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Inhibitory and Excitatory Networks Balance Cell Coupling in the Suprachiasmatic Nucleus: a Modeling Approach

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Abstract

Neuronal coupling contributes to circadian rhythms formation in the suprachiasmatic nucleus (SCN). While the neurotransmitter vasoactive intestinal polypeptide (VIP) is considered essential for synchronizing the oscillations of individual neurons, γ -aminobutyric acid (GABA) does not have a clear functional role despite being highly concentrated in the SCN. While most studies have examined the role of either GABA or VIP, our mathematical modeling approach explored their interplay on networks of SCN neurons. Tuning the parameters that control the release of GABA and VIP enabled us to optimize network synchrony, which was achieved at a peak firing rate during the subjective day of about 7 Hz. Furthermore, VIP and GABA modulation could adjust network rhythm amplitude and period without sacrificing synchrony. We also performed simulations of SCN networks to phase shifts during 12h:12h light-dark cycles and showed that GABA networks reduced the average time for the SCN model to resynchronize. We hypothesized that VIP and GABA balance cell coupling in the SCN to promote synchronization of heterogeneous oscillators while allowing flexibility for adjustment to environmental changes.

Keywords

circadian rhythms; coupling; gamma-aminobutyric acid; mathematical modeling; suprachiasmatic nucleus

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Supporting Material

Materials and methods, seven supporting figures, a supporting table, and supporting references are available in the supporting material document.

Author Contributions

NJK participated in simulation design, performed the simulations, analyzed the data, produced the images, and wrote the paper. SRT and MAH participated in simulation design, data analysis, and writing the paper.

Conflicts of Interest

The authors declare no conflict of interest in the publishing of this work.

1. Introduction

The way organisms anticipate the timing of daytime and nighttime, referred to as circadian rhythms, is essential for good health and optimal timing of metabolic processes and behavior (Kondratova and Kondratov, 2012; McClung, 2007; Xu et al., 2005). Circadian rhythms may be disrupted in otherwise healthy individuals by a variety of perturbations such as jet lag, social jet lag, rotating shift work and seasonal changes (Sack et al., 2007). Psychiatric disorders such as schizophrenia (Boivin, 2000; Wulff et al., 2012) and neurodegenerative disorders such as Alzheimer's disease (Satlin et al., 1995; Wu and Swaab, 2007) are also characterized by loss of circadian rhythms. Those afflicted with circadian disruption suffer from sleep loss and erratic wake times (Buysse et al., 2005). Reduced cognitive performance has also been exhibited by employees whose jobs require shift rotation or flight crew members with over eight hours of jet lag per week (Cho et al., 2000; Rouch et al., 2005; Viitasalo et al., 2014). Maladies such as obesity, diabetes, and heart attacks have also been correlated to human social jet lag (Roenneberg et al., 2012) and knockout mice lacking circadian rhythmicity (Shi et al., 2013; Turek et al., 2005). Therefore, circadian disruption represents a serious public health concern.

In mammals, the suprachiasmatic nucleus (SCN) of the hypothalamus generates these rhythms and responds to cues such as light and feeding (Klein et al., 1991). Mice with their SCN surgically ablated have their circadian rhythms abolished, while the SCN confers host rhythmic behavior to transplant recipients (Sujino et al., 2003). Remarkably, a coherent signal is produced by the SCN despite it being composed of 20,000 heterogeneous neural oscillators (Herzog et al., 2004; Webb et al., 2009; Welsh et al., 1995). In order for these cells to develop a consensus circadian rhythm, they form networks to exchange information about their individual and collective oscillatory patterns (Reppert and Weaver, 2002). Robust rhythms are necessary for ensuring that regular sleep and other behavioral schedules are followed even when external cues are absent; however networks must also be flexible so that organisms may adjust their schedule to seasonal changes or time shifts (Herzog, 2007; Meijer et al., 2010; Pfeuty et al., 2012).

The neurotransmitter vasoactive intestinal peptide (VIP) has been shown to be essential for synchronizing SCN neurons (Aton et al., 2005). VIP secretion follows a circadian pattern with peaks during the subjective day (Shinohara et al., 1995). Mice lacking genes for VIP or its receptor, VPAC₂, have highly disrupted circadian rhythms (Bechtold et al., 2008; Maywood et al., 2006), while doses of VIP have entrained or phase shifted SCN tissue oscillations in vitro (Reed et al., 2001; Watanabe et al., 2000). Furthermore, when the dorsolateral SCN shell, which lacks VIP secreting cells, is separated from the ventromedial SCN core, which contains many VIP secreting cells, the core remains synchronized while rhythms are not observed in the shell (Belenky et al., 2008; Yamaguchi et al., 2003). VPAC₂ activation, in conjunction with cytosolic calcium oscillations, stimulate Per1 and Per2 gene expression through the cAMP response element-binding protein (CREB) signaling cascade (Nielsen et al., 2002; Tischkau et al., 2003). Therefore, VIP is likely paramount among neurotransmitters for connecting SCN cells into a functional network.

Meanwhile, GABA is the principal inhibitory neurotransmitter in the brain and is also pervasive throughout the SCN where its functionality is controversial (Castel and Morris, 2000; Moore and Speh, 1993). GABA has been reported to synchronize (Liu and Reppert, 2000) or desynchronize (Freeman Jr. et al., 2013) SCN neurons. A potential link between VIP and GABA is also strongly suggested by the increase in GABA secretion from SCN neurons administered exogenous VIP (Itri and Colwell, 2003). One study has shown that while GABA opposes VIP-mediated synchrony during steady-state conditions, it facilitates resynchronization from antiphase conditions induced by long days (Evans et al., 2013). Although GABA, unlike VIP, has no known mechanism for directly influencing the molecular core clock of SCN neurons (Aton et al., 2006), it does open GABA_A chloride ion channels (Itri et al., 2004) and its concentration oscillates throughout the day in phase with VIP. Whether GABA networks are excitatory or inhibitory has been shown experimentally (Wagner et al., 1997) and in models (Vasalou et al., 2011) to depend on the intracellular chloride concentration, but in the SCN core GABA networks seem to only be inhibitory (Albus et al., 2005). The aim of our modeling study was therefore to explore new ways of how GABA-induced inhibitory post-synaptic currents (IPSCs) could modulate network properties in conjunction with VIP signaling.

To discover new relationships between VIP and GABA networks in the SCN, we performed computational analyses with a modified version of a heterogeneous multicellular model previously developed by our group (Vasalou et al., 2011). Heterogeneous cell populations were of particular interest because of the wide phenotypic behavior that exists, even within classically defined regions such as the core or the shell. Our network population consisted of neurons that in the absence of neurotransmitter signaling exhibited either sustained, damped, or no oscillations (Vasalou and Henson, 2010). Neurons with different intrinsic properties have different requirements for entrainment (Abraham et al., 2010), and so a network of heterogeneous oscillators would ideally include local differences in neurotransmitter concentrations that are responsive to receptor cell feedback (Fig. 1). This responsiveness is similar to what was observed by Itri and Colwell when they found that GABA was released by neurons in the presence of high levels of exogenous VIP (Itri and Colwell, 2003). When entraining to periods different from their intrinsic periods, we expected weak oscillators to require excitatory positive feedback networks for generating robust rhythms. Meanwhile we expected inhibitory negative feedback networks to weaken strong oscillators, having the effect of increasing their range of entrainment (Abraham et al.). So while VIP is ultimately necessary for rhythmic coupling, we hypothesized that too strong of an excitatory signal could push a cell into circadian disruption whereas a subtler influence would more effectively shift the neuron to the consensus periodicity. Therefore, we expected that a system where the strongest oscillators also released the highest levels of inhibitory GABA (thus slowing VIP and GABA release from cells in its local network) would achieve this neuroexcitatory balance locally and confer improved synchronization globally.

