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The Role of Orexin Receptors in Diurnal Variations in Learning and Memory

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THE ROLE OF OREXIN RECEPTORS IN DIURNAL VARIATIONS IN LEARNING AND MEMORY

by

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Abstract

The brain's ability to learn and remember is a topic of extensive debate and research. Mammals share many similarities, including the way in which information from the outside world is processed and stored. Mammalian circadian rhythms have long been thought to be essentially involved with these processes. Orexin, a neurotransmitter in the brain, plays a crucial role in arousal and circadian rhythm. This study is designed to explore the brain's ability to process and store information at different times of the circadian period, and to explore the role of orexin by comparing the performance of normal (wild-type) mice and abnormal (knockout) mice lacking a receptor for orexin in a spatial navigation task. Based on the results of previous research and the known roles of orexin in the brain, I predicted that wild-type mice that learn during the dark phase—which is their active phase—will perform better in a spatial learning and memory task than their counterparts that learn during the light phase, while knockout animals deficient in orexin receptor signaling will not be influenced by the diurnal timing of testing. I tested this prediction by comparing performance in a water-filled radial maze and by analyzing hypothalamic orexin neuron activation in animals tested at the beginning or at the end of their active period, using cFos immunohistochemistry. Our results point to a possible role of orexin in the diurnal variation of learning and memory.

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Table of Contents

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Introduction

Circadian rhythms affect the body's numerous systems, due to their role in metabolism and arousal. The brain's control center for the circadian rhythm is the suprachiasmatic nucleus (SCN) of the hypothalamus. Termed the "master clock," this important structure runs on a 24-hour light-dark cycle and is controlled by information that it receives from its surroundings, such as light. Its location gives the SCN an influential position over the control of changes in the body through the use of hormones. In fact, besides receiving input from the external surroundings, the SCN is also able to gather information from the status of the body's internal environment (Zelinski et al., 2014a). Dispersed throughout the body in different organs, secondary circadian clocks function in harmony with the SCN. Feedback loops mediate the transcription and translation of key genes associated with the circadian oscillations. Disruptions of these rhythms lead to serious impairments correlated with sleep and wakefulness.

Disruptions of sleep-wake oscillations affect many systems that the SCN influences. Much of my understanding about circadian disruptions has come from research in animal models, but humans offer intriguing avenues for investigation as well. Shift work, for example, has been linked to disease (Morris et al., 2016). Specifically, researchers explored the mechanisms behind the changes in blood pressure and inflammation associated with working night shifts. Participants stayed in a sleep laboratory during the experiment, where sleep patterns were analyzed and markers for blood pressure and inflammation were measured. After taking into account other contributing factors among the test subjects, the researchers were able to conclude that disruptions to circadian rhythms lead to cardiovascular disease.

In animal models, circadian rhythms are studied by controlling the light-dark cycle of the subjects' habitation. Common methods include shifting the cycles, incorporating more cycles, and using constant illumination. Disruptions in circadian rhythm have been shown to influence memory formation, through disruption of hippocampal processes such as neurogenesis, plasticity, and long-term potentiation. In an experiment using a Morris water maze, shifts in the cycles caused impaired performance in the test subjects (Zelinski et al., 2014b). A good indicator of memory acquisition and retrieval, the Morris water maze is highly dependent on the hippocampus for optimal performance.

Other research has shown that circadian rhythm disruptions lead to other cognitive and behavioral impairments as well (De Bundel et al., 2013). Cryptochrome, one of the genes

involved with circadian feedback loops and oscillations, was manipulated in an experiment with mice. Testing wild type and knockout subjects in an object recognition task, the researchers cast a possible role for the cryptochrome gene, and thus the circadian clock, in memory formation. Another cognitive function affected by manipulation of this gene is anxiety. Knockout mice were less eager to explore the open arms of an elevated plus maze and didn't spend as much time as their wild type counterparts in the center area of an open field. However, the researchers were unable to support a link between cryptochrome and depression or substance abuse.

Another study examined the effects of meal timing on learning and memory (Loh et al., 2015). Specifically, the researchers fed their experimental group of test subjects in the middle of their inactive phase to see how this would affect their cognitive ability. The experimental group performed worse on both fear conditioning and object recognition tests, when compared to the group that was fed on a regular schedule. At the physiological level, long-term potentiation was also disrupted in the experimental group. Interestingly, evidence showed that the hippocampal circadian clock, and not the SCN clock, was affected by the misaligned feeding.

