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Pooja Addala April 10, 2023

The Role of Blue LED Light Exposure in Basolateral Amygdalar Serotonin Innervation and Anxiety-Like Behavior

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Abstract

The Role of Blue LED Light Exposure in Basolateral Amygdalar Serotonin Innervation and Anxiety-Like Behavior

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Blue LED light has been thought to impact affective behavior by activating intrinsically photosensitive retinal ganglion cells (ipRGCs). Past literature has suggested that frequent long-term activation of ipRGCs will continually over-activate anxiety networks in the limbic system leading to the development of anxiety psychopathologies. Literature has indicated that nocturnal blue light exposure negatively impacts mood, whereas diurnal blue light exposure has been used as a treatment modality for seasonal affective disorder (SAD). Additionally, long-term altered light has been correlated with differences in monoamine levels and behavioral changes. Thus, the present study aimed to see if this pattern holds true with blue light, specifically looking at serotonin the basolateral amygdala. Therefore, the present study aimed to investigate whether nocturnal or diurnal blue light exposure creates changes in both serotonin innervation in the basolateral amygdala and anxiety-like behavior.

Adolescent Mongolian gerbils (*Meriones uniguiculatus)* were either assigned blue LED light enrichment for four weeks, at morning or night where they received 12:12 hours of light and dark, with blue LED light enrichment turned on two hours before or after the colony lighting. Following, the gerbils underwent the Elevated Plus Maze (EPM) and Social Approach tasks. Once reaching adulthood, the gerbils were euthanized, and serotonin innervation of the basolateral amygdala was tested through immunohistochemistry using the SERT antibody.

It was hypothesized that the gerbils who received evening light enrichment would show lower levels of social approach and greater time in the enclosed arms of the EPM levels, indicating anxiety-like phenotypes and behaviors. Additionally, it was expected that these gerbils would have a lower SERT density in the basolateral amygdala compared to gerbils with morning or no enrichment. Therefore, it was expected that the evening lighting conditions mimic behavioral and neurochemical changes similar of anxiety pathologies.

There was not significant data suggesting that blue LED lighting during the adolescent development period is correlated with either serotonin innervation or anxiety-like behaviors. This may indicate that low levels of blue light do not actually cause detrimental harm, despite the common belief. This may suggest that there may be confounding variables that attribute to increased psychopathologies, and there may not be a direct mechanism involving iPRGCs. However, this interpretation should be considered preliminary due to the small sample size and the limited areas of interest and behavioral phenotypes studied.

Keywords: Blue LED light exposure, chronobiology, anxiety psychopathology, serotonin

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The Role of Blue LED Light Exposure in Basolateral Amygdalar Serotonin Innervation and Anxiety-Like Behavior

In our modern era, a significant portion of the population is increasingly exposed to blue-enriched lighting, throughout the 24-hour, day-night cycle, due to the use of novel technologies, with their continual advancements, emitting this light. The natural solar day to night and light to dark cycles help regulate the circadian rhythm and affective behavior (Tähkämö et al., 2019). Exposure to blue-enriched lighting differs intrinsically from exposure to solar lighting not only in its timing, but also in its spectral composition (Tähkämö et al., 2019); therefore, it is imperative to recognize, and understand, any possible social and neuroanatomical long-term effects to affective behavior from this light exposure. Literature indicates that altered lighting affects social behavior through changes in affective behavior and monoamine concentrations; however, there is a paucity of research focusing on whether there are differences based on the timing of the exposure. Understanding the relationship between blue light exposure, neurochemical changes, and affective behavior is important to determine whether blue light plays a role in the development of anxiety-like and depressive pathologies which have increased in urban settings (Green et al., 2015). Additionally, the prevalence of adolescent psychopathology has increased and there is probable relationship with technology usage (Twenge, 2020). The present research tests whether exposure to either morning or evening blue lighting mediates anxiety-like behavior and serotonin innervation in the basolateral amygdala in an adolescent rodent model. The present research will progress literature concerning novel anxiety-like pathology risk factors from recent human developments.

Psychophysiological Impacts of Lighting

In humans, exposure to light has psychophysiological implications for processes including cognition, sleep, arousal, and rhythms such as the circadian rhythm (Wright et al., 2013). The circadian clock uses light as a signal to regulate the sleep-wake cycle and is the most sensitive to light during the night (Aschoff, et al., 1971).

Mammalian light perception occurs in the retina using photoreceptors which contribute to both image and non-image forming perception. Light is used in image forming photoreception via phototransduction, a process whereby light is transformed to electrical impulses. Photoreceptors contain photopigments which are proteins that activate when light hits them, leading to neural impulses in the retinal ganglion cells after several stages of retinal processing . Examples of photoreceptors include cones and rods which contain the rhodopsin and photopsin photopigment respectively.