Our model was particularly useful for testing these hypotheses because it included not only the effect of VIP on the core molecular clock (Leloup and Goldbeter, 2003) but also an electrophysiological component that captured the effect of GABA on the resting membrane potential and firing rate (Vasalou and Henson, 2010). Since the two neurotransmitters influenced different components of the model, their combined action would be indirectly

rather than directly antagonistic for a coupled network of heterogeneous SCN neurons. Our modeling studies were therefore designed to determine if GABA signaling could counterbalance high concentrations of secreted VIP to improve network performance. We used two scenarios to test this hypothesis, one where the network synchronized in the dark and another where the network resynchronized to an imposed light shift.

2. Results

2.1. GABA and VIP coordinate to change network properties

By modulating the maximum rates of release of VIP and GABA (v_{VIP} and v_{GABA} , respectively), the model predicted that VIP and GABA had differential roles in determining network properties. The heat maps in Fig. 2 show the effects of these modulations on network synchrony, mean peak firing rate, amplitude, and period, with the values of v_{VIP} and v_{GABA} centered on previously published values (Vasalou et al., 2011). We also reported variability of these values across five simulations performed with different network topologies but with the same release rate combinations (S1 Table). VIP and GABA had contrasting roles in regulating peak firing rates of individual neurons in the network: VIP acted as an excitatory agent at all levels of v_{GABA} , and GABA was inhibitory at all levels of v_{VIP} (Fig. 2A). This prediction is in agreement with data showing that VIP is excitatory (Pakhotin et al., 2006) and that increasing GABA always generated greater IPSC in the core of the SCN (Albus et al., 2005). GABA depletion increased firing rates in agreement with experimental studies (Gribkoff et al., 2003; Shirakawa et al., 2000). That GABA was always inhibitory in our model was attributable to the highest mean intracellular chloride level for any neuron in any simulation never exceeding 23.9mM, whereas we previously showed that the threshold mean intracellular chloride concentration for GABA to be excitatory during the circadian day was about 26mM (Vasalou et al., 2011). This study also suggested that increased chloride levels, which reduced GABA-induced IPSC similarly to a decrease in GABA levels, would also result in a loss of network synchrony. The present study increased the range of tested GABA levels to more clearly demonstrate the role of GABA on network properties.

If v_{VIP} and v_{GABA} were both increased or both decreased, we could achieve peak firing rates similar to the nominal case (center square) value of 7.33 ± 0.11 Hz. The network was more sensitive to changes in v_{VIP} than to changes in v_{GABA} . By maintaining the peak firing rate of the network close to the value achieved in the nominal case, GABA and VIP coordinated through mutual influence to bring about high levels of synchrony in the heterogeneous network (Fig. 2B). To quantify the degree of synchronization, we calculated the synchronization index (SI) using the MATLAB® toolbox WAVOS (Harang et al., 2012) to compare phase over time of each neuron's *Per*mRNA oscillations. For the SI measure, a value of unity represented complete synchrony and a value of zero represented total asynchrony. Critically, our results showed that GABA lowered the SI when VIP levels were low but increased the SI when VIP levels were high.

The SI is a good measurement for phases differences between neurons in a given topology but does not directly report the number of cells that failed to adopt a stable rhythm. The percentage of cells that failed to adopt stable rhythms (defined as exhibiting greater than 5%

variation in amplitude and greater than 0.2h variation in period) in each topology varied considerably across the 125 simulations performed in this study, but this percentage of cells was usually less than 1% as long as the mean peak firing rate ranged between 6.0 Hz and 8.5 Hz (n=99 simulations). This increased to 5% among networks with a mean peak firing rate of less than 6.0 Hz (n=13 simulations). For networks with a reported mean peak firing rate greater than 8.5 Hz (n=13 simulations), 36% on average and as much as 81% of neurons had unstable rhythms. This set of results from the model simulations further demonstrated that a moderate mean peak firing rate, as modulated by VIP and GABA, may be very important for allowing heterogeneous populations of cells to synchronize to stable rhythms.

Overall, VIP decreased network synchrony when GABA levels were zero but improved synchrony when v_{GABA} was at its highest value. Supported by experiments that have shown that SCN cells release more GABA when VIP is applied exogenously (Itri and Colwell, 2003) and that GABA was particularly effective in desynchronizing VIP null SCN explants (Freeman Jr. et al., 2013), we hypothesize that this mechanism of VIP-GABA control could be responsible for homeostatic regulation of neuronal firing rates and might help explain seemingly inconsistent experimental results concerning the role of GABA in SCN synchronization (Freeman Jr. et al., 2013; Liu and Reppert, 2000).

GABA and VIP also had a strong influence on the amplitude of each cell in the network as reflected by mean *Per* mRNA levels (Fig. 2C). We examined the mean amplitude of individual neurons rather than the network amplitude to compare with the degree of synchronization, which was reported in Fig. 2B. As expected, the amplitude increased with v_{VIP} since *Per* mRNA transcription was stimulated by CREB directly through the action of VIP in the model. We also expected that GABA would reduce amplitude because it reduced firing rates and in turn reduced the release of VIP, and indeed GABA affected amplitude analogously to its influence on peak firing rate. These results generally are in agreement with experimental data showing that GABA can control the amplitude of circadian rhythms (Aton et al., 2006) and that GABA can counterbalance VIP through *Per* mRNA suppression (Ehlen et al., 2006). Meanwhile, VIP and GABA also coordinated to control period length (Fig. 2D). The effect of GABA was large when v_{VIP} was at its highest value and GABA had almost no impact when v_{VIP} was low. Furthermore, the network was most sensitive to VIP when GABA levels were high. These heat maps show that VIP-GABA balance allowed the heterogeneous networks to be highly synchronous over a range of periods. It is interesting to note that the period increased in the direction of both increasing VIP and increasing GABA, which was different from the directionality of changes in peak firing rate and amplitude. Namely, firing rate and amplitude were highest at high VIP and low GABA levels while the period was longest at high VIP and high GABA levels and shortest when either VIP or GABA was low. Remarkably, high levels of synchrony could be achieved across a range of periods, emphasizing the benefit of independent modulation of GABA and VIP release rates. If such a qualitative result were observed experimentally, the SCN would have the versatility to achieve oscillations with both a specific period and amplitude. This sensitivity analysis for the effect of VIP and GABA on period provided the clearest evidence that there was coordination (rather than a simple additive dependency) stemming from how the two neurotransmitters acted on different components of the cellular machinery, and in turn each other's release and local cellular dynamics.