The biological clock is influenced by aging processes and can be related to cognitive dysfunctions in aging. Evidence of this was contributed by a group of scientists studying the effects of age and time period on learning and memory (Winocur & Hasher, 2004). Old and young rats underwent a water maze task at the start and the conclusion of their active phase. An especially noteworthy result obtained by the researcher was that there not only were differences between animals of different age, but that there were differences between groups in the two time periods. The younger rats performed better near the end of their active phases, whereas the older rats achieved their highest performance at the beginning of their active phases. This has tremendous implications for the role of rhythmicity in learning and memory throughout the lifespan and on improving performance by timing activities based on individual circadian rhythms.

Many neurotransmitter systems are influenced by circadian rhythms. Orexin, one of these neurochemicals that will be examined in this study, is produced in cell bodies of the lateral hypothalamus, and was first associated with feeding behavior. Two separate research groups are credited with its discovery, one naming it orexin and the other hypocretin (De Lecea et al., 1998; Sakurai et al., 1998). Now it is known that it also influences bodily processes related to arousal, such as wakefulness and stress, due to its widespread projections throughout the brain, including

the hippocampus, locus coeruleus, and raphe nuclei (Nambu et al., 1999). Narcolepsy, a sleep disorder characterized by extreme daytime sleepiness, has been shown to be a result of orexin deficiency in the brain (Chemelli et al., 1999). Orexin's role is to excite specific brain nuclei that mediate the control of wakefulness through important neurotransmitter systems such as dopamine, norepinephrine, and acetylcholine. Accordingly, its absence leads to decreased arousal and abnormal brain activity. Narcolepsy can also be observed with the absence of orexin receptors or the administration of orexin receptor antagonists. While it is yet unclear the precise role of each receptor, studies show that type 2 antagonists reduce time spent awake and increase NREM sleep in mice (Etori et al., 2014).

Indeed, orexin appears to aid in the sustainment and enhancement of wakefulness throughout the active period, especially under motivating circumstances (España & Scammell, 2011). Cleaved from the same precursor molecule, there are two types of these neuropeptide hormones, and they each bind to two G-protein coupled receptors. Orexin-A has been shown to bind to both receptors, while Orexin-B chiefly binds to orexin receptor type 2. It is important to note that both types of receptors are expressed in differing locations in the brain. For instance, type 1 receptors are found in the hippocampus, whereas type 2 receptors reside in the cerebral cortex.

The role of the orexin system in cognitive functions is still under investigation. Previous research studies both support and reject a significant role of this neurotransmitter in these important processes. Two separate groups have used injections as an experimental design to determine the role of orexin in learning and memory. The first group is credited for research involving orexin administration on rats that suggests this neurochemical improves long-term potentiation, a mechanism necessary for learning and memory (Wayner et al., 2004). Specifically, the experimenters surgically placed bilateral cannulae into a part of the rats' hippocampus called the dentate gyrus and infused orexin-A. After administration of the neuropeptide, electrodes were put into place and the effects of orexin were visualized. Using different concentrations, the researchers found that orexin increases long-term potentiation. It is interesting to note that in their discussion, they explain that this result was contrary to their previous finding that orexin negatively affects water maze performance.

The second study analyzed rats' performance in a water maze, and found that orexin has a significant function in spatial learning and memory (Akbari et al., 2007). Researchers placed

cannulae into the dentate gyrus of the brains, and targeted the type 1 orexin receptors with an antagonist, SB-334867-A. To test its effects on memory acquisition, three out of the four groups of mice received different doses of the drug before their trials. The same was done for the mice being tested for consolidation and retrieval, but they were injected after their initial set of trials and examined 24 hours later for the amount of time spent in the correct arm of the maze. The experimental groups of mice were significantly impaired in terms of their ability to find the hidden platform while learning the maze task. The researchers concluded that the orexin receptor antagonist led to impaired memory acquisition and consolidation, but that it had no effect on spatial memory retrieval.

Learning and memory processes rely on several brain circuits, such as the hippocampus, amygdala or basal ganglia. Learning is the acquisition of knowledge, while memory is the successful use of the knowledge. Long-term potentiation is the physiological mechanism for memory storage. It involves the strengthening of synapses through repeated activity. The hippocampus, an area of the brain distinguished by long-term potentiation and memory, has been the focus of many research projects that have wanted to dissect its part in circadian rhythms. Through the use of electrophysiology, researchers have found that potentiation is also controlled by circadian oscillations (Chaudhury et al., 2005). Upon stimulation with a current from an electrode, the sliced brains of groups of mice were examined for differences in excitability. The slices that were tested during the active phase of the test subjects consistently exhibited potentiation that was greater in magnitude than that found in slices tested during the inactive phase. The decay of the potentiation was slower during the active phase as well. Essentially, this implies that the hippocampus runs on a time clock, with learning and memory occurring with more success and stability during the active phase.