Traditionally, rods and cones were thought to be the only photoreceptors in the retina (Paul et al., 2009). However, Foster et al.(1991) noted that retinally degenerate mice, lacking both rods and cones, still had comparable circadian rhythm functioning to normal mice, therefore indicating the possibility of another photoreceptor involved in circadian rhythm functioning. Further studies discovered melanopsin, the photopigment responsible for circadian rhythm functioning, which ultimately led to the discovery of intrinsically photosensitive retinal ganglion cells (iPRGCs) (Paul et al., 2009).

Intrinsically photosensitive retinal ganglion cells (iPRGCs) have been known to project to the suprachiasmatic nucleus (SCN), a region of the hypothalamus responsible for maintaining the circadian rhythm (Paul et al., 2009). More recently, iPRGCs have been discovered to project

towards mood-regulating regions of the limbic system including the lateral habenula and amygdala (Schmidt et al., 2011). Additionally, molecular biology techniques have revealed a potential correlation with having a missense melanopsin mutation and incidence of seasonal affective disorder (Roecklein et al., 2008). The above noted literature seems to suggest that light plays a role in mood regulation and affective behavior.

The Amygdala's Role in Affective Behavior

The amygdala is a set of nuclei in the limbic system with a primary focus on emotion and emotional memory processing. The amygdala is associated with reward learning, and responses to both fear inducing and positive stimuli (Murray, 2007; Bocchio et al., 2016). For instance, many fMRI studies have noted the amygdala's activation even to low-arousing, fearful stimuli such as upon viewing certain facial expressions (Whalen et al., 2002). Lesions to the bilateral amygdala as seen in Kluver-Bucy syndrome have resulted in visual agnosia, hypo emotionality, and hypersexuality (Olucha-Bordanau et al., 2015). The amygdala receives inputs from both unimodal and multimodal sensory structures and directs projections to structures in the hypothalamus and brainstem (Olchua-Bordanau et al., 2015). Beesdo et al. (2009) reported that individuals with anxiety and co-morbid depressive disorders had higher amygdala activation to both high-arousing and low-arousing fearful stimuli compared to healthy participants. This pattern of findings indicates connectivity of these pathways and structures in emotional processing.

The present study focuses specifically on the basolateral amygdalar nuclei (BLA), which are a pallial region in the amygdala. Alterations of neurochemical circuitry in the basolateral amygdala have been correlated with both depressive and anxiety disorders (Whalen et al., 2002).

As such, it has been hypothesized that serotonin firing differs in the amygdala during fearful conditions (Whalen et al., 2002). This indicates that serotonin innervation in the amygdala may modulate fearful responses and memory consolidation. The BLA is innervated by many modulatory systems that use specific neurotransmitters and neurohormones including norepinephrine and serotonin (Sadikot & Parent, 1990). Previous studies have noted that altered circuitry of both modulatory and inhibitory neurotransmitters in the BLA has correlated with anxiety-like pathology; for example, Sajdyk & Shekhar (1997) observed that when GABA was blocked in the BLA, there was an increase of anxiety-like behaviors. Furthermore, Bruchas et al (2009) identified CRFI-1 (corticotropin-releasing hormone) release in the kappa opioid system in the rat basolateral amygdala as a mediator for anxiety-like behavior. However, John & Currie (2012) observed a decrease in anxiety-like behavior following the addition of N-arachidonoyl- serotonin, a serotonin antagonist, from the BLA. Understanding neurochemical interactions in the BLA, especially of serotonin, is fundamentally important for understanding the development of anxiety pathologies.

Serotonin System in the Central Nervous System

The present study focuses on the neurotransmitter serotonin (5-Hydroxytryptamine or 5-HT) which is an indolamine neurotransmitter derived from tryptophan. The role of serotonin in psychopathology has been grossly simplified to having a direct role in pathology; however, there is no well-defined mechanism for the effects of serotonin transmission on typical or pathological behaviors (Cowen, 2008). Instead, 5-HT modulates many mechanisms that have varying effects and purposes in different brain regions.

To produce serotonin in the brain, tryptophan hydroxylase (TPOH2) converts tryptophan to 5-hydroxytryptophan, which is then converted to serotonin by aromatic L-amino acid decarboxylase (AADC) (Charnay, 2010). 5-HT is initially transported by vesicular monoamine transporters (VMAT) to vesicles for storage until release (Daubert & Condron, 2010). There are seven families of serotonin receptors, and apart from the 5-HT_3 family, they are all G protein linked receptors (Daubert & Condron, 2010). Serotonin is degraded by monoamine oxidase (MAO), an enzyme which catalyzes the oxidation of monoamines.

The dorsal raphe is the major serotonin-producing region in the midbrain. There are many pathways originating from the dorsal raphe that innervate the central nervous system (Figure 1). The dorsal and medial ascending pathways innervate the globus pallidus and substantia nigra respectively (Steinbusch et al.,2021). The ventral ascending pathway is responsible for innervating many brain regions of the limbic system including the amygdala, hippocampus, and hypothalamus(Steinbusch et al., 2021). Psychopathologies including depressive, anxiety, and psychotic disorders are associated with limbic system dysfunction (Figure 1), and selective serotonin reuptake inhibitors (SSRI), a class of medication, which blocks serotonin reuptake, is a common treatment modality (Hensler, 2006).

ipRGCs project to the dorsal raphe nucleus, (Li, Ren, Huang, Lin, Pu, Pickard & So, 2015) therefore, alterations in concentrations in monoamines, like serotonin, may indicate dysfunction in circadian rhythm activity (Flyktman et al., 2017). Additionally, iPRGCs have projections to various limbic system structures including the amygdala. Thus, understanding the relationship between iPRGCs, serotonin, and amygdala may provide a clearer insight into the anxiety pathologies.