2.2. Electrophysiology coordinates heterogeneous neurons

We sought to determine how the behavior of individual neurons contributed to the changes in network properties that were observed by modulating VIP and GABA release rates. To do so, we studied how each neuron controlled oscillations related to either the molecular “core clock” mechanism or the electrophysiology component of the model. We compared the peak times of *Per* mRNA oscillations (a common indicator of core clock behavior) to the peak times of oscillations of VIP receptor saturation (S_{VIP}) (an indicator of the electrophysiological behavior of the VIP network) for each cell in the heterogeneous network. These two indicators were chosen for demonstrative purposes because their peak times were relatively close compared to other indicators such as calcium (which peaked on average 7h before PER protein (Brancaccio et al., 2013; Enoki et al., 2012) as shown in this model previously (Vasalou and Henson, 2010)). Individual cells were plotted against their intrinsic periods and amplitudes since these are generally model-independent attributes of oscillators. Oscillator parameters were distributed so that most cells would be arrhythmic in the absence of neurotransmitter signaling, as observed in low density dispersed cell cultures (Webb et al., 2009) and modeled previously (Vasalou and Henson, 2011). We were able to characterize these cells nonetheless by running separate simulations where the free-running neuronal population was uncoupled and each neuron was forced to signal itself with both VIP and GABA. The calculated periods and amplitudes from these simulations were a strong indicator of “optimal peak firing rate”, i.e. the network peak firing rate at which the S_{VIP} and *Per* mRNA peak times came closest together for a given neuron (Fig. S1 A). Furthermore, 98% of the total neuron population had an optimal peak firing rate between 5.7Hz and 8.5Hz, which could explain why networks firing with this range of mean frequencies all had SI values greater than 0.88. This suggested that maintaining a precise phase relationship between these two intracellular oscillations correlated with the degree of synchronization for an individual cell to its global network.

Fig. S1 A also showed that cells with higher intrinsic amplitudes and/or shorter periods required more GABA and/or less VIP to reduce the firing rate and achieve the optimal VIP/GABA balance. High intrinsic amplitude was highly linearly correlated with high values of v_{sPO} (the heterogeneous parameter controlling the baseline *Per* mRNA synthesis rate; calculated correlation coefficient, $r=0.89$), and v_{sPO} in turn has been shown previously in this model to correlate with oscillator strength (Vasalou and Henson, 2011), so high intrinsic amplitude neurons can be generally understood to be the population’s strong oscillators. Conversely, cells with smaller amplitudes and/or longer periods (weak oscillators) required less GABA and/or more VIP to achieve this balance (for statistical significance, see Fig. S2 A). Mechanistically, VPAC₂ receptor saturation governed *Per* mRNA transcription through CREB signal transduction, but CREB was also influenced by intracellular calcium levels. In this way, modulating v_{VIP} and v_{GABA} could influence the *Per* mRNA/ S_{VIP} phase difference since VIP largely influences core clock activity while GABA directly affects only cell electrophysiology. Up-regulating GABA or down-regulating VIP could reduce the phase difference when S_{VIP} was advanced relative to *Per* mRNA, while up-regulating VIP or down-regulating GABA could reduce the phase difference when S_{VIP} was delayed relative to *Per* mRNA. This relationship demonstrated the broader concept that VIP and GABA could coordinate the timing of components’ oscillations to facilitate population synchronization.

To examine this point further, we explored these peak time differences specifically for simulations performed at v_{VIP} - v_{GABA} value pairs leading to high, moderate, or low mean peak firing rates. A direct finding was that a neuron that was hyper-excited, meaning it was connected in a network firing faster than was optimal for that cell, exhibited *Per*mRNA oscillation that peaked ahead of the VIP signal, suggesting early anticipation of the signal. Conversely, a cell that was under-excited had *Per*mRNA oscillations that consistently lagged behind the VIP signal, resulting in negative phase differences between the two oscillations. We found that the networks with the least number of high magnitude phase differences amongst its cell population also had the highest values for synchronization index. Whether a cell was under- or over-, or properly excited at a given v_{VIP} - v_{GABA} value pair depended largely on its intrinsic period and amplitude (oscillator strength). Neurons with long intrinsic periods and small intrinsic amplitudes (weaker oscillators) synchronized well and many high amplitude and long period cells were actually phase delayed in networks with high mean firing rates (Fig. S1 B). The median case produced the greatest network synchrony (Fig. S1 C). For systems with low firing rates, only neurons with short intrinsic periods and high intrinsic amplitudes (stronger oscillators) synchronized well (Fig. S1 D).

Modulating VIP and GABA maximum release rates could also destabilize the oscillations of certain individual neurons, making them unable to entrain to the collective network rhythm (Fig. S3). While almost all cells in our constructed networks maintained stable oscillations for the nominal v_{VIP} and v_{GABA} values (100% in 4 out of the 5 topologies tested), networks with extreme values of v_{VIP} and/or v_{GABA} often contained cells that consistently exhibited unstable oscillations regardless of topology. For this analysis, stable oscillations were defined as having period lengths that varied by no more than 0.2h or amplitudes that varied by no more than 5% over time when network behavior was accessed. In the most extreme case of network over-excitation tested, $70.0\% \pm 9.2\%$ of neurons failed to generate stable oscillations. In this case, however, increasing the maximum GABA release rate to 0.25 nM/h reduced the number of asynchronous neurons to $1.9\% \pm 1.7\%$. Meanwhile, for the most under-excited case, $11.7\% \pm 17.5\%$ of neurons in a given simulation failed to entrain with the collective network period. The network topology contributed substantially to which neurons failed to produce stable oscillations during a given simulation. We found that neurons least likely to entrain to the network (marked by black asterisks) at v_{VIP} - v_{GABA} value pairs associated with low mean peak firing rates were mostly in the region of the period-amplitude field map associated with being phase advanced relative to the collective network rhythm (bottom right quadrant; high amplitude/short period). GABA was therefore critical for balancing electrophysiological oscillations with molecular clock oscillations at high levels of VIP.

2.3. GABA Assisted Phase Shifting Networks Entrained to Light

In constant darkness, inhibitory GABA signaling in the simulations improved network synchronization at high VIP levels but reduced synchronization at low VIP levels. To study the potential benefits of GABA for entrainment to environmental cues where strong coupling might also have negative consequences, we entrained the multicellular model to 12h:12h light-dark (LD) cycles and then implemented a 12h phase shift to determine how rapidly the network could adjust. Light input was modeled as complete saturation of VPAC₂ receptors

by VIP ($S_{VIP}=1$) as well as complete saturation of NMDA receptors to allow the maximum influx rate of extracellular calcium into the neuron (Vasalou and Henson, 2011). These simulations were performed with or without GABA signaling to examine how individual neurons within the network re-entrained after the light shift.

To adjust to the 12h phase shift, some neurons in the network phase delayed while other neurons phase advanced. Furthermore, some neurons switched from being an “advancer” to being a “delayer” in the presence of GABA signaling. Among these “switchers,” no neuron changed from being a delayer to an advancer in the presence of GABA, underscoring the critical role of GABA in promoting and facilitating phase delays. To illustrate these behaviors, *Per* mRNA traces of representative neurons of each switching phenotype were displayed alongside plots showing how the *Per* mRNA oscillation peak location changed relative to the light period over time (Fig. 3). We compared the peak time prior to the light shift to the peak time during the transition to the new light phase, and we defined the time for re-entrainment as the time at which the phase relationship with the *Zeitgeber* (light stimulus) was restored to within 0.5h of the original value. The *Per* mRNA traces with and without GABA were almost identical prior to the light shift, and the shift initially caused the amplitude to drop. Amplitude restoration was achieved at about the same time as when the system completed its re-entrainment to the new light schedule. GABA helped delayers by reducing their time for re-entrainment; e.g. from seven days to five days in a representative neuron (Fig. 3A). This neuron was significantly more phase delayed throughout the transition with GABA than without GABA, and the recovery was smoother and deviated less across the simulations. A representative switcher (Fig. 3B) took one less day to re-entrain without GABA, but the transition was marked by a dramatic decrease in amplitude and greater variability in the absence of GABA. A representative advancer (Fig. 3C) also showed longer re-entrainment time with GABA but the difference from the GABA free case was not statistically significant. During the transition, GABA caused the advancer to initially phase delay with phase advances only coming after more extreme amplitude reduction. Infradian oscillations in oscillation amplitude and peak time after re-entrainment were present both with and without GABA.