The aim of the current study is to examine the link between circadian rhythms and behavioral performance, using a spatial learning task. I am interested in how circadian rhythms affect behavior and performance, and whether it is more effective to learn in the morning or in the evening. Of equal importance is what diurnal variations in brain chemistry occur and how they influence learning and memory processes. With this is mind, my focus will be on the role of orexin. A comparison of performance at the beginning or at the end of the active period, using mice deficient in the orexin receptor (knockout mice) and wild-type mice will add valuable information to the research that has already been done on this neurotransmitter.

I hypothesize that circadian rhythms influence learning and memory via orexin-related pathways. Specifically, I predict that wild-type mice in the maze learning and memory task will learn quicker and commit fewer errors at the beginning of their active period (dark) compared to wild-type mice that learn at the end of their inactive period (light), due to increased arousal. The circadian timing of testing (Zeitgeber time) will not influence knockout animals. Wild-type mice tested during their inactive period are expected to show greater orexin neuronal activation than those tested during the active phase, since they are awakened from sleep and orexin secretion is highest upon waking (Lee et al., 2005). Neuronal activation will be measured through the analysis of expression of the immediate early gene cFos, using immunohistochemical methods and confocal microscopy.

Methods – Experiment 1

Subjects

Subjects were 44 C57 BL/6 male mice that were 3-4 months old. They were housed in groups of two to four. Their cages were kept in a room with a constant 12-hour light/dark cycle (8 AM – 8 PM) that was temperature-controlled. Water and food were given ad libitum. All experimental procedures followed ethical standards for the use of laboratory animals. Additionally, this study was approved by the Institutional Animal Care and Use Committee (IACUC).

Apparatus

The radial water maze consists of eight numbered arms and four platforms, submerged under water to ensure that the mice cannot see the contents of each arm. In the experiment room, three walls that have unique patterns and serve as extra-maze spatial cues surround the maze. The experimenter acts as the fourth cue. The maze is filled with water at room temperature to a level 1 cm above the platforms. Yellow paint is added to the water to make it opaque, and both water and paint are added throughout the duration of the experiment as needed.

Phase 1: Maze Training and Experiment with Data Analysis

In this experiment, the mice were divided into morning (n=23) and evening (n=21) groups. The morning mice were run at 6 AM (2 hours before the end of the active dark period), and the evening mice were run at 6 PM (2 hours before the beginning of the active dark period). All mice were run in the maze at the same, specific times every day for 21 days. Each mouse received a unique combination of platforms, which remained constant for the duration of the

experiment. However, the task became increasingly difficult from trial to trial. At the beginning of daily testing, the maze contained four platforms. When the mouse found a platform, that platform was removed and the mouse was given 30 seconds under a heating lamp in order to dry and recover. The next trial, the mouse had to use its spatial memory to find the remaining platforms. That continued until only one platform remained and the mouse located it, ending the daily trials. Thus, as the mouse progressed through the trials, the chances of finding the correct platform gradually decreased from 4 platforms: 8 total arms to 1 platform: 8 total arms. Upon completion of the trials, the mouse was allowed to sufficiently dry off in a cage with a heating lamp and pad.

All conditions, including water temperature, extra-maze cues, and room temperature were recorded and kept constant. The experimenter running the mice kept track of all maze arms entered by each mouse in every trial. An entry of half an arm or less was counted as a partial entry. Data was gathered and analyzed in the categories of working memory correct, working memory incorrect, and reference memory. Reference memory errors mean the mouse entered an arm that never had a platform, working memory correct errors mean the mouse entered an arm that had previously held a platform, and working memory incorrect errors mean the mouse returned to an arm that never had a platform. The data was recorded by the experimenter each day and plotted in an Excel worksheet to display how the different groups compare in regard to learning and memory.

Phase 2: Brain sectioning, staining, and image capture

When each group finished the experiment (within 90 min from the beginning of the test for each mouse), mice were deeply anesthetized with Isoflurane and transcardially perfused with 4% paraformaldehyde in 0.1M Phosphate Buffer, pH 7.4. Then their brains were collected and postfixed overnight in paraformaldehyde solution. After that, the brains were sectioned on a vibratome (coronal cuts, 50 μm sections) to best obtain the region of interest.