Serotonin Transporter (SERT)

The present study utilizes the presence of SERT, a serotonin transporter, to detect and measure serotonergic fibers in the amygdala. SERT is found on both the soma, axon, and axon terminals of serotonergic cells (Shi et al., 2010). SERT facilitates the reuptake of serotonin using an alternate access mechanism (Murphy et al., 2004). Thus, only one side of the binding site is accessible for transmission at a given time (Murphy et al., 2004). Na^+, Cl^-, and K^+ must all bind to the extracellular binding site of SERT to allow for 5-HT to be transported back into the pre-synaptic neuron (Murphy et al., 2004).

Polymorphisms, or variations, in the SERT gene have been correlated with depression, anxiety, and other such related pathologies (Nemeroff & Owens, 2009). Additionally, the combination of early childhood trauma and the functional SERT polymorphism has been associated with one known etiology of depression. (Vergene & Nemeroff, 2006). This pattern of findings seem to suggest that differences in the SERT gene may result in psychopathologies as well as differences in serotonin levels. For instance, Storvik et al. (2007) reported in a study using radiography, that there is a negative correlation among patients with alcoholism who exhibit dysfunctional emotional regulation, and SERT density in the amygdala.

SSRIs, a common treatment modality for depression and anxiety disorders, are SERT antagonists, meaning they block SERT functioning (Hensler, 2006). This inhibition of serotonin reuptake would lead to a greater amount of serotonin in the synapse (Hensler, 2006). One proposed mechanism for the therapeutic effects of SSRIS is that normal serotonin reuptake blocks SERT phosphorylation and internalization leading resulting in higher SERT density (Ramamoorthy & Blakley, 1999). SSRIs, would, then work by reducing the firing rate of tonic, or sustained,

serotonin, thereby leading to the downregulation of SERT, increased phosphorylation, and internalization of SERT (Ramamoorthy & Blakley, 1999). The eventual result would be a higher density of SERT after the usage of SSRI. Furthermore, Stein & Andrews (2015) reviewed that mice with the knock-out SERT gene have higher levels of anxiety-like behavior. Hence, literature indicates that a decreased, or absent, density of SERT correlates with psychopathological phenotypes.

The Mongolian Gerbil Model and Relevant Animal Behavior

The present study used a Mongolian gerbil (*Meriones uniguiculatus)* rodent model to examine the effects of altered lighting conditions. In studying visual neuroscience and potential translational implications, it is essential to pick a model that closely mimics both human physiology and behaviors. More traditional laboratory rodents including mice and rats are mostly nocturnal, and this means that their mechanisms of photoreception differ greatly from humans (Refinetti, 2006). Mongolian gerbils also have greater variability in showing diurnal and nocturnal patterns, which is more like human behavior than other rodent models (Refinetti, 2006). Like humans, gerbils have both a very robust cone system, and dichromatic blue-green color vision (Govardovskii et al., 1991). Furthermore, like humans, Mongolian gerbils are social animals. For example, they are known to form colonies of which they are territorial (Shimozuru et al., 2008). Both sexes are involved in pup rearing, which is rare amongst the known rodent species, but is generally typical in humans(Shimozuru et al., 2008). Additionally, past literature indicates that anxiety-like behavior in gerbils typically arises during challenges of socialization (Shimozuru et al., 2008). Thus, the Mongolian gerbil model serves as an appropriate animal model to understand how environmental changes, such as altered lighting, may possibly mediate the development of psychopathology.

Anxiety-like behavior is assessed differently in humans and non-humans as the emotional state and thought processes of non-humans cannot be assessed. Therefore, in the present research, anxiety-like behavior refers to maladaptive behavior towards ambiguous, or possibly threatening stimuli (Lawther et al., 2020). Notably, animal models are frequently used to test phenomena which may be applied to humans, but they cannot precisely predict human behavior. Specifically, anxiety is usually divided into trait or state anxiety. Trait anxiety refers to anxiety-like behavior independent of an apparent stressor, whereas state anxiety refers to anxiety dependent on a stressor or event (Ohl, 2003). Animal tests cannot directly measure trait (pathological) anxiety because these tests only measure behaviors that are evoked by a situation (i.e.: exposure to a novel stimulus) (Ohl, 2003). Typically, state anxiety tests measure avoidance and vigilance behaviors (Lawther et al., 2020).

