The biological clock in mammals controls many important behavioral functions, one of which is sleep and wake patterns. It has been well established that this circadian pacemaker is located in the suprachiasmatic nucleus (SCN) of the hypothalamus. Lesions of the SCN have been shown to entirely eliminate circadian patterns of sleeping and waking, indicating that the SCN is completely necessary for circadian regulation of sleeping and waking in mammals.

Neurotransmitters play a very important role in the SCN and how it controls sleeping and waking; namely, neurotransmitters mediate circadian signaling from the SCN and act within the SCN to influence the activity of SCN neurons. Mathematical modeling has previously been done to analyze the sleep wake patterns of mammals, specifically rodents, and how these patterns are controlled by different neurotransmitters. These models have represented the difference in light and dark sleep and wake patterns with different parameter values. Modeling has yet to delve into the role that the circadian clock in the SCN plays in the sleeping and waking of rodents. We have developed an SCN firing rate model that explores the circadian regulation of the SCN and consequently, the circadian regulation of sleeping and waking over a 24 hour period. The model takes into consideration projections to and from the SCN and their excitatory and/or inhibitory effects on sleeping and waking.

Circadian variation of SCN firing rate

Experimental evidence shows that SCN firing activity is higher during the light period and lowering during the dark period. In nocturnal rodents, this corresponds to higher SCN activity during the inactive period and lower SCN activity during the active period. This overall trend in SCN firing rate represents a pseudo-sinusoid curve over a 24 hour period. In addition to this more general trend, it has also been shown that SCN activity will briefly increase and decrease in accordance with vigilance state, independently of the circadian phase. In particular, SCN firing rate will increase each time a rodent enters a REM or wake phase, and decrease each time a rodent enters a sleep phase. This behavior and SCN response is interesting because while the generally higher activity of the SCN during the day seems to inhibit wake and excite sleep in nocturnal animals, the more specific SCN firing rate has been found to be higher during waking and lower during sleeping [Figure 1]. This vigilance state-dependent variation results from feedback from the sleep-wake network onto the SCN. This feedback consists of

Figure 1 SCN activity, slow wave activity, and vigilance states. SCN activity follows a general circadian trend over the 24 period. In addition, higher activity is seen during REM sleep than NREM sleep, representing the dependence of SCN neuronal activity on vigilance states.

Taken without permission from [3]
Projections to the SCN
The SCN receives projections of acetylcholine (ACh) from the laterodorsal tegmental nucleus (LDT) and the pedunculopontine tegmental nucleus (PPT) and projections of serotonin (5HT) from the dorsal raphe (DR). Activation of the LDT and the PPT promote wake and rapid eye movement (REM) sleep in rodents. In accordance to figure 1, SCN neuronal activity will be higher during REM sleep, explaining the excitatory effects of cholinergic projections onto the SCN. The same follows for serotonergic projections, as SCN neuronal activity is higher during wake states as well. Refer to figure 2 for a visualization of SCN projections.

Projections from the SCN
The SCN is a GABAergic population. This means that the primary neurotransmitter released by the SCN is gamma amino butyric acid (GABA). The SCN projects to the locus coeruleus (LC) and DR, populations controlling waking, the ventrolateral preoptic nucleus (VLPO), an important population for the control of sleep, and the LDT/PPT, populations that contribute to the control of REM sleep. The mechanisms for these projections are indirect, but generally have net excitatory or inhibitory effects.

In its projections to the LC and DR, the SCN first projects GABA to the subparaventricular zone (sPVz), which projects to the dorsomedial hypothalamic nucleus (DMH), which projects glutamate to the lateral hypothalamus (LH). The LH then projects orexin to the LC. While the signs on each of these projections are not completely determined for the nocturnal rat, the net effect of the projections from the SCN to the LC and DR is inhibitory. This can be understood by referring to figure 1 and noticing that overall SCN activity is higher during the subjective day, meaning that overall SCN activity is higher during sleep-dominated time in nocturnal animals. The net inhibitory effect of SCN GABA on the waking populations, LC and DR, correlates with this observation. Projections from the SCN to the LDT/PPT follow the same pathway as those to the LC and DR, and have a net inhibitory effect as well.

Projections from the SCN to the VLPO have a few different possible mechanisms that may be taking place. Similarly to the LC, DR, and LDT/PPT, there has been evidence that shows projections first to the sPVz, then to the DMH, and then to VLPO. Each of these populations is GABAergic. Projections to the VLPO directly from the sPVz, skipping over the DMH as well as projections that skip over the sPVz have been shown. Additionally, experimental evidence has pointed towards sparse direct projections from the SCN to the VLPO, but this is still unclear. While the direct projection from the SCN to the VLPO has been experimentally determined to contain both excitatory and inhibitory elements, we chose a
net excitatory effect of the SCN projection to the VLPO to be consistent with experimental observations in which higher SCN neuronal activity during the subjective day corresponds to higher VLPO activity in nocturnal rodents.