The sections from the hypothalamus, the region of interest, were immunostained using antibodies against orexin (to identify orexinergic neurons) and cFos (to identify activated neurons). The first stage of immunohistochemistry was blocking and permeabilization, in which sections were incubated for 2 hours at room temperature with a 10% Donkey Serum, 0.3% Triton X -100 in phosphate-buffered saline (PBS) solution. Next, the blocking solution was removed and primary antibodies were applied: rabbit anti-cFos (Calbiochem, PC38, 1:1000) and goat anti-

Orexin A (Santa Cruz Biotechnology, SC8070, 1:100); the sections were incubated overnight at 4° C (17-18h). The next day, the primary antibodies were removed and all slices were washed with PBS and 0.1% Tween-20. Sections were incubated with secondary antibodies: donkey anti-goat (Jackson ImmunoResearch, Alexa 594, 1:100) and donkey anti-rabbit (Jackson ImmunoResearch, Alexa 488 conjugated, 1:100), at room temperature for 2 h. The secondary antibodies were subsequently removed and the slices were washed with PBS and 0.1% Tween-20 before being placed on slides with Prolong Gold (Invitrogen), to keep the stains from fading.

Images were acquired and analyzed using ZEN software on a Zeiss 710 confocal microscope with appropriate excitation and emission filters (for Alexa 488 Donkey anti Rabbit – cFos and for Alexa 594 Donkey anti Goat – Orexin). Brain sections of five subjects from the morning group and six subjects from the evening group were used. These images were then analyzed and categorized into one of four groups based on their relative location to Bregma in the brain. Cell counts were performed for each image using Adobe Photoshop and the data from each group was compared using a one-way ANOVA, with $p < 0.05$.

Methods – Experiment 2

Subjects

Subjects were 27 OXR2 KO male mice that were 3-4 months old. The rest of the protocol for Subjects followed that of Experiment 1.

Apparatus

The same radial maze was used as in Experiment 1.

Phase 1: Maze Training and Experiment with Data Analysis

In this experiment, the mice were divided into morning (n=13) and evening (n=14) groups. The rest of the protocol for Phase 1 followed that of Experiment 1.

Results

To examine the link between circadian rhythms and behavioral performance in a spatial learning task I used laboratory mice (C57BL/6). The mouse has become ubiquitous as a test subject due to its similarity of genome and physiology to us. Mice can suffer from almost identical diseases as human beings, making them ideal candidates for research and application. This mammalian species also breeds relatively quickly and is easily cared for and maintained. Adding to this, the genome of the C57BL/6 strain has been successfully and completely documented. The only potential drawback is that mice are nocturnal, meaning that in a study of

circadian rhythms, their habits are opposite to ours. However, correlations can still be made after taking this into account. To produce a knockout strain, genes of embryonic stem cells are specifically targeted through homologous recombination; a mutation is introduced into the gene, making its product nonfunctional.

For behavioral studies I used an eight-arm radial water maze. This particular type of maze is ideal for my purposes because it aptly tests both working memory and reference memory in the context of spatial memory (Penley et al., 2013). Traditional land mazes rely on food to motivate the animals to learn and remember and animals need to be food-deprived. With a water maze, the animals are driven to use their memory in order to escape, which is a substantial source of motivation. In addition, neither food deprivation nor pre-training is required for the test subjects. The design of this maze involves taking out platforms once they are found, so the demand for the animals to use their working memory to find the remaining platforms is significantly increased. Also, due to the design of the maze, the test subjects have eight definite choices they can make to find a platform. The person running the trials can note errors and correct choices. The maze itself is maintained with little effort and allows for the behavior of the test subjects to be easily assessed by the experimenter.

In the first experiment, male C57 BL/6 mice (BL6, n=44) were separated into two groups that were run either in the evening (2h before the beginning of their active phase) or in the morning (at the end of their active phase). The brains of these mice were used for analysis of neuronal activation. In Experiment 2, orexin receptor 2 deficient mice (OXR2 KO, n=27) were divided into morning and evening groups and trained in the maze. The OXR2 KO mice lack receptors for orexin but exhibit a very mild narcolepsy and have proven useful in performing experiments to determine the receptor's function (Willie et al., 2003).

The physiological portion of this project relies on immunohistochemistry to analyze the area of interest in the brain of the mice. Essentially, my goal is to obtain a snapshot of neuronal activation in the lateral hypothalamus in the timeframe of the last maze trial. Thin sections of mouse brains fixed with paraformaldehyde, a chemical cross-linker, are required to assess protein expression of individual neurons. Indirect immunostaining, the method I used in my experiment, involves the use of primary and secondary antibodies. The primary antibody forms a bond with the target antigen of the brain tissue, and the secondary antibody with its fluorescent tag attaches to the primary antibody to allow visualization of the protein of interest.

The targets of my staining are orexin and cFos. A common way to examine activation of neurons (Kawashima et al., 2014), cFos staining enables visualization of activation present in the orexin neurons. The gene for cFos is a proto-oncogene, meaning that it can lead to cancer if mutated. The product of the gene is a transcription factor associated with changes in the neuron's phenotype, as in during an action potential (Kovács, K.J., 1998). Different structures in the brain exhibit diversity in their cFos stimulation threshold, but usually an accurate correlation can be made between the presence of cFos and the activity of the neuron in the pathway under examination.