Past literature indicated changes in anxiety-like behaviors due to lighting manipulations in a range of non-human subjects. For example, Mongolian gerbils experiencing an 8:16 light/dark cycle show lower locomotor activity compared to gerbils experiencing 12:12 light/dark cycles (Juarez-Tapia et al., 2015). Similar results were noted in a study which exposed mice to constant light, as those mice showed increased anxiety and learned helplessness responses (Fonken et al., 2009).

Purpose of Present Study

The incidence rates of anxiety pathologies have increased; thus, it is imperative to understand how novel environmental factors, such as blue light, mediate pathology development. The present research aims to understand one of the underlying mediating mechanisms specifically whether nocturnal, or diurnal, blue light exposure creates alterations in serotonin innervation, as

measured by SERT density in the basolateral amygdala, and its possible resultant changes in anxiety-like behavior (Figure 2).

Wirz-Justice & Terman (2022) recognized that patients diagnosed with depressive, (ie: seasonal affective) and anxiety-like (ie: generalized anxiety), disorders have responded well to early morning, white light therapy. Blue light therapy has become increasingly popular but, as Do et al.(2022) noted in their work there needs to be comprehensive and ongoing research to fully understand its role and efficacy. In contrast, Joshi (2022) surveyed college students on their cell phone use at night and reported that students with greater cell phone use before bed had more sleep disruptions. Joshi (2022) also found a positive correlation between cell phone use before bed and lower psychological well-being. This suggests that blue light may have temporally dependent therapeutic or detrimental effects.

Additionally, melanopsin is the most sensitive to blue light among all the monochromatic lights (Bailes & Lucas 2013). iPRGCs, which contain melanopsin, project to the limbic system and the dorsal raphe (Paul et al., 2009). Therefore, blue light may have the most direct impact on affective behavior and variations in neurotransmitter levels in comparison to other monochromatic lights.

Based on previous literature, we assigned adolescent Mongolian gerbils to blue LED light enrichment in the morning, or at night, where they received 12:12 hours of animal colony light and dark, with blue LED light enrichment turned on two hours before or after the colony lighting. The gerbils began receiving the lighting treatment around the commencement of adolescence. Following exposure to the LED lights, the gerbils underwent behavioral testing including the Elevated Plus Maze (EPM) and Social Approach tasks to test for avoidance behaviors and blunted sociability respectively. Towards the end of puberty, the gerbils were euthanized, and

serotonin innervation of the basolateral amygdala was visualized through immunohistochemistry and SERT density was quantified and analyzed in comparison to anxiety-like behaviors exhibited by the gerbils earlier in the study (Figure 3).

It was hypothesized that the gerbils in the evening condition would show lower levels of social approach and greater time in the enclosed arms of the EPM levels compared to the gerbils in the morning condition. These behaviors indicate anxiety-like phenotypes. Additionally, it was predicted that SERT would be less dense in the basolateral amygdala of the gerbils under the evening condition compared to the morning and control conditions. Therefore, it was expected that the evening lighting conditions mimic behavioral and neurochemical changes like anxiety pathologies.

Method

The study utilized a between-subjects, experimental design (Figure 4). The gerbils were randomly assigned to either morning lighting, an evening, lighting, and or control lighting condition (n=6 per condition). All three conditions had 12-hour light and 12-hour dark cycle at 150 lux. The light was turned on and off at 7:00 AM EST and 7:00 PM EST respectively. The morning condition was exposed to 310 lux Blue LED light two hours before colony lighting while the evening condition was exposed two hours after colony lighting. Behavioral testing began on the fourth week after the gerbils were administered experimental lighting. The gerbils were euthanized, and brains were perfused and harvested by H.R Rodman following behavioral testing.

Animals and Rearing Conditions

The sample consisted of 18 adolescent female Mongolian gerbils, *Meriones uniguiculatus,* Charles River Laboratories. The gerbils arrived at the lab between 28-32 postnatal day (PND). The gerbils were acclimated to their colony for 10 days. Following acclimation, the gerbils were placed in their respective lighting for 3.5 weeks and then returned to colony lighting. The gerbils were housed two per cage for the first three weeks of the experiment, and then were housed one per cage for the final week of the experiment. The gerbils had weekly cage cleanouts and sand baths for cleanliness. Food and water were provided ad libitum. The gerbils were 67-72 PND at the commencement of behavioral testing. All behavioral testing began at 12:00 pm and ended by 5:00 pm EST. Following behavioral training and testing, the gerbils were humanely euthanized, and their brains were removed for further neuroanatomical analyses. All care and use of the animals were in accordance with protocols approved by the Emory University Institutional Animal Care and Use Committee (IACUC). Behavioral training and testing, and animal maintenance were performed by P. Moon, M. Li, and H.R. Rodman.

Behavioral Training and Testing

Social Approach Task The social approach task is a behavioral task designed to test blunted sociability, an indicator of depressive and anxiety-like psychopathologies (Nadler et al., 2004). The social approach task used a two chambered sociability apparatus, where the test gerbil is placed in the left-most chamber and can freely move between the different chambers in the apparatus. The right-most chambers contained a "stimulus" gerbil. Sociability is indicated when the test gerbil spends time with the novel, "stimulus" gerbil. "Test" gerbils refer to those exposed to experimental conditions.