Projections to and from the SCN are summarized in figure 2. The retinal hypothalamic tract (RHT) projection shown in this figure is glutamatergic and possible modeling uses for this projection will be discussed later in the paper.

**Methods**

The overall goal of this model is to explore how SCN firing rate affects sleep-wake patterns and, in turn, how sleep-wake patterns feed back and affect SCN firing rate. In addition to sleep-wake inputs, the SCN firing rate will be controlled by the 24 hour circadian clock. The basic scheme of the model is depicted in figure 3A and the more specific projections between the SCN and S/W can be seen in figure 2.

A standard firing rate model is used to simulate the firing rate of the SCN over a period of 24 hours. The equations for the SCN firing rate are as follows:

\[
\frac{df_{SCN}}{dt} = \frac{(f_{SCN \_inf}(c(t)+sw(t))-f_{SCN})}{\tau_{fSCN}}
\]

\[
f_{SCN \_inf}(x) = \max_{SCN} \times 0.5 \times (1 + \tanh((x-SCN_{ceth})/SCN_{slope}))
\]

where \(x = c(t)+sw(t)\)

\[
c(t) = \sin(t*2*\pi/(24*3600))
\]

\[
sw(t) = gACh_{SCN} \times cACh + g5HT_{SCN} \times c5HT
\]

The sole input initially was the circadian clock, which for now remains as a simple sine curve scaled to a period of 24 hours (in seconds). This firing rate model was then incorporated into Diniz Behn and Booth’s previous sleep wake model\(^2\) (scheme shown in Figure 3B), accounting for the feedback of the SCN projections from the LDT/PPT and the DR of ACh and 5HT, respectively, and the feed-forward of the SCN to the LC, DR, VLPO, and LDT/PPT. The firing rate of each of these populations was modeled in a similar way as the SCN equations shown above.

Excitatory terms (positive signs) were placed into the SCN firing rate equation, representing the excitatory qualities of both cholinergic and serotonergic projections to the SCN. These terms, shown above, have positive scaling constants multiplied by the state-dependent varying concentrations of ACh and 5HT, respectively.

Next, a differential equation was added to represent the concentration of GABA that the SCN releases. The equations for the concentration of GABA are as follows:
The concentration of GABA projected by the SCN varies with SCN activity. That is, it is higher during the subjective day and lower during the subjective night. This is represented by the differential equation. Additionally, the feedback from the sleep-wake network will also affect GABA release by modulating SCN firing rate.

This new SCN GABA gave way to the addition of SCN projections to the sleep-wake network. The specific nature of these projections was explained previously. While multiple neurotransmitters are involved in the indirect projection of the SCN to the LC, DR, and LDT/PPT (GABA, glutamate, and orexin), we have simplified this in our model and represented the net effect of these projections with a direct GABA projection. As per previous discussion, the net effect of this projection will be excitatory to the VLPO and inhibitory to the LC, DR, and LDT/PPT. Terms for this effect were inserted into the differential equations for the LC, DR, LDT/PPT, and VLPO with a scaling constant $g$ (positive for excitatory and negative for inhibitory) multiplied by the concentration of GABA released by the SCN. The relevant parameters and variables and their biological significances are displayed in Table 1.

<table>
<thead>
<tr>
<th>Parameters/Variables</th>
<th>Biological Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>fSCN</td>
<td>SCN firing rate</td>
</tr>
<tr>
<td>fLC</td>
<td>LC firing rate (wake promoting)</td>
</tr>
<tr>
<td>fDR</td>
<td>DR firing rate (wake promoting)</td>
</tr>
<tr>
<td>fR</td>
<td>REM firing rate</td>
</tr>
<tr>
<td>fVLPO</td>
<td>VLPO firing rate (NREM sleep promoting)</td>
</tr>
<tr>
<td>cGSCN</td>
<td>GABA released from the SCN</td>
</tr>
<tr>
<td>cACh</td>
<td>ACh released from LDT/PPT</td>
</tr>
<tr>
<td>c5HT</td>
<td>5HT released from DR</td>
</tr>
<tr>
<td>gAChSCN</td>
<td>Measurement of release of ACh to the SCN</td>
</tr>
<tr>
<td>g5HTSCN</td>
<td>Measurement of release of 5HT to the SCN</td>
</tr>
<tr>
<td>gGSCNLC</td>
<td>Measurement of release of GABA to the LC from the SCN</td>
</tr>
<tr>
<td>gGSCNDR</td>
<td>Measurement of release of GABA to the DR from the SCN</td>
</tr>
<tr>
<td>gGSCNVLPO</td>
<td>Measurement of release of GABA to the VLPO from the SCN</td>
</tr>
<tr>
<td>gGSCNR</td>
<td>Measurement of release of GABA to the LDT/PPT from the SCN</td>
</tr>
</tbody>
</table>