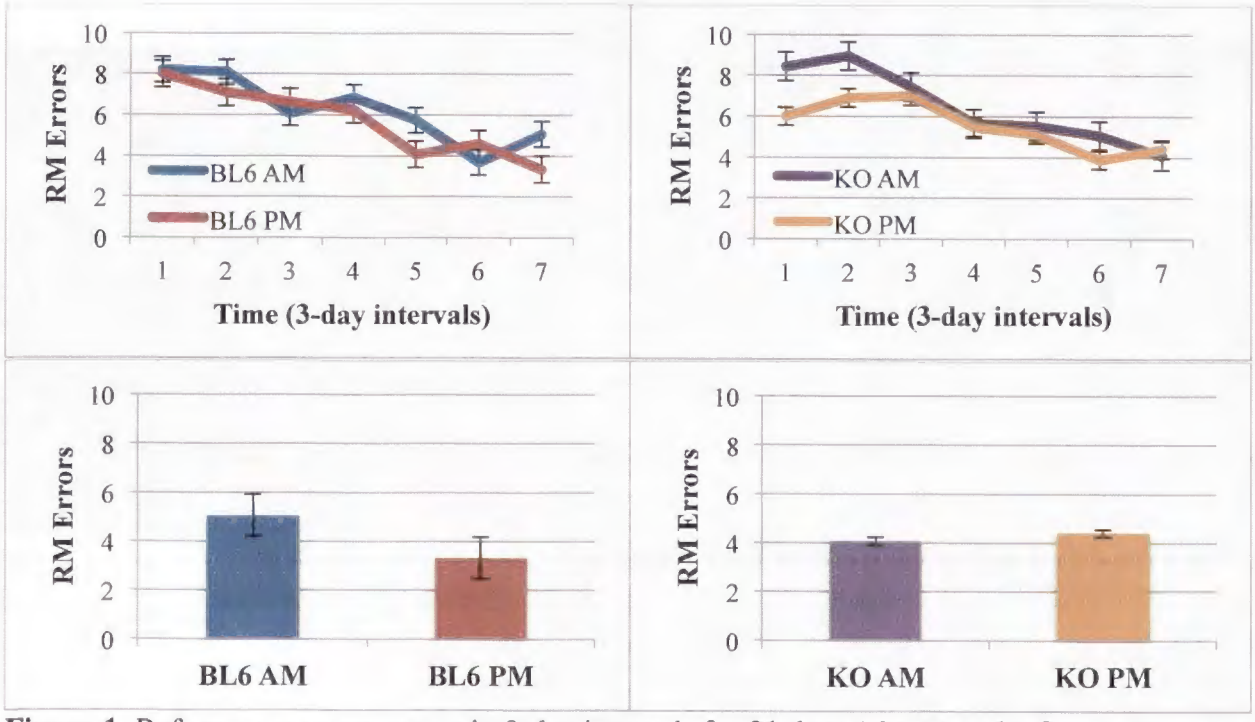
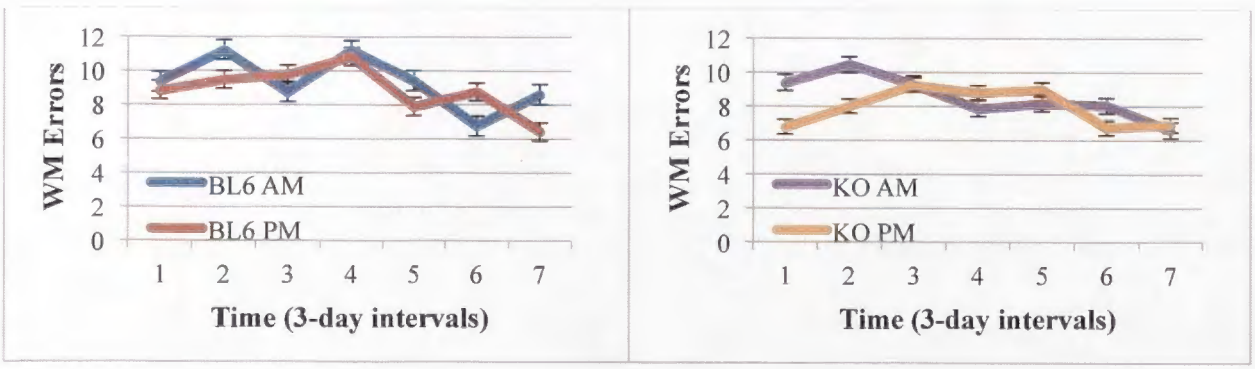


Figure 1. Reference memory errors in 3-day intervals for 21 days (above) and reference memory errors in the last 3-day interval (below).