Prior to the start of the social approach task, the gerbils were given 10 minutes in the staging area and 3 minutes in the sociability chamber to acclimate to the changed environment and apparatus respectively. During the acclimation period, the gerbils learned the structure of the sociability chamber, specifically that they could move between the chambers. Following the acclimation period, the test gerbil was trapped under a glass beaker in the middle chamber to avoid movement, while the "stimulus" gerbil was placed in the right-wing chamber. The test gerbil was released, and their movements and time spent in each chamber was recorded for 5 minutes. The social approach task was coded by M. Li.

Nadler et al., 2004 of the University of North Carolina, Chapel Hill and the National Institute of Mental Health originally created the sociability chamber.

Elevated Plus Maze The elevated plus maze (EPM) is a task designed to test avoidance behavior, an indicator of anxiety-like behaviors in gerbils. The EPM uses an apparatus shaped as a "plus" with two arms of the plus being enclosed with high walls and the other two arms being open. The gerbils are free to move between the different arms of the maze. Anxiety-like behavior is calculated by the number of entries and time spent in each of the arms of the apparatus .

The day after the social approach task, the gerbils underwent the elevated plus maze (EPM). The gerbils were given three minutes in the EPM apparatus to acclimate and learn the

structure of the apparatus. After, the gerbils were placed in the middle of the apparatus to begin the test.

P. Addala coded the EPM task. P. Addala placed a transparency screen over a computer screen and measured each arm of the EPM using a ruler and divided each arm into three equal parts. The timing of the gerbils' entrances and exits, as well as the time spent in each part of the EPM was recorded for five minutes. An entrance was only measured if all four paws of the gerbil crossed a new area of the EPM.

Handley and Mithani (1984) of the University of Aston, Birmingham originally created the elevated plus maze.

Neuroanatomical Analysis and Histological Processing

Histological Processing Immunohistochemical processing was used to visualize the location of serotonergic fibers in the basolateral amygdala. The week following the EPM, the gerbils were humanely euthanized and brain tissue was harvested by H.R. Rodman. Coronal sections (30μ) of the brain were prepared using a Microm microtome. Reference sections series (every sixth section) were prepared using Gallyas silver myelin and cresyl violet staining procedures consistent with other work in the field (Figure 5).

All immunohistochemical work utilizing the SERT antibody followed procedures consistent with the field. A 10% normal goat serum was used as the blocking agent and an anti-rabbit primary antibody (AB9726, Millipore-Sigma) was used against serotonin transporter with a dilution of 1:10,000. An anti-rabbit biotinylated immunoglobin G (BIgG) was used as the secondary antibody with a dilution of 1:200. The ABC method was used, and diaminobenzidine (DAB) was used as a chromogen to visualize the serotonergic fibers. The sections were counterstained with a Giemsa staining solution which aided in intensifying the DAB, and visualizing structures. The sections were cover-slipped using Permount (Figure 6).

Scoring and Data Reduction

Behavioral Testing and Training For the social approach task and elevated plus maze, the timing of the gerbil's entrances and exits among various parts of the apparatus and the time spent in the chamber were used to measure sociability and anxiety-like behavior respectively. Data from both the social approach task and elevated plus maze were statistically analyzed by one-factor analyses of variance (ANOVA).

Quantification of 5-HT Innervation Serotonin innervation of the basolateral amygdala was assessed by quantifying SERT-immunoreactive bouton density in two sections representing the anterior and posterior portions of this structure. These sections corresponded to plates 26 and 28 of the Radtke-Schuller et al. (2016) atlas, respectively (Figure 7 & 8). The BLA was identified through its clear demarcation by the intercalated nuclei of the amygdala, the size of its cells, and overall shape of the nucleus. A Nikon Optiphot microscope was used to take photomicrographic images centered on the BLA captured at a total magnification of 400x. The densities of SERT-positive boutons were in counting frames of 15m by 15m centered within sampling boxes of a grid for a total of 25 sampling boxes per image within a grid area of 230m x 160m. The SERT-positive fibers were e -visualized and photographs in a standard focal plane corresponding to the plane within the tissue just above that in which the cell somas closest to or within the counting frames are in focus. The photographs were uploaded to Microsoft Power-point and magnified at 200x. A scale was created with ratings assigned from 1-5, with one reflecting weak density and five reflecting strong density (See Appendix A). The boutons out of the frame and out of focus were not accounted for. Additionally, overlapping boutons were only counted as one bouton. Any frames with blood vessels or significant artifacts were omitted. The average score of the frames were calculated per picture. Two raters (P. Addala and H. R. Rodman) independently rated density of test sections to assess the reliability of the counting scheme. Data were statistically analyzed using one-factor analyses of variance (ANOVA).