Table 1

This initial model was tested to match the experimental data seen in Blanco-Centurion et al. 2007 for number and length of wake, NREM and REM bouts. We adjusted the parameters accordingly to match the simulations to this experimental data.
Results

The initial problem encountered when parameter fitting this model was that there weren't longer wake bouts seen during the subjective night, as were seen in experiments. The major parameter change to fix this was raising the VLPO threshold, making the VLPO more difficult to activate, thus resulting in longer wake bouts. Additionally, we created a circadian varying function, stim(t), which is the noise variable representing outside stimulus. This varied in such a way that it would be smaller during the subjective day and larger in the subjective night, resulting in the necessary longer wake bouts at night. Put simply, more outside stimulus during the subjective night would make it more difficult for NREM sleep to occur, thereby increasing the length of wake bouts.

Parameter searching resulted in the reproduction of experimentally determined sleep-wake patterns [Table 2], with small error [Figure 6]. The 24 hour cycle of the SCN firing rate emulated the overall circadian pattern as well as the response to vigilance state, as discussed in Deboer et al 2007. This can be seen in figure 4, which portrays phase- and state-dependent variations in SCN firing rate. Figure 5 shows more specific behavior during the peak of the subjective day and during the peak of the subjective night. During the subjective day (lights on), more NREM and REM sleep is seen, and the wake bouts are significantly shorter. During the subjective night (lights off), very long wake bouts are seen, with intermittent NREM and very little REM sleep.

<table>
<thead>
<tr>
<th>Subjective Day</th>
<th>Mean Duration of Bouts (minutes)</th>
<th>Number of Bouts</th>
<th>Percentage in State</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wake</td>
<td>1.5099</td>
<td>167</td>
<td>34.96%</td>
</tr>
<tr>
<td>NREM</td>
<td>2.1677</td>
<td>164</td>
<td>49.22%</td>
</tr>
<tr>
<td>REM</td>
<td>1.1404</td>
<td>80</td>
<td>15.67%</td>
</tr>
<tr>
<td>Subjective Night</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wake</td>
<td>8.8831</td>
<td>67</td>
<td>82.44%</td>
</tr>
<tr>
<td>NREM</td>
<td>1.5809</td>
<td>64</td>
<td>14.09%</td>
</tr>
<tr>
<td>REM</td>
<td>0.7651</td>
<td>24</td>
<td>2.54%</td>
</tr>
</tbody>
</table>

Table 2  Average of ten simulations of sleep-wake patterns from the model. This can be directly compared to the data seen in Blanco-Centurion 2007 as per Figure 6.
To gain a greater understanding of the intricacies of this model, we performed a few simulations in which feedback from the LDT/PPT and the DR to the SCN was blocked. First, the cholinergic and serotonergic projections were blocked individually, while the other remained intact. Next, both projections were blocked to eliminate all feedback from the sleep-wake network to the SCN.

During each of these simulations, polyphasic activity was eliminated during the subjective night, as the LC was activated for almost the entire dark period. The feedback was reduced more when ACh was blocked as opposed to when 5HT was blocked [Figure 6]. This could be due to the fact that ACh is projected by both the REM populations and the wake-REM populations, while 5HT is projected solely by the DR [Figure 3]. This means that the normalized concentration of ACh would reach two, while the

![Figure 5](image-url)  
*Figure 5* SCN Firing rate, LC activity, VLPO activity, and REM activity during the subjective day and subjective night. Each graph is over a time scale of four hours, subjective day represented by hours 4-8 and subjective night represented by hours 16 to 20 (over a 24 hour day total.) More NREM and REM sleep during the subjective day, as well as more wake during the subjective night can be observed. (Yellow=LC (wake), Red = VLPO(NREM), Blue=REM)
normalized concentration of 5HT would only reach one. Therefore, blocking ACh would cause a greater decrease in feedback.

Discussion

Conclusions

Ultimately, this model shows the dependence of sleep/wake behavior on the variation of SCN firing rate. SCN firing rate cycles sinusoidally from high to low during the subjective day and night, respectively. In doing so, the SCN and its projections control sleep and wake patterns in rodents, promoting sleep during the subjective day and wake during the subjective night. Feedback from the sleep/wake cycle onto the SCN accounts for the slightly higher SCN firing rate during wake states and slightly lower SCN firing rate during sleep states. Simulations of the model reproduce observations made in Deboer et al. (2003): SCN firing rate increases when LC and REM activity increases, and decreases when VLPO (NREM) activity decreases [Figure 5].