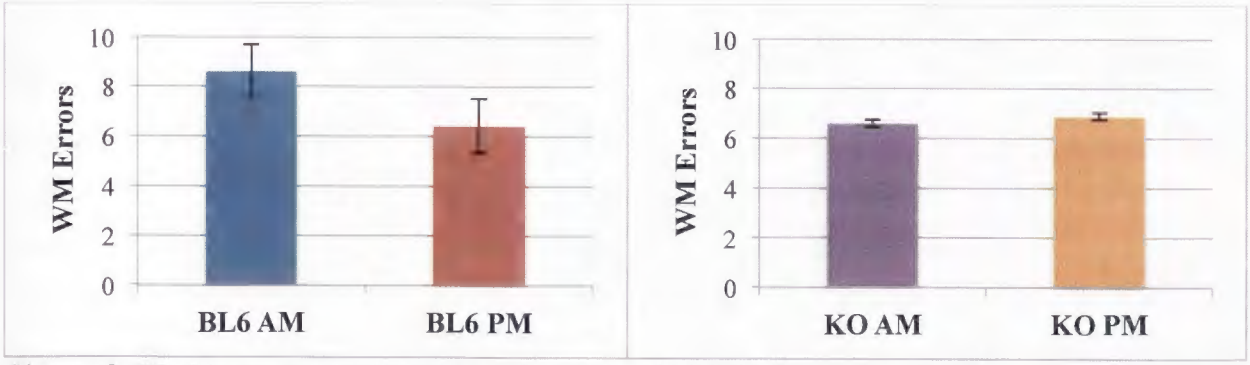


Figure 2. Working memory errors in 3-day intervals for 21 days (above) and working memory errors in the last 3-day interval (below).

Figure 3. Immunofluorescence in the lateral hypothalamus of C57 BL/6 mice in morning (left) and evening (right) groups.

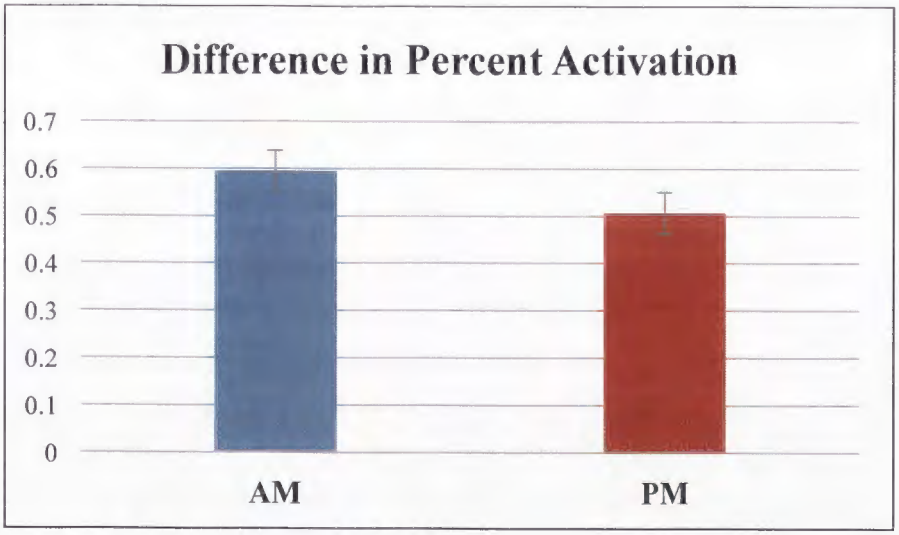


Figure 4. Percent activation of orexin neurons in morning and evening groups of C57BL/6 mice.

Figure 1 depicts the average number of reference memory errors made by the mice throughout the experiment. The formula used for this type of error was the sum of working memory incorrect errors and reference memory errors. Similarly, Figure 2 illustrates the average number of working memory errors made by the mice during the 21 days of the experiment. The formula for this error was the sum of working memory incorrect errors and working memory correct errors. Taken in intervals of three days, the average number of errors for each group shows the overall learning of the test subjects. For both groups of mice, acquisition stretched over the first few days while they grew accustomed to the testing environment. After the initial learning was complete and during the course of the project, evidence that learning is taking place in all groups of mice can be seen from the downward trend of the graphs.