We explored how individual neuron characteristics affected network adjustment to 12h light shifts by identifying the number of advancers or delayers in each simulation. Networks without GABA had $12.3\% \pm 1.3\%$ of neurons as advancers, while this number decreased to $4.3\% \pm 0.5\%$ for networks with GABA. This attribute made GABA effective in uniformly adjusting the network to the light shift. The average effect with and without GABA on 400 heterogeneous cells connected by different topologies is shown in Fig. S4. The advancers were located in the far right of Fig. S4 A, which corresponded to cells with the highest intrinsic amplitudes (strongest oscillations). The region of switchers was located between the advancers and delayers, which seemed to correspond to lower intrinsic periods. We also compared the time for re-entrainment for the entire network distribution without GABA (Fig. S4 B) or with GABA (Fig. S4 C). The re-entrainment time ranged from three to seven days for both cases (not including the two cells that never resynchronized), and the neurons that required the longest times to adjust had smaller intrinsic periods and mid-to-low amplitudes while neurons that required the shortest times had either high periods or very high amplitudes. Without GABA, the average time for re-entrainment was 4.90 ± 0.07 days

and with GABA the time decreased significantly to 4.60 ± 0.05 days. In the networks with GABA, 61% of neurons reduced their average resynchronization time compared to networks without GABA. This population is to be compared with the 8.5% of neurons for which the average resynchronization time increased, the 30% of neurons which exhibited no change, and 0.5% of neurons that never resynchronized with or without GABA.

We found strong evidence that GABA produced faster network re-entrainment by facilitating delays, both by causing more cells to delay and by allowing most delayers to delay faster. We plotted the decrease in time for re-entrainment of each cell so that the neurons most positively affected by GABA could be identified (Fig. S4 D). Without GABA, cells with the longest intrinsic periods required the least amount of time to re-entrain through delaying. The cells that benefited most from GABA signaling were also predominantly delayers. Cells with short periods and small amplitudes required up to 7 days to re-entrain by delaying without GABA, while with GABA these cells shortened their time for re-entrainment by up to 2 days. In all simulations, delayers almost exclusively produced some improvement. The only neurons that consistently required more time to re-entrain with GABA were switchers and advancers, which were relatively rare compared to delayers. Overall, the ability of GABA to cause more cells in the network to delay made the system generally more uniform in how it adjusted to the light shift, resulting in more rapid shifts.

Up-regulated values of VIP increased re-entrainment times. As the VIP maximum release rate (v_{VIP}) was increased, the network became less able to quickly re-synchronize to the 12h phase advance, requiring as long as 12 days. GABA had the most significant effect in reducing the time needed for re-entrainment when VIP was sufficiently high while also not saturating the system (Fig. S5 A). The percentage of advancers in the network increased as VIP was up-regulated as well (Fig. S5 B). At higher VIP levels, the location of the switchers was located much closer to the center of the distribution and advancers occupied a much larger region of the low intrinsic period, high intrinsic amplitude space. The cells most strongly affected by GABA were switchers and cells adjacent to that region on the field map; the adjacent advancers moderately increased their time of re-synchronization by about 2 days, while adjacent delayers exhibited dramatic improvements of up to 7 days. Meanwhile, the switchers themselves improved their time of re-synchronization by an average of 3 days. Therefore, GABA also could reduce re-entrainment times in networks of VIP up-regulated neurons by enhancing phase delaying capabilities of individual cells. The efficacy of GABA signaling depended strongly on the VIP level, with GABA able to provide the necessary counterbalance only at low and moderate VIP release rates.

To understand when GABA was most strongly exerting its influence during the light shift, we developed velocity response curves (VRCs), previously shown to be an effective tool for assessing circadian entrainment dynamics (Taylor et al., 2010). Dark pulses were used to develop these VRCs as a simple way to resolve the differential role of GABA on neurons adjusting to changing light schedules. Note that dark pulses rather than light pulses were necessary in these simulations because light was modeled to completely saturate the VIP receptor VPAC2. This way of modeling light was physically appropriate due to high secretion levels of pituitary adenylate cyclase-activating peptide (PACAP), another VPAC2 agonist, observed during light stimulation experiments (Morin and Allen, 2006). The

consequence is that GABA in the model does not affect cellular behavior during light phases or sudden light pulses but would influence the simulation results for sudden dark pulses or phase shifts.

We determined the instantaneous rate of change in phase velocity for one-hour-long dark pulses imposed at different times on single cells entrained to 12h:12h LD cycles to generate a VRC that represented the average cell response. The neurons were also self-signaling, either with VIP and GABA or with just VIP. Our dark-dark modeling studies discussed above showed that GABA could beneficially reduce the firing rate of neurons that were hyper-excited from excess VIP signaling. We suspected that these hyper-excited states were analogous to those states experienced by neurons entrained to expect light at a given time but instead receive a dark pulse, motivating us to analyze the impact of GABA during these dark pulses.

In the absence of GABA signaling, the dark pulse reduced the phase velocity during the first half of the light period (ZT=0h–5h) but induced a small phase velocity increase during the second half of the light period (ZT=6h–11h) (Fig. 4A). The introduction of GABA signaling increased the size of the delay region by as much as 9% but had no significant effect on the advance region (Fig. 4B). The impact of GABA was more evident when the velocity response curve without GABA was subtracted from the curve obtained with GABA to generate a differential velocity response curve (Fig. 4C). This GABA-induced increase in the delay region area could be one reason why GABA helped some neurons shift from advancers to delayers. Meanwhile, the negligible effect on the advance region area might have explained why GABA never shifted neurons from delayers to advancers. Moreover, Fig. 4B showed that GABA signaling allowed delayers to shift faster by increasing the width of the delay region, in agreement with previous research showing that shortened re-entrainment times could be explained by increases in the area of velocity response curves (Webb et al., 2012). Overall, GABA significantly increased the area of the delay region by 8.4% while decreasing the advance region area by 2.1%.

The dark pulse response curves were used to estimate the effect of phase advances or phase delays of different lengths by integrating the area under the curve (Taylor et al., 2008). By progressing forward through the VRC starting at ZT=0h (Fig. 4B), this analysis showed that all phase delays would cause cells in the network to adjust by decreasing their phase velocity. Even if the new period was delayed by longer than 6 hours, thus entering the advance region, the integrated area under the advance region was always smaller than the area under the delay region so that the net effect would always be phase delay. Cells would phase advance universally if a phase advance was six hours or less, as can be visualized by integrating the VRC, starting at ZT=11h and progressing left along the time axis. For a 12h phase advance, which was the case for the network in the previous example, one would determine the effect of unexpected darkness during the light period by integrating the entire VRC (ZT=0h–11h). The result is that the 12h of darkness initially causes phase delays in the network and the strength of this delay is greater when GABA signaling is present.