Results

SERT Innervation Analyses

The Serotonin Transporter (SERT) was used to visualize serotonergic fibers, and therefore measure serotonin innervation in the basolateral amygdala. Composite scores were created for: Average Left Hemisphere Density (left anterior and posterior sections), Average Right Hemisphere Density (right anterior and posterior sections); and Combined Average Whole Brain Density (Average Left Hemisphere Density and Average Right Hemisphere Density). The data were exported to SPSS to analyze differences among mean SERT densities among different brain hemispheres and different experimental groups.

One-way ANOVAs were performed to test the hypothesis that the gerbils in the different conditions would differ in their BLA SERT density for the right and left hemispheres. In the left hemisphere, the one-way ANOVA revealed that there was not a statistically significant difference in SERT density between the experimental groups, $F(2,15) = 1.20$, $p = .328$, $\omega^2 = .011$. In the right hemisphere, the one-way ANOVA revealed that there was not a statistically significant difference in SERT density between the experimental groups, $F(2,15) = 1.87$, $p = .832$, $\omega^2 = -.047$. To compare differences of mean serotonin density between the two hemispheres, a paired samples *t*-Test was performed. The mean of the left hemisphere SERT density ($M = 2.35$, $SD = .347$) was not significantly lower than the right hemisphere SERT density ($M = 2.43$, $SD = .287$), $t(17) = -1.21$, $p = .120$.

Anxiety-Like Behavior Analyses

The elevated plus maze (EPM) and the social approach tasks were used to measure avoidant behavior and blunted sociability respectively as both are indicators of anxiety-like behavior. Microsoft Excel was used to prepare and process the data for both tasks.

Respective composite scores for time spent in the open and enclosed arms were created for the EPM. To test the hypothesis that the gerbils in different conditions would spend different amounts of time in the enclosed arms of the EPM, a one-way ANOVA was performed. The one-

way ANOVA revealed that there was no statistically significant difference in the amount of time spent in the open arms between the experimental groups, $F(2,33) = .850$, $p = .437$, $\omega^2 = .081$. Additionally, there was not a statistically significant difference in the amount of time spent in the enclosed arms between the experimental groups, $F(2,33) = .169$, $p = .846$, $\omega^2 = .022$.

For the social approach task, composite scores were created to sum all time spent in the stimulus and experimental gerbil sides of the sociability chamber respectively. Additionally, the number of times the experimental gerbil crossed to the stimulus gerbil's side was measured. The one-way ANOVA revealed that there was not a statistically significant difference in the amount of time spent in the stimulus or experimental gerbil's side between the experimental groups, $F(2,33) = .410$, $p = .667$, $\omega^2 = -.017$ and $F(2,33) = .361$, $p = .699$, $\omega^2 = -.018$ respectively. Furthermore, there was not a statistically significant difference in the amount of crossing between the experimental groups, $F(2,33) = 1.72$, $p = .195$, $\omega^2 = .020$.

Exploratory Analyses

Exploratory analyses were performed to assess a possible relationship between SERT density in the right hemisphere of the BLA, and time spent in the open and closed arms of the EPM, without respect to their lighting conditions. A Pearson correlation was used to assess a relationship between right hemisphere SERT density and time spent in the open and closed arms respectively. There was not a significant correlation between right hemisphere SERT density and time spent in the open arms or closed arms respectively, $r(18) = .051$, $p > 0.01$ and $r(18) = -.193$, $p > 0.01$.

Discussion

The present study aimed to answer whether there is a relationship between the timing of ambient blue lighting in either early morning or late-night hours, and its suspected association with anxiety pathology, measured in SERT density of the basolateral amygdala and exhibited anxiety-like behaviors. It was hypothesized that exposure to blue lighting at abnormal times would be related to anxiety pathology. In modern times, it has become increasingly important to not only examine, but also to comprehend how advancements in modern technology may be contributing to the increasing prevalence of psychopathologies (Hidaka, 2012). Disturbances to the circadian rhythm, brought on by mistimed blue light have been found to increase the prevalence of mood disorders such as anxiety and depression (Bedrosian & Nelson, 2017). Furthermore, blue light directly stimulates iPRGCs that project to limbic structures like the amygdala (Bedrosian & Nelson, 2017). For this reason, it is crucial to understand how altered blue lighting may impact mechanisms and structures related to anxiety pathologies.

The results of the present study did not support the original hypothesis as that there was not a significant difference between the timing and presence of blue light exposure and anxiety pathology (Figures 9-13). However, the present study may help build upon understanding, and defining, the altered mechanisms that result from mistimed blue light exposure. These findings differ from previous studies which have suggested that exposure to mistimed light has resulted in changes in affective behavior (Juarez-Tapia, 2015). However, many previous researchers have exposed their rodents to higher levels of blue light in comparison to the present study. For instance, Bilu et al. (2019) found a positive correlation in fat sand rats, *Psammomys Obesus*, with blue lighting and anxiety-like behavior measured in a combination of behavioral tests including the elevated plus maze. However, their rodents were exposed to 1,300 lux blue light for three

weeks, whereas the present study used 310 lux blue light, and sunrise intensity is 400 lux. Similarly, Wu et al.(2021) found that in C57BL/6 mice, 780 lux blue light exposure, for four weeks, at night correlated with sleep disturbances and consequent pathological behavior. This may indicate a threshold where blue light can be correlated with anxiety pathology. However, both studies used a nocturnal rodent model with different visual systems than the Mongolian Gerbil. Thus, the animal's visual system could be a possible confounding variable in these results.