At the conclusion of the behavioral portion of Experiment 1, the evening group of wild-type mice outperformed the morning group, as is shown by the bar graph. To determine statistical significance, an independent-samples t-test was conducted to compare performance between the two groups. There was a significant difference in reference memory between the evening group ($M = 3.3254$, $SD = 2.07463$) and the morning group ($M = 5.0580$, $SD = 3.0607$); $t(39) = 2.2143$, $p = 0.0327$. However, in terms of working memory, there was not a statistical difference between the evening group ($M = 6.4127$, $SD = 3.7685$) and the morning group ($M = 8.6014$, $SD = 4.3504$); $t(42) = 1.7876$, $p = 0.0811$.

In contrast, at the conclusion of Experiment 2, the morning group of knockout mice outperformed the evening group, but not by a margin of statistical significance. For reference memory, the independent-samples t-test with the morning group ($M = 4.0641$, $SD = 2.2033$) and the evening group ($M = 4.3810$, $SD = 2.7754$) resulted in $t(24) = -0.3297$ and $p = 0.7445$. For working memory, the independent-samples t-test with the morning group ($M = 6.5897$, $SD = 3.8155$) and the evening group ($M = 6.8810$, $SD = 4.3385$) resulted in $t(25) = -0.1855$ and $p = 0.8543$.

Levels of hypothalamic orexin neuron activation in subjects run at the end of their active phase (AM) and before the beginning of the active phase (PM) are illustrated in Figure 3. First, orexin neurons (red) were identified and counted. Next, orexin neurons that also stained for cFos (green) were counted. Images from 11 subjects were used, 5 from the morning group and 6 from the evening group. The number of activated orexin neurons (cFos positive) was calculated as a proportion of the total number of orexin neurons. On average, 59.44% of the orexin neurons

were activated in the morning group, whereas 50.66% of the orexin neurons were activated in the evening group (ANOVA, $p = 0.037$).

Discussion

I was able to support my hypothesis that learning and memory are influenced by orexin-related pathways related to circadian rhythms. Due to differences in arousal and brain activation before and after the dark period, I expected the mice that learned at the beginning of their active period to perform better than the mice that learned at the end of their active period. The results of the behavioral aspect of Experiment 1 indicated that wild-type mice perform better in a spatial memory task near the beginning of their active period. The data shows evidence that learning occurred in both groups, but the evening group of wild-type mice outcompeted the morning group by finding the platform with fewer errors. This result was statistically significant when analyzed for differences in reference memory errors between the PM and AM groups.

Analysis of activated orexin cells of the lateral hypothalamus of both groups of wild-type mice showed that the mice that learned during their active phase (AM) experienced greater neuronal activation than those run during the inactive phase (PM). Thus, the degree of neuronal activation was not correlated with performance in the maze: the morning group of mice benefited from greater activation of orexin neurons, but the evening group committed fewer errors than the morning group.

The results of Experiment 2 further illustrate the relationship between orexin and learning. While learning occurred in both groups of knockout mice, at the end of the experiment there was no statistical difference in the number of reference memory and working memory errors made between the evening and morning groups. In this regard, my prediction that the time of day would not influence learning and memory in OXR2 KO mice was correct.

Differential learning and memory in the PM and AM groups may be the result of not only the circadian timing, but also by their relationship to sleep. Studies show that sleep is critical to the formation of long-term memories. Interestingly, diverse parts of sleep play a role in memory enhancement. For example, rapid-eye movement (REM) sleep leads to greater long-term potentiation because more acetylcholine is available for synaptic transmission and there are elevated levels of transcription in genes associated with synaptic plasticity. In an experiment with 5-HT receptor knockout mice, the test subjects found a hidden platform in a similar water maze in shorter amounts of time (Graves et al., 2001). In the absence of this receptor, mice

experience amplified levels of REM sleep, which would lead to improved memory consolidation and learning. Thus, the morning group of mice would have experienced better memory consolidation due to learning the task at the end of their active period, followed by sleep.

The results of the neuronal activation analysis which were not consistent with my prediction may have been negatively affected by the small number of subjects analyzed: I were able to use brains sections from only 11 subjects, and the images I obtained were made up unequally between the two groups—two-thirds of the images were from the evening group. A larger sample size may be required, as would analysis of activated neurons in the hippocampus.

My behavioral results support the role of orexin in hippocampal learning and memory. This project was motivated, in part, by the question of what period of the day it is best for learning. While some light has been shed, many questions still remain. As behavioral results do not seem to be correlated with hypothalamic neuron activation measured by cFos, further research, possibly involving other methods and/or larger samples, is necessary.