To further explore how GABA's effect on the VRC influences network behavior, additional phase shifting experiments with light were performed on the 400-cell coupled network. A

12:12h LD cycle was shifted by 12 hours implemented with an interval of 24h constant darkness (Fig. S7), rather than the interval of 24h light that was used to generate the results presented in Fig. S4. The distribution of delayers and advancers across the network were virtually the same in both cases (Fig. S7A). This also did not appreciably change the re-entrainment time for most of the networks' cells (Fig. S7B–C) nor did it alter the degree with which GABA was beneficial in reducing these times (Fig. S7D). We expect that the direction of the phase shift did not change a cell's delayer-advancer phenotype because the area under the VRC is the same whether it is integrated forwards or backwards. In another simulation, a 9h phase advance was implemented by truncating a 12h day to 3h and then continuing with the 12:12 LD cycle (Fig. S8). As predicted by the VRC, this split the network more evenly into advancers and delayers, with region constituting about 5% of the network switching to delaying from advancing in the presence of GABA (Fig. S8A). These switchers exist because GABA enlarged the area of the VRC delay zone. Simply because of the larger number of hours shifted through (15h for delayers vs. 9h for advancers), delayers took about two days longer on average than advancers to re-entrain (Fig. S8B–C). GABA was beneficial in reducing the time for re-entrainment for 25% of the network, compared with 10% that took longer to re-entrain (Fig. S8D). Moreover, GABA reduced the number of ambivalent cells (taking more than twice the network average to re-entrain) in the network from 11% to 6%.

3. Discussion

A core tenet of this study was that proper functioning of the SCN required a balance between excitatory and inhibitory networks. This hypothesis was derived from the importance of excitatory VIP to intercellular coupling [25] and the overall prevalence of inhibitory GABA in the SCN [32]. Our multicellular model predicted that the SCN network had an optimal peak firing rate for synchronization that could be achieved by an appropriate balance between the VIP and GABA maximum release rates, with the increase in GABA levels in response to high VIP levels as observed by Itri and Colwell [39] being necessary in order to compensate for increasing VIP levels. As demonstrated by the sensitivity analyses presented in this study, GABA can either synchronize or desynchronize a system depending on the level of VIP in the network. Although it is difficult to infer the relative amounts of VIP present in the studies that show either GABA synchronizing (Liu and Reppert, 2000) or desynchronizing (Evans et al., 2013; Freeman Jr. et al., 2013), it is reasonable to postulate that levels of VIP or some other factor affecting interconnectivity could produce this disparity. Similarly, our simulations agree with experimental findings that GABA promotes re-synchronization when different subsets of the system are in antiphase, and that the effect of GABA during a re-synchronizing transition would be phase dependent (Evans et al., 2013). That VIP and GABA play complementary roles and that both may act as a synchronizing force was also recently highlighted, and our modeling results suggest that their relative concentrations modulate each neurotransmitter's functionality (Evans et al., 2013). Our model results suggested that these were especially important because of the differential demands for synchronization between higher amplitude strong oscillators and lower amplitude weak oscillators in a heterogeneous network.

However, the system dynamics were considerably more complicated than just direct competition to control the firing rate since VIP also contributes to phase shifting the molecular clockwork (Tischkau et al., 2003), while bound GABA receptors directly contribute to chloride currents and related electrophysiological changes. We found that highest synchronization indices were achieved when the peak firing rate (on average across the SCN core) was confined to a narrow range. The model provided strong evidence that a precise balance between VIP and GABA signaling was required to achieve this balance due to their opposing effects of increasing and decreasing firing rates, respectively. Meanwhile, the two neurotransmitters did not directly oppose each other (because of their influence on different components of the model) but rather coordinated to modulate network properties, as shown by the increase in period observed at high values of both VIP and GABA. Experimental validation of this balance could be performed through the use of transcriptional modulators for VIP and GABA or through the application of neurotransmitter receptor agonists and antagonists. One of our results explored how neurotransmitter firing both adapts a cell to synchronize with its network by coordinating the timing of its molecular core clock oscillations. Another results showed how GABA in particular facilitated the time for network resynchronization by allowing individual strong oscillations to phase shift by delaying, in concert with the majority of the network. Overall, our approach of simulating how excitatory and inhibitory networks work in collaboration to control a variety of network properties (such as firing rate, synchrony, amplitude, and period) generated new insights compared with experimental studies focused on how VIP or GABA works in isolation (An et al., 2013; Freeman Jr. et al., 2013; Shen et al., 2000).

We predicted that when ionic currents and intracellular clock components are synchronized, cells are better able to anticipate firing from other cells in the network. Our study has shown that the phase difference between VIP receptor saturation and *Per* mRNA was dependent on the intrinsic oscillatory properties of a neuron and could change as VIP and GABA signaling was modulated. While controlled timing of the VIP signal relative to the *Per* mRNA oscillation was not necessary for cells to adopt the same period, the phases of all neurons were more closely aligned when this phase relationship was reduced throughout the network. Otherwise, many cells anticipated peaks in the firing rate too early in the day or the network contained a large number of cells with *Per* mRNA oscillations peaking well after stimulation with VIP.

The importance of synchronizing these two components' oscillations was due to the role of VIP in controlling the core molecular clock through *Per* mRNA oscillations. Alternatively, the results could have been represented in terms of calcium oscillations, which also directly influence the core clock through CREB. Meanwhile for GABA, which does not have a known mechanism for directly influencing the clock, the primary role would be to control the timing between calcium and *Per* mRNA oscillations by modulating neuron electrophysiology. For model validation, exploration of how phase differences between electrophysiological components such as calcium currents, shown to be an important circadian output (Aguilar-Roblero et al., 2015), and clock components such as *Per* mRNA vary when the efficiency of coupling is modulated will continue to be the subject of future work (Casado and Morillo, 2015; Yasunaga et al., 2015). Meanwhile, our results showed that synchronization index is highest when *Per* mRNA and the reception of the VIP

neurotransmitter oscillate in phase on average throughout the network. Modeling results demonstrating the importance of excitatory neurotransmitters in phase tuning to synchronize coupled SCN networks have been presented previously (Ananthasubramaniam et al., 2014), while our results suggest that inhibitory networks (coupled through GABA) would have an important role in phase tuning as well. Furthermore, those studying the phase relationships between ionic currents and clock components should be aware that phases might be highly dependent on VIP and GABA signaling.