Notably, Carlsson et al. (1980) noted that in human post-mortem brain tissue, there were greater amounts of serotonin in people who passed during the summer months in comparison to the winter months. With the assumption that there is greater light availability in the summer months, this may indicate that light does play a role in serotonin innervation. Thus, subtle mistimed blue light exposure may not lead directly to previously noted detrimental effects, thus potentially giving the opportunity to set new parameters for the effective use of blue light, without deleterious consequences, which could be revealed with further research

It is important to consider confounding variables which may have led to increases in anxiety pathologies in human studies. For example, most of the blue LED exposure, at atypical times, comes from the use of technologies such as cell phones and laptops. These devices allow for social connection twenty-four hours a day, thereby resulting in chronic socioemotional arousal (Joshi, 2022). Additionally, late night notifications, such as texts and phone calls, create disturbances to the sleep-wake cycle which has been correlated with pathological behaviors (Li et al., 2015). Lepp et al (2014) have also noted that increased cell phone usage has a negative correlation with physical activity and academic performance, and a positive correlation with anxiety and life dissatisfaction. However, they also note that these devices do allow for multi-tasking

and social networking, further implying many confounding variables could contribute to pathological behaviors.

An exploratory analysis was performed to look at whether there are differences in serotonin laterality in the basolateral amygdala. While the result was not significant, our mean values indicated that the left hemisphere had lower SERT density compared to the right hemisphere. Many previous studies have noted the right amygdalar hemisphere of the brain is greater in volume and has a stronger association with emotional processing. Anderson & Teicher (1999) had significant results indicating that increased right amygdalar serotonin innervation was directly related to anxiety-like behavior measured by the elevated plus maze. Additionally, Murphy et al. (1987) noted the right basolateral amygdala was significantly larger than the left. Thus, this may imply that increased SERT density in the right hemisphere may mediate emotional responses. Additionally, further exploratory analyses were performed to look at a possible relationship between SERT density in the right hemisphere and behavior in the EPM (Figures 14 & 15). Although there were not significant correlations, it should be noted that Pearson coefficients was positive for right hemisphere SERT density and time spent in the open arms, and negative for right hemisphere SERT density and time spent in the closed arms. These findings differ from previous literature which did indicate a positive correlation between SERT density and behavioral responses (Anderson & Teicher, 1999).

Limitations and Future Directions

The present study had several limitations which should be considered in interpreting the findings. Additionally, continued studies focusing on the intersection of chronobiology, behavioral neuroscience, and clinical psychology are necessary to further understand how blue light may impact affective behavior.

Using the SERT antibody in immunohistochemistry was novel to the lab, thus requiring some experimentation to determine optimal buffer solutions, parameters of antibody dilution,

and timing of final steps of the immunohistochemistry. Thus, there were differences in staining quality and consistency across the samples. Additionally, as this was a pilot study, there was a small sample size with only six gerbils per condition. This may have led to type II errors, so further research in the lab will expand the sample size.

It is impossible to study certain mechanisms of psychopathology on humans, thus animals are often used. However, as we cannot study the emotional, subjective state or thought processes of animals, it is merely impossible to have true face and construct validity in translational research using an animal model (Baumman & Schumann, 2018). For instance, the Elevated Plus Maze (EPM) is often interpreted as a measure anxiety of open spaces and the social approach task measures sociability. However, both depressive and anxiety-like pathologies have varied phenotypical expressions, thus it is hard to predict whether an animal's behavior is pathological or not. Therefore, utilizing other paradigms which measure anxiety such as the light/dark box and the Vogel lick suppression tasks, which measure anxiety and anhedonia respectively, may be useful for a broader understanding anxiety-like phenotype (Harro, 2018). Future researchers should continue to create a comprehensive scale for anxiety and depression based on previous paradigms. It would be useful to understand how an analysis combining multiple paradigms would alter data analyses.

As previously noted, there could be confounding variables such as increased emotional arousal contributing to increases in psychopathologies in human populations. Therefore, using a human model would be beneficial to greater understand any confounding factors. For instance, in a human follow up study, an investigator can ask different participants to stop using any blue light emitting technology and various times before bed and measure anxiety using tests for clinical anxiety, like the General Anxiety Disorder-7 (GAD-7) scores. A clinical interview could be useful to understand why someone uses technologies before bed, and if there are any changes in mood due to these devices.

Conclusion

With the increasing prevalence rates of psychopathology, it is critical to understand how modern environmental influences, such as blue LED light may mediate this development. Our results have indicated that at the adolescent development period, low amounts of blue LED lighting are not correlated with anxiety pathology nor SERT density in the basolateral amygdala. Thus, low levels of blue light alone, even at night, may not be inherently problematic for anxiety pathology both behaviorally and neurochemically. Furthermore, this indicates the possibility of confounds mediating the relationship between blue LED light exposure and psychopathologies.