Word count: 1,026

Reflection

I enjoyed the research I was able to perform in my undergraduate career. While helping me become a better candidate for medical school, it exposed me to the world of research and gave me an excellent relationship with professors and peers. Even though it was a large task, the capstone project has been an excellent way to review everything I have done and to get to know what else is being done in my field of research. It feels great to have a finished project that sums up around two years of work. One of the main challenges I have run into with my project has been working with a department other than my own. The psychology department is amazing, but it would have been more convenient to have all my work contained within the biology department. I can't complain too much about this aspect of my work, however, because I was very lucky to have been a part of a psychology laboratory. And in all reality, neuroscience, which was my area of work, is very correlated with biology. Besides having to become acquainted with a few elements in the field of psychology, I did not encounter more difficulty making the transition between it and my own field. In terms of actually completing the capstone project, the writing portion was obviously the most time-consuming. The project I completed was very intricate and complicated, but when it came to describing what I did and what happened, I found that it took a small amount of words. As can be seen with the finished project, the introduction is mostly a literature review and makes up half of my capstone project. Having applied for both a SURCO grant and a URCO grant has been a tremendous asset in a number of ways. First, the proposals involved a significant portion of writing. And even though this part was a lot smaller for the proposals, it was in the same format and required knowledge of the literature. Second, I had to present my research at a formal conference due to receiving a grant. This motivated me to ensure I had mastered my project and its implications. Explaining it to people at the conference allowed me to know how to help others without a background in my topic to understand what I did and why it's important. Before closing my discussion on my evaluation of the written part of my project, I need to give a lot of credit to Utah State University's library program. Because of its article database, I was able to have much-needed access to scientific papers that would both help introduce and support my own research. The database also helped me find awesome articles, and it's something I would hope all students take advantage of.

My advice to those who would like to do a capstone project is to plan it out as early as possible. Be aware of deadlines and set personal goals as to when things need to be accomplished. Honors is great because it gets you thinking ahead, so it would be great to have an idea of what you wouldn't mind spending a considerable amount of time on and what you want to represent your undergraduate experience. That is one thing I would definitely emphasize—do what you want and what you are interested in. Although many options are available for potential contracts, I am very glad that a lot of my Honors credits came from continuing the research that became my capstone project. This kept me in touch with my mentors and encouraged me to follow through with what I had started. The greatest thing it did for me was making it so that by the time my senior year came, the experimental portion of my capstone project was already done. This took away a lot of stress that many students have as they try to come up with a project and complete the proposal by the beginning of senior year. All I have had to do is put all of my work together and make the finished product. The last semester of my senior year has been very relaxing, which has allowed me to finish what I need to and to start focusing on where my education is headed. With that in mind, I think students should do as much as they can with their research. They should write proposals for grants, present at conferences or symposiums, and become familiar with other research related to their chosen field of interest. Students should make sure that they have an effective team on their side before they begin their capstone projects. It wouldn't be too difficult to find mentors and committee members that would be willing to sign the proposal agreement. However, it's important to have professors on your side that will give you feedback and work with you to accomplish the task. My mentors have given me tremendous assistance with how the written portion of my project should look, and they've given me ideas as to what to include. Those involved in the capstone process need to be kept informed on the project as well. Especially if you plan to publish your work, your mentors need to be aware of this so they can plan accordingly. I say this because my mentors would like to publish my work before it is made public, in order to receive full credit for the contributions we've made to our area of study and to not allow other researchers to duplicate what we've already done. I will also say that completing an Honors capstone project has been worth every minute I've put into it. I'm currently receiving three credits during my last semester of college for doing something interesting pretty much on my own. It's also something that has the potential to be published. It's been a great way to

reflect on my education and what I have actually learned. And looking back, I'm proud of what I've been able to do and very appreciative of the support that I have received from the Honors program. I would recommend that Honors students complete a capstone project.

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Professional Author Biography

Son of Scott and Patricia, Jacob Blotter grew up in Centerville, Utah and went to Viewmont High School. After one year of college at Utah State University, he served an LDS mission in the Dominican Republic from July 2011 to July 2013. He came back to USU and completed his degree in Biology with an emphasis in Human Biology and a minor in Chemistry. He received an URCO grant for his research and was able to present at the Biology Symposium and the Student Research Symposium on campus. He was an Honors UTF for Dr. David Peak's Complexity and the Arts class for two consecutive school years, and he was awarded the UTF of the Year for the Honors Program in 2015. In that same year, he was nominated to receive the Bill E. Robins Award. Along with his work in the Honors Program, he has served as a UTF for many courses in the Biology Department, including Human Anatomy and Advanced Human Physiology. At the conclusion of his senior year, Jacob received the Deans Scholar Award in the College of Science because of his overall 4.0 GPA. After graduation, he plans to attend medical school at the Uniformed Services University of the Health Sciences.