The balance between firing and the molecular clock must also be fine-tuned when networks entrained to light undergo phase shifts, and here again GABA was shown to be beneficial, as it was shown previously for reconfiguration from long days and explored both in SCN slices and in computational multiscale models of neurons with heterogeneous chloride concentrations (DeWoskin et al., 2015; Evans et al., 2013; Myung et al., 2015). Neurons can anticipate when to fire and secrete VIP because they have been exposed to steady daily patterns and have the molecular machinery to maintain robust rhythms. In the cases of a dark pulse or phase shift, these neurons may resynchronize more quickly if they can more effectively repress this anticipatory VIP secretion during sudden periods of darkness. This type of situation was exactly when one would expect GABA to be beneficial since we showed that GABA could balance excessive VIP signaling when the VIP maximum release rate was up-regulated. Therefore, increases in VIP coupling strength would likely make GABA even more important for resynchronization. Experimental studies are needed to show the effect of GABA and GABA blockade on re-entrainment times for networks with different degrees of excitatory coupling. We have used our model to study potential purposes for GABA secretion in the SCN and in the process found potential roles for inhibitory networks in synchronization and re-entrainment. We believe that the concept of coordination between excitatory and inhibitory neuronal networks could play a pivotal role in devising effective treatment strategies for individuals with disrupted circadian rhythms.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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References

- Abraham U, Granada AE, Westermarck PO, Heine M, Kramer A, Herzel H. Coupling governs entrainment range of circadian clocks. *Molecular systems biology*. 2010; 6:1–19.
- Aguilar-Roblero, R.; Díaz-Muñoz, M.; Báez-Ruiz, A.; Quinto-Muñoz, D.; Lundkvist, G.; Michel, S. Mechanisms of Circadian Systems in Animals and Their Clinical Relevance. Springer; 2015. Intracellular Calcium as a Clock Output from SCN Neurons; p. 115-132.
- Albus H, Vansteensel MJ, Michel S, Block GD, Meijer JH. A GABAergic mechanism is necessary for coupling dissociable ventral and dorsal regional oscillators within the circadian clock. *Current Biology*. 2005; 15:886–893. [PubMed: 15916945]
- An S, Harang R, Meeker K, Granados-Fuentes D, Tsai CA, Mazuski C, Kim J, Doyle FJ, Petzold LR, Herzog ED. A neuropeptide speeds circadian entrainment by reducing intercellular synchrony. *Proceedings of the National Academy of Sciences*. 2013; 110:E4355–E4361.

- Ananthasubramaniam B, Herzog ED, Herzel H. Timing of neuropeptide coupling determines synchrony and entrainment in the mammalian circadian clock. *PLoS computational biology*. 2014; 10:e1003565. [PubMed: 24743470]
- Aton SJ, Huettner JE, Straume M, Herzog ED. GABA and Gi/o differentially control circadian rhythms and synchrony in clock neurons. *Proceedings of the National Academy of Sciences*. 2006; 103:19188–19193.
- Aton SJ, Colwell CS, Harmar AJ, Waschek J, Herzog ED. Vasoactive intestinal polypeptide mediates circadian rhythmicity and synchrony in mammalian clock neurons. *Nature neuroscience*. 2005; 8:476–483. [PubMed: 15750589]
- Bechtold DA, Brown TM, Luckman SM, Piggins HD. Metabolic rhythm abnormalities in mice lacking VIP-VPAC2 signaling. *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology*. 2008; 294:R344–R351.
- Belenky MA, Yarom Y, Pickard GE. Heterogeneous expression of γ -aminobutyric acid and γ -aminobutyric acid-associated receptors and transporters in the rat suprachiasmatic nucleus. *Journal of Comparative Neurology*. 2008; 506:708–732. [PubMed: 18067149]
- Boivin DB. Influence of sleep-wake and circadian rhythm disturbances in psychiatric disorders. *Journal of Psychiatry and Neuroscience*. 2000; 25:446. [PubMed: 11109296]
- Brancaccio M, Maywood ES, Chesham JE, Loudon AS, Hastings MH. A Gq-Ca²⁺ axis controls circuit-level encoding of circadian time in the suprachiasmatic nucleus. *Neuron*. 2013; 78:714–728. [PubMed: 23623697]
- Buyse D, Monk T, Carrier J, Begley A. Circadian patterns of sleep, sleepiness, and performance in older and younger adults. *Sleep*. 2005; 28:1365–1376. [PubMed: 16335481]
- Casado J, Morillo M. A model of calcium-mediated coupling between membrane activity and clock gene expression in neurons of the suprachiasmatic nucleus. *arXiv preprint*. 2015 arXiv: 1503.00908.
- Castel M, Morris JF. Morphological heterogeneity of the GABAergic network in the suprachiasmatic nucleus, the brain's circadian pacemaker. *Journal of anatomy*. 2000; 196:1–13. [PubMed: 10697283]
- Cho K, Ennaceur A, Cole JC, Suh CK. Chronic jet lag produces cognitive deficits. *J Neurosci*. 2000; 20:1–5. [PubMed: 10627575]
- DeWoskin D, Myung J, Belle MD, Piggins HD, Takumi T, Forger DB. Distinct roles for GABA across multiple timescales in mammalian circadian timekeeping. *Proceedings of the National Academy of Sciences*. 2015:201420753.
- Ehlen JC, Novak CM, Karom MC, Gamble KL, Paul KN, Albers HE. GABAA receptor activation suppresses Period 1 mRNA and Period 2 mRNA in the suprachiasmatic nucleus during the mid-subjective day. *European Journal of Neuroscience*. 2006; 23:3328–3336. [PubMed: 16820022]
- Enoki R, Kuroda S, Ono D, Hasan MT, Ueda T, Honma S, Honma K-i. Topological specificity and hierarchical network of the circadian calcium rhythm in the suprachiasmatic nucleus. *Proceedings of the National Academy of Sciences*. 2012; 109:21498–21503.
- Evans JA, Leise TL, Castanon-Cervantes O, Davidson AJ. Dynamic interactions mediated by nonredundant signaling mechanisms couple circadian clock neurons. *Neuron*. 2013; 80:973–983. [PubMed: 24267653]
- Freeman GM Jr, Krock RM, Aton SJ, Thaben P, Herzog ED. GABA networks destabilize genetic oscillations in the circadian pacemaker. *Neuron*. 2013; 78:799–806. [PubMed: 23764285]
- Gribkoff VK, Pieschl RL, Dudek FE. GABA receptor-mediated inhibition of neuronal activity in rat SCN in vitro: pharmacology and influence of circadian phase. *Journal of neurophysiology*. 2003; 90:1438–1448. [PubMed: 12750413]
- Harang R, Bonnet G, Petzold LR. WAVOS: a MATLAB toolkit for wavelet analysis and visualization of oscillatory systems. *BMC research notes*. 2012; 5:163. [PubMed: 22448897]
- Herzog ED. Neurons and networks in daily rhythms. *Nature Reviews Neuroscience*. 2007; 8:790–802. [PubMed: 17882255]
- Herzog ED, Aton SJ, Numano R, Sakaki Y, Tei H. Temporal precision in the mammalian circadian system: a reliable clock from less reliable neurons. *Journal of biological rhythms*. 2004; 19:35–46. [PubMed: 14964702]

