. (2006) Synchronization and Maintenance of Timekeeping in Suprachiasmatic Circadian Clock Cells by Neuropeptideergic Signalling. Current Biology 16: 599–605.

3. Herzog ED (2007) Neurons and networks in daily rhythms. Nat Rev Neurosci 8: 790–802.

4. Liu AC, Webb DK, Ko CH, Tran HG, Zhang EE, et al. (2007) Intercellular Coupling Contributes to Antagonism in the SCN Circadian Clock Network. Cell 129: 605–616.

5. Webb DK, Takahashi JS, Kay SA (2010) Suprachiasmatic Nuclei: Cell Autonomy and Network Properties. Annual Review of Physiology 72: 551–577.

6. Colwell CS, Michel S, Ini J, Rodrigues W, Tan J, et al. (2003) Disrupted circadian rhythms in VIP- and PHI-deficient mice. American Journal of Physiology - Regulatory, Integrative and Comparative Physiology 285: R939–R949.

7. Vosku AM, Schroeder A, Lish DH, Colwell CS (2007) Vasoactive Intestinal Peptide and the Mammary Gland at Different Times of the Day. Am J Physiol Endocrinol Metab 293: E541–E547.

8. Harmar AJ, Marston HM, Shen S, Pratt C, West KM, et al. (2002) The VIPAC2 Receptor Is Essential for Circadian Function in the Mouse Suprachiasmatic Nucleus. Cell 109: 497–508.

9. Maywood ES, Chesham JE, O’Brien JA, Hastings MH (2011) A diversity of circadian pacemakers. Proceedings of the National Academy of Sciences 108: 14306–14311.