The mechanisms for the feedback from the sleep-wake network to the SCN remain virtually unknown. This model predicts that this feedback is necessary to achieve experimental sleep-wake cycles in rats. The results of this model imply that cholinergic and serotonergic projections to the SCN may function as a negative feedback loop contributing to the polyphasic behavior of nocturnal rats.

Limitations

There are a few inconsistencies between the data produced by this model and the experimental data found in Blanco-Centurion et al. 2007. The two main differences are that in the model, there is slightly less REM sleep in both the subjective day and the subjective night due to shorter bouts compared the experimental data [Figure 6]. Also, in the model, the average lengths of all bouts are slightly shorter than that of the experimental data, wake bouts during the subjective night showing the largest difference of about a minute. The difference in REM bout lengths can be attributed to the addition of an inhibitory term representing the SCN projection to the LDT/PPT. This term primarily reduces the amount of REM bouts during the subjective day.
A tradeoff for this reduction in REM bouts is an increase in the bout length of NREM sleep because there are no longer too many REM bouts breaking up the NREM bouts. Originally, a problem with the model was the NREM bout length during the subjective day. Adding the REM-inhibitory term solved this problem, with a small tradeoff.

The slightly shorter bouts in this model can be attributed to the noise that is present in this model. The main purpose of noise in this model is to account for the variation seen in real rodent sleep patterns. Noise may cause the sleep-wake cycle to be more broken up than it should be, explaining the slightly shorter bouts. It should be noted that there are somewhat more wake and NREM bouts seen during the subjective day in my model, which logically accompany the shorter duration of bouts due to noise.

**Future Study**

Currently, this model represents the circadian clock as a simple sine curve. However, there is much experimental evidence pointing towards the idea that light, as well as certain neurotransmitters, causes phase shifts in the circadian clock. To account for these phase shifts, the circadian clock should be modeled in a more dynamic way.

In other studies, a dynamic model for the circadian clock and the effects of light on the circadian clock has been represented by a classic van der Pol oscillator. So far, the van der Pol oscillator model has only been used to model the human circadian pacemaker, and has successfully accounted for light pulses and their phase-shifting effect on the clock. The van der Pol oscillator presents a viable option for exploring a different, more dynamic model of the circadian pacemaker in rodents. This oscillator will have to be modified to be representative of the circadian pacemaker in rodents instead of humans, accounting for the differences in nocturnal and diurnal responses to light. Rodents do not respond to light during the subjective day, accounting for a “dead zone” seen in the rodent phase response curve to light. Humans, however, respond to light at all circadian phases. This is one of the major differences that would have to be accounted for when applying the van der Pol oscillator to rodents.
A more dynamic model of the circadian pacemaker would facilitate potential explorations of how certain inputs (i.e. light) affect the circadian clock, and indirectly how these inputs affect the SCN firing rate and sleep/wake patterns.

Another area that is almost entirely uncertain experimentally, but quite important in painting the whole picture of sleeping and waking, is the effect of SCN firing rate on the clock. Schwartz et al. 1987 injected tetrodotoxin (TTX) into the SCN to prevent spiking in SCN neurons and observe the resultant effects on the circadian pacemaker. Interestingly, the intrinsic circadian pacemaker kept oscillating, but the normal phase-shifting effects of light on the circadian clock were not seen. This implies that there must be some effect of SCN firing rate on the clock, but the mechanism of this effect remains unknown. Modeling in this area would be a good way to form educated predictions about the effects of SCN firing rate on the clock and how this might affect sleep wake patterns in mammals.

Lastly, the SCN is a GABAergic network, meaning its neurons both project GABA and receive projections of GABA. A term could be added in to the model to represent the effect of this intrinsic SCN GABA on firing rate. Experiments on the nature of this GABA are still fairly uncertain, as they have been shown to contradict each other. Wagner et al. 1997 found that SCN GABA is excitatory during the day and inhibitory at night in nocturnal rats, meaning it exaggerates the circadian function of the SCN so that it is even higher during the day and lower at night. Another study, performed by De Jeu and Pennartz on the nocturnal rat, found that GABA is uniformly inhibitory during the day and excitatory at night. These results predict that GABA may act as a filter for light inputs to the SCN. That is, GABA allows for light to affect the SCN during the subjective night and does not allow light to affect the SCN during the subjective day. This correlates with the experimental effects of light on the rat discussed above.

This model can be fine-tuned and later applied to human circadian rhythms, providing an excellent framework with which to study and predict the human sleep wake cycle. Understanding how phase shifting affects the circadian clock and sleeping and waking will give way to a greater understanding of puzzling phenomena such as jet lag and shift work.

Acknowledgements
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