- Itri J, Colwell CS. Regulation of inhibitory synaptic transmission by vasoactive intestinal peptide (VIP) in the mouse suprachiasmatic nucleus. *Journal of neurophysiology*. 2003; 90:1589–1597. [PubMed: 12966176]
- Itri J, Michel S, Waschek JA, Colwell CS. Circadian rhythm in inhibitory synaptic transmission in the mouse suprachiasmatic nucleus. *Journal of neurophysiology*. 2004; 92:311–319. [PubMed: 14973316]
- Klein, DC.; Moore, RY.; Reppert, SM. *Suprachiasmatic nucleus: the mind's clock*. Oxford University Press; 1991.
- Kondratova AA, Kondratov RV. The circadian clock and pathology of the ageing brain. *Nature Reviews Neuroscience*. 2012; 13:325–335. [PubMed: 22395806]
- Leloup J-C, Goldbeter A. Toward a detailed computational model for the mammalian circadian clock. *Proceedings of the National Academy of Sciences*. 2003; 100:7051–7056.
- Liu C, Reppert SM. GABA synchronizes clock cells within the suprachiasmatic circadian clock. *Neuron*. 2000; 25:123–128. [PubMed: 10707977]
- Maywood ES, Reddy AB, Wong GK, O'Neill JS, O'Brien JA, McMahon DG, Harmar AJ, Okamura H, Hastings MH. Synchronization and maintenance of timekeeping in suprachiasmatic circadian clock cells by neuropeptidergic signaling. *Current Biology*. 2006; 16:599–605. [PubMed: 16546085]
- McClung CA. Circadian rhythms, the mesolimbic dopaminergic circuit, and drug addiction. *The Scientific World Journal*. 2007; 7:194–202. [PubMed: 17982593]
- Meijer JH, Michel S, VanderLeest HT, Rohling JH. Daily and seasonal adaptation of the circadian clock requires plasticity of the SCN neuronal network. *European Journal of Neuroscience*. 2010; 32:2143–2151. [PubMed: 21143668]
- Moore RY, Speh JC. GABA is the principal neurotransmitter of the circadian system. *Neuroscience letters*. 1993; 150:112–116. [PubMed: 8097023]
- Morin L, Allen C. The circadian visual system, 2005. *Brain research reviews*. 2006; 51:1–60. [PubMed: 16337005]
- Myung J, Hong S, DeWoskin D, De Schutter E, Forger DB, Takumi T. GABA-mediated repulsive coupling between circadian clock neurons in the SCN encodes seasonal time. *Proceedings of the National Academy of Sciences*. 2015; 112:E3920–E3929.
- Nielsen HS, Hannibal J, Fahrenkrug J. Vasoactive intestinal polypeptide induces per1 and per2 gene expression in the rat suprachiasmatic nucleus late at night. *European Journal of Neuroscience*. 2002; 15:570–574. [PubMed: 11876785]
- Pakhotin P, Harmar AJ, Verkhatsky A, Piggins H. VIP receptors control excitability of suprachiasmatic nuclei neurones. *Pflügers Archiv*. 2006; 452:7–15. [PubMed: 16283205]
- Pfeuty B, Thommen Q, Corellou F, Djouani-Tahri EB, Bouget FY, Lefranc M. Circadian clocks in changing weather and seasons: Lessons from the picoalga *Ostreococcus tauri*. *BioEssays*. 2012; 34:781–790. [PubMed: 22806346]
- Reed HE, Meyer-Spasche A, Cutler DJ, Coen CW, Piggins HD. Vasoactive intestinal polypeptide (VIP) phase-shifts the rat suprachiasmatic nucleus clock in vitro. *European Journal of Neuroscience*. 2001; 13:839–843. [PubMed: 11207820]
- Reppert SM, Weaver DR. Coordination of circadian timing in mammals. *Nature*. 2002; 418:935–941. [PubMed: 12198538]
- Roenneberg T, Allebrandt KV, Mrosovsky M, Vetter C. Social jetlag and obesity. *Current Biology*. 2012; 22:939–943. [PubMed: 22578422]
- Rouch I, Wild P, Ansiau D, Marquié J-C. Shiftwork experience, age and cognitive performance. *Ergonomics*. 2005; 48:1282–1293. [PubMed: 16253945]
- Sack RL, Auckley D, Auger RR, Carskadon MA, Wright KP Jr, Vitiello MV, Zhdanova IV. Circadian Rhythm Sleep Disorders: Part I, Basic Principles, Shift Work and Jet Lag Disorders An American Academy of Sleep Medicine Review: An American Academy of Sleep Medicine Review. *Sleep*. 2007; 30:1460. [PubMed: 18041480]
- Satlin A, Volicser L, Stopa EG, Harper D. Circadian locomotor activity and core-body temperature rhythms in Alzheimer's disease. *Neurobiology of aging*. 1995; 16:765–771. [PubMed: 8532109]

- Shen S, Spratt C, Sheward WJ, Kallo I, West K, Morrison CF, Coen CW, Marston HM, Hamar AJ. Overexpression of the human VPAC2 receptor in the suprachiasmatic nucleus alters the circadian phenotype of mice. *Proceedings of the National Academy of Sciences*. 2000; 97:11575–11580.
- Shi, S-q; Ansari, TS.; McGuinness, OP.; Wasserman, DH.; Johnson, CH. Circadian disruption leads to insulin resistance and obesity. *Current Biology*. 2013; 23:372–381. [PubMed: 23434278]
- Shinohara K, Honma S, Katsuno Y, Abe H, Honma K. Two distinct oscillators in the rat suprachiasmatic nucleus in vitro. *Proceedings of the National Academy of Sciences*. 1995; 92:7396–7400.
- Shirakawa T, Honma S, Katsuno Y, Oguchi H, Honma Ki. Synchronization of circadian firing rhythms in cultured rat suprachiasmatic neurons. *European Journal of Neuroscience*. 2000; 12:2833–2838. [PubMed: 10971625]
- Sujino M, Masumoto K-h, Yamaguchi S, van der Horst GT, Okamura H, Inouye S-IT. Suprachiasmatic nucleus grafts restore circadian behavioral rhythms of genetically arrhythmic mice. *Current biology*. 2003; 13:664–668. [PubMed: 12699623]
- Taylor SR, Gunawan R, Petzold LR, Doyle FJ. Sensitivity measures for oscillating systems: Application to mammalian circadian gene network. *Automatic Control, IEEE Transactions on*. 2008; 53:177–188.
- Taylor SR, Webb AB, Smith KS, Petzold LR, Doyle FJ. Velocity response curves support the role of continuous entrainment in circadian clocks. *Journal of biological rhythms*. 2010; 25:138–149. [PubMed: 20348465]
- Tischkau SA, Mitchell JW, Tyan S-H, Buchanan GF, Gillette MU. Ca²⁺/cAMP response element-binding protein (CREB)-dependent activation of Per1 is required for light-induced signaling in the suprachiasmatic nucleus circadian clock. *Journal of Biological Chemistry*. 2003; 278:718–723. [PubMed: 12409294]
- Turek FW, Joshu C, Kohsaka A, Lin E, Ivanova G, McDearmon E, Laposky A, Losee-Olson S, Easton A, Jensen DR. Obesity and metabolic syndrome in circadian Clock mutant mice. *Science*. 2005; 308:1043–1045. [PubMed: 15845877]
- Vasalou C, Henson MA. A multiscale model to investigate circadian rhythmicity of pacemaker neurons in the suprachiasmatic nucleus. *PLoS computational biology*. 2010; 6:e1000706. [PubMed: 20300645]
- Vasalou C, Henson MA. A multicellular model for differential regulation of circadian signals in the core and shell regions of the suprachiasmatic nucleus. *Journal of theoretical biology*. 2011; 288:44–56. [PubMed: 21871462]
- Vasalou C, Herzog ED, Henson MA. Multicellular model for intercellular synchronization in circadian neural networks. *Biophysical journal*. 2011; 101:12–20. [PubMed: 21723810]
- Viitasalo K, Puttonen S, Kuosma E, Lindström J, Härmä M. Shift rotation and age–interactions with sleep–wakefulness and inflammation. *Ergonomics*. 2014:1–10.
- Wagner S, Castel M, Gainer H, Yarom Y. GABA in the mammalian suprachiasmatic nucleus and its role in diurnal rhythmicity. *Nature*. 1997; 387:598–603. [PubMed: 9177347]
- Watanabe K, Vanecsek J, Yamaoka S. In vitro entrainment of the circadian rhythm of vasopressin-releasing cells in suprachiasmatic nucleus by vasoactive intestinal polypeptide. *Brain research*. 2000; 877:361–366. [PubMed: 10986351]
- Webb AB, Angelo N, Huettner JE, Herzog ED. Intrinsic, nondeterministic circadian rhythm generation in identified mammalian neurons. *Proceedings of the National Academy of Sciences*. 2009; 106:16493–16498.
- Webb AB, Taylor SR, Thoroughman KA, Doyle FJ III, Herzog ED. Weakly circadian cells improve resynchrony. *PLoS computational biology*. 2012; 8:e1002787. [PubMed: 23209395]
- Welsh DK, Logothetis DE, Meister M, Reppert SM. Individual neurons dissociated from rat suprachiasmatic nucleus express independently phased circadian firing rhythms. *Neuron*. 1995; 14:697–706. [PubMed: 7718233]
- Wu Y-H, Swaab DF. Disturbance and strategies for reactivation of the circadian rhythm system in aging and Alzheimer's disease. *Sleep medicine*. 2007; 8:623–636. [PubMed: 17383938]
- Wulff K, Dijk D-J, Middleton B, Foster RG, Joyce EM. Sleep and circadian rhythm disruption in schizophrenia. *The British Journal of Psychiatry*. 2012; 200:308–316. [PubMed: 22194182]