10. Nielsen HS, Hannibal J, Fahrenkrug J (2002) Vasoactive intestinal polypeptide induces per1 and per2 gene expression in the rat suprachiasmatic nucleus late at night. European Journal of Neurosciences 15: 570–574.
19. Ueda HR, Hayashi S, Chen W, Sano M, Machida M, et al. (2005) System-level identification of transcriptional circuits underlying mammalian circadian clocks. Nature Genetics 37: 187–192.
20. Korenčík A, Bordyugov G, Košler R, Rozman D, Golčík M, et al. (2012) The Interplay of co-Regulatory Elements Rules Circadian Rhythms in Mouse Liver. PLoS ONE 7: e46835.
21. Forger DB, Peskin CS (2003) A detailed predictive model of the mammalian circadian clock. Proceedings of the National Academy of Sciences USA 100: 14096–14101.
22. Leloup JC, Goldbeter A (2003) Toward a detailed computational model for the mammalian circadian clock. Proceedings of the National Academy of Sciences 100: 7407–7412.
23. Bernard S, Gonze D, Cajavec B, Herzel H, Kramer A (2007) Synchronization-Coupled Circadian Oscillators. Biophysical Journal 89: 120–129.
24. Bernard S, Gonze D, Cajavec B, Herzel H, Kramer A (2007) Synchronization-induced rhythminess of circadian oscillators in the suprachiasmatic nucleus. PLoS Computational Biology 3: e60.
25. To TL, Henson MA, Herzog ED, Doyle III JF (2007) A Molecular Model for Intercellular Synchronization in the Mammalian Circadian Clock. Biophysical Journal 92: 3792–3803.
26. Vaasoulou C, Herzog ED, Henson MA (2009) Small-World Network Models of Intercellular Coupling Predict Enhanced Synchronization in the Suprachiasmatic Nucleus. Journal of Biological Rhythms 24: 245–254.
27. Hauten M, Koeppl H, Gonze D (2012) Effect of network architecture on entrainment and entrainment properties of the circadian oscillations in the suprachiasmatic nucleus. PLoS Computational Biology 8: e1002419.
28. Wehbi AB, Taylor SR, Thoroughman KA, Doyle JF, Herzog ED (2012) Weakly Circadian Cells Improve Resynchrony. PLoS Computational Biology 8: e1002779.
29. Vaasoulou C, Herzog ED, Henson MA (2011) Multicellular Model for Intercellular Synchronization in Circadian Neural Networks. Biophysical Journal 101: 11–20.
30. Azcarón JA, Boullia LL, Pérez Vicente CJ, Rítor F, Spigler R (2005) The Kuramoto model: A simple paradigm for synchronization phenomena. Reviews of Modern Physics 77: 157–165.
31. Indic P, Schwatz WJ, Paydarfar D (2008) Design principles for phase-splitting behaviour of coupled cellular oscillators: clues from hamsters with split circadian rhythms. Journal of The Royal Society Interface 5: 873–883.
32. Ueda HR, Hirose K, Lin M (2002) Intercellular Coupling Mechanism for Synchronized and Noise-Resistant Circadian Oscillators. Journal of Theoretical Biology 216: 501–512.
33. Herzog ED, Atan SJ, Numano R, Sakaki Y, Tei H (2004) Transient Precision in the Mammalian Circadian System: A Reliable Clock from Less Reliable Neurons. Journal of Biological Rhythms 19: 35–46.
34. Kuramoto Y (1975) Self-entrainment of a population of coupled non-linear oscillators. In: Araki H, editor, International Symposium on Mathematical Problems in Theoretical Physics, Springer Berlin Heidelberg, volume 39 of Lecture Notes in Physics pp. 420–442 doi:10.1007/BFb0013365.
35. Kuramoto Y, Nishikawa I (1987) Statistical macrodynamics of large dynamical systems. Case of a phase transition in oscillator communities. Journal of Statistical Physics 49: 569–605.
36. Abrahamsson EE, Moore RY (2001) Suprachiasmatic nucleus in the mouse: retinal innervation, intrinsic organization and efferent projections. Brain Research 916: 172–191.
37. Shen S, Sprat C, Sheward VJ, Walsh M, West K, et al. (2000) Overexpression of the human VPAC2 receptor in the suprachiasmatic nucleus alters the circadian phenotype of mice. Proceedings of the National Academy of Sciences 97: 11573–11580.
38. Tranvickova-Bendova Z, Cermakian N, Reppert SM, Sassone-Corsi P (2002) Bimodal regulation of mPeriod promoters by CREB-dependent signaling and CLOCK/BMAL1 activity. Proceedings of the National Academy of Sciences of the United States of America 99: 7728–7733.
39. Bar Y, Shigeyoshi Y, Okamura H (1997) Development of Vasooactive Intestinal Peptide mRNA Rhythm in the Rat Suprachiasmatic Nucleus. The Journal of Neuroscience 17: 3920–3931.
40. Krajnik K, Kashon MI, Rosewell KL, Wise PM (1986) Sex Differences in the Daily Rhythm of Vasooactive Intestinal Polypeptide But Not Arginine Vasopressin Messenger Ribonucleic Acid in the Suprachiasmatic Nuclei. Endocrinology 119: 4189–4196.
41. Ono D, Honna S, Honna K (2013) Cryptochromes are critical for the development of coherent circadian rhythms in the mouse suprachiasmatic nucleus. Nature Communications 4: 1666.
42. Freeman Jr GM, Krock RM, Atan SJ, Thaben P, Herzog ED (2013) GABA Networks Destabilize Genetic Oscillations in the Circadian Pacemaker. Neuron 80: 796–806.
43. Evans JA, Leise TL, Castanon-Cervantes O, Davidson AJ (2013) Dynamic Interactions Mediated by Nonredundant Signaling Mechanisms Couple Circadian Clock Networks. Neuron 80: 973–983.
44. Ban Y, Shigeyoshi Y, Okamura H (1997) Development of Vasooactive Intestinal Polypeptide But Not Arginine Vasopressin Messenger Ribonucleic Acid in the Suprachiasmatic Nuclei. Endocrinology 119: 4189–4196.
45. Krajnik K, Kashon MI, Rosewell KL, Wise PM (1986) Sex Differences in the Daily Rhythm of Vasooactive Intestinal Polypeptide But Not Arginine Vasopressin Messenger Ribonucleic Acid in the Suprachiasmatic Nuclei. Endocrinology 119: 4189–4196.
46. Yamaguchi Y, Suzuki T, Mizoro Y, Kori H, Okada K, et al. (2013) Mice Genetically Deficient in Vasopressin V1a and V1b Receptors Are Resistant to Jet Lag. Science 342: 85–90.
47. Brancaccio M, Maywood ES, Chesham JE, Loudon AS, Hastings MH (2013) A Gq/Ca2+-Axis Controls Circuit-Level Encoding of Circadian Time in the Suprachiasmatic Nucleus. Neuron 78: 714–729.
48. Enoki R, Kuroda S, Ono D, Hasan MT, Ueda T, et al. (2012) Topological specificity and hierach-chiral network of the circadian calcium rhythm in the suprachiasmatic nucleus. Proceedings of the National Academy of Sciences 109: 21498–21503.
49. O’Neill JS, Maywood ES, Chesham JE, Takahashi JS, Hastings MH (2008) cAMP-Dependent Signaling as a Core Component of the Mammalian Circadian Pacemaker. Science 320: 949–953.
50. Olivier K, Impy S, Smith D, Athos J, Sturm DR (1999) Circadian Regulation of AMP Response Element-Mediated Gene Expression in the Suprachiasmatic Nuclei. Journal of Biological Chemistry 274: 17748–17756.
51. Cagampang FRA, Sheward WJ, Harmar AJ, Piggins HD, Coen CW (1998) Circadian changes in the expression of vasoactive intestinal peptide 2 receptor mRNA in the rat suprachiasmatic nucleus. Molecular Brain Research 54: 108–112.
52. Koike N, Yoo SH, Huang HC, Kumar V, Lee C, et al. (2012) Transcriptional Architecture and Chronatin Landscape of the Core Circadian Clock in Mammals. Science 335: 341–344.
53. Yan L, Karatsoreos I, LeSauter J, Welsh D, Kay S, et al. (2007) Exploring Spatiotemporal Orga-nization of SCN Circuits. Cold Spring Harbor Symposium on Quantitative Biology 72: 527–541.
54. Welsh AB, Angelo N, Huerta JE, Herzog ED (2009) Intrinsic, nondeuteriministic circadian rhythm generation in identified mammalian neurons. Proceedings of the National Academy of Sciences 106: 16493–16498.
55. Sutoru D, Ying P, Jose A, Michael R (2004) Coupled oscillators control morning and evening locomotor behaviour of Drosophila. Nature 431: 862–868.
56. Reppert SM, Weaver DR (2002) Coordination of circadian timing in mammals. Nature 418: 935–941.
57. Yamaguchi S, Isejima H, Matsuo T, Okura R, Yagita K, et al. (2003) Identification of transcriptional circuits underlying mammalian circadian clocks. Nature Genetics 36: 1408–1412.
58. Lauffenburger DA, Linderman JJ (1996) Receptors: Models for Binding, Trafficking, and Signaling. Oxford University Press. 21498–21503.
59. Reppert SM, Weaver DR (2002) Coordination of circadian timing in mammals. Nature 418: 935–941.
60. Yamaguchi S, Isejima H, Matsuo T, Okura R, Yagita K, et al. (2003) Identification of transcriptional circuits underlying mammalian circadian clocks. Nature Genetics 36: 1408–1412.
61. Lauffenburger DA, Linderman JJ (1996) Receptors: Models for Binding, Trafficking, and Signaling. Oxford University Press. 21498–21503.