- Xu Y, Padiath QS, Shapiro RE, Jones CR, Wu SC, Saigoh N, Saigoh K, Ptá ek LJ, Fu Y-H. Functional consequences of a CKI δ mutation causing familial advanced sleep phase syndrome. *Nature*. 2005; 434:640–644. [PubMed: 15800623]
- Yamaguchi S, Isejima H, Matsuo T, Okura R, Yagita K, Kobayashi M, Okamura H. Synchronization of cellular clocks in the suprachiasmatic nucleus. *Science*. 2003; 302:1408–1412. [PubMed: 14631044]
- Yasunaga M, Murotomi K, Abe H, Yamazaki T, Nishii S, Ohbayashi T, Oshimura M, Noguchi T, Niwa K, Ohmiya Y. Highly sensitive luciferase reporter assay using a potent destabilization sequence of calpain 3. *Journal of biotechnology*. 2015; 194:115–123. [PubMed: 25528501]

Highlights

- Predicted how changes in VIP and GABA release rates influence circadian networks.
- A balance between inhibitory and excitatory networks was required for synchronization.
- Over-excitation increased the time required for adjustment to changing light schedules.
- Increased GABA network activity could assist with light shifts for high VIP levels.

Fig. 1. Diagram of model neurotransmitter signaling

The connections between two cells in the multicellular model of the SCN core are depicted, one of which secretes VIP and one of which does not secrete VIP. The model was based on the assumption that 20% of cells are VIP producers while all cells secrete GABA. VIP is a key driver of molecular core clock oscillations (waves), making this neurotransmitter essential for synchronization. The neurons are heterogeneous, so the intrinsic period and amplitude of their molecular core clocks will vary. The molecular clock oscillations drive firing rates (lightning bolts) which in turn drive neurotransmitter release. Firing rates are inhibited by GABA reception for all SCN core neurons. Therefore, the GABA network can be conceptualized as a negative feedback mechanism. The overlapping VIP and GABA feedback mechanisms across networked heterogeneous cells influence each other's concentrations and local cellular dynamics and as such form the basis of the hypothesis of coordination tested in these studies.

Fig. 2. Sensitivity analyses around VIP and GABA demonstrate their influence on various network properties

Shown are heat maps representing the effects of modulating the maximum release rate of VIP (v_{VIP}) (rows) and the maximum release rate of GABA (v_{GABA}) (columns). (A–D) The influence of modulating v_{VIP} and v_{GABA} on mean peak firing rate, synchronicity, mean neuronal amplitude, and period, respectively, are shown for a 400-cell model of the mammalian SCN ventral core. For each v_{VIP} - v_{GABA} value pair, results were averaged over five simulations with each simulation connecting the same population of heterogeneous

neurons through a different set of random connections. Synchronization Index (SI) values are scaled from 0 to 1, with 1 representing total synchrony and 0 representing total asynchrony. Standard deviations and some statistical significance information are available in S1 Table.

Fig. 3. GABA played an important role in the process and timing of re-entrainment

Per mRNA rhythms and phase shifting dynamics are shown for 12:12 LD cycles before and after a 12h light phase shift (starting at the dotted line) for three representative neurons at $v_{VIP}=0.5$ nM/h. The light periods are indicated by yellow rectangles. The time difference between the *Per* mRNA peak time and the end of the *Zeitgeber* period (ZT=12), both before (left axis) and after (right axis) the light shift, was plotted over time. Advancer neurons have peak time differences from ZT_{after} that are greater than 24h to reflect that advancers perform an additional cycle more than delays to resynchronize to the new light period. Dotted lines

indicate the end of the day in which the light shift was implemented. The arrow for each case indicates the day re-entrainment was achieved, defined as being within 0.5h of their stable phase relationship before the light shift. **(A, D)** GABA reduced the time for re-entrainment for a delayer neuron. **(B, E)** GABA switched a delayer neuron to an advancer neuron, which could result in an increase (displayed) or a reduction in the time for re-entrainment. **(C, F)** GABA caused an advancer neuron to advance more slowly, also resulting in an increase in re-entrainment time. Results and error bars in (D–E) are for the same cell connected by different GABA and VIP topologies over five independent simulations. Results and error bars in (F) are from three independent simulations and exclude two simulations in which the cell switched from being an advancer to being a delayer with GABA. Asterisks in (B) denote significant differences with or without GABA from a one-tailed *t*-test.

Fig. 4. The effect of GABA on velocity response curves

GABA significantly enlarged the delay region but had a negligible effect on the advance region of the velocity response curve (VRC) generated by applying dark pulses to individual SCN neurons entrained to 12:12 LD cycles. A single one hour dark pulse was applied at different phases of a 12h light period. The instantaneous change in phase velocity was plotted against the *Zeitgeber* time (ZT) at which the 1h dark pulse began to generate VRCs. From the original network, 40 neurons were randomly selected but the analyses were performed only with the 32 neurons that maintained a stable phase velocity prior to the dark

pulse. The error bars present standard deviations about the mean calculated from the 32 cell ensemble. The dotted lines represent the singularity between the advance and delay regions. **(A)** Each neuron signaling itself with VIP and no GABA ($v_{VIP}=0.50$ nM/h; $v_{GABA}=0$ nM/h) exhibited a delay region from ZT=0h–6h and a smaller advance region from ZT=6–12h. **(B)** Each neuron signaling itself with VIP and GABA ($v_{VIP}=0.50$ nM/h; $v_{GABA}=0.50$ nM/h) exhibited a larger delay region and a similar advance region compared to the case without GABA. **(C)** The effect of GABA on the phase velocity was calculated by subtracting the VRC with GABA (B) from the VRC without GABA (A). Note that the scale of the y-axis in (C) is an order of magnitude less than in (A–B). Overall, the delay region with GABA was 8.4% greater than the delay zone without GABA whereas the advance region was 2.1% smaller with GABA.