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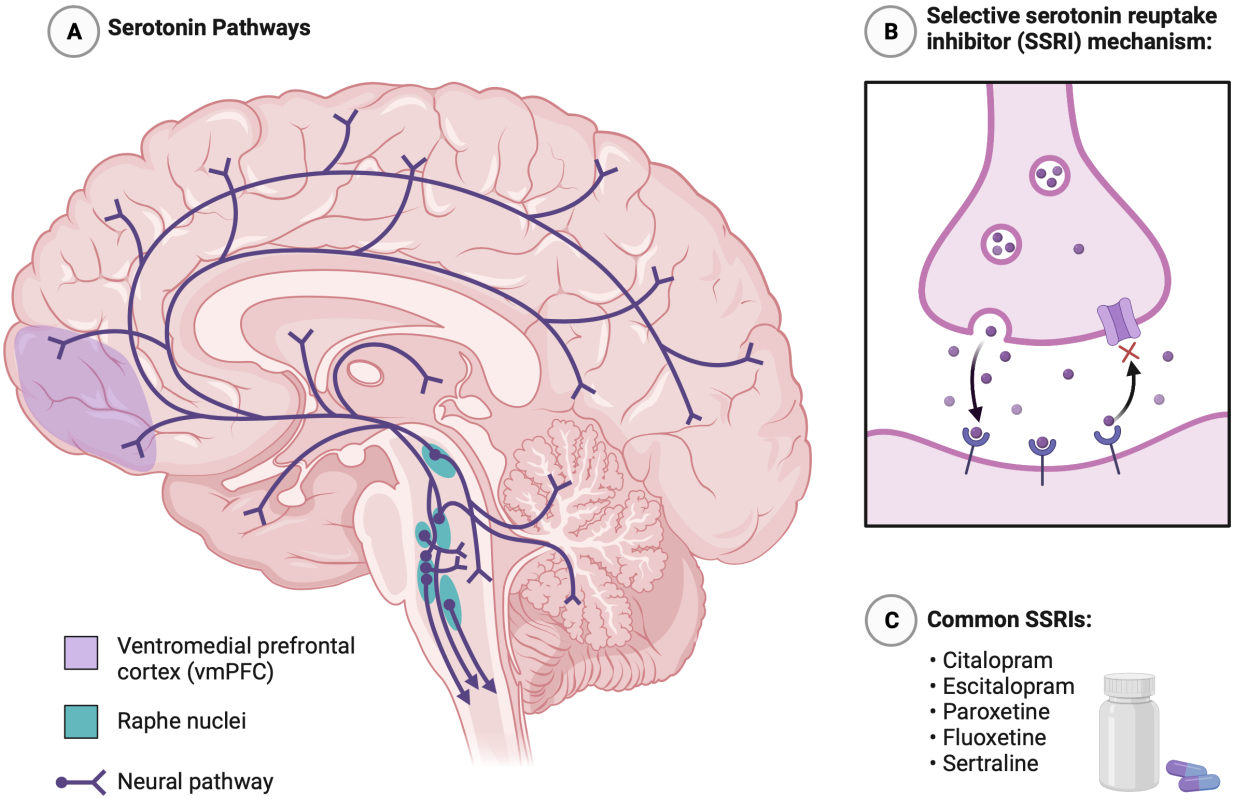
Figure 1
Sertonin System In the Central Nervous System: Section A of this diagram shows serotonin pathways which originate from the dorsal raphe nucleus, the main production site of serotonin. Section B of this diagram shows the mechanism of how SSRI affect the reuptake of serotonin. Section C names common SSRIs used for the treatment of psychopathologies in humans.



Created with BioRender.com

Figure 2: Proposed Blue Light and IPRGC mechanism: This diagram shows the proposed mechanism for how blue light may affect mood and social behaviors through IPRGC projections.

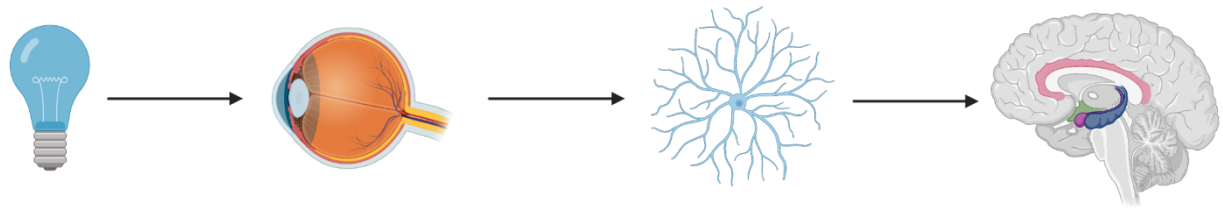


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Figure 3: Proposed Study: This diagram shows the different variables in the present study, and possible interactions of these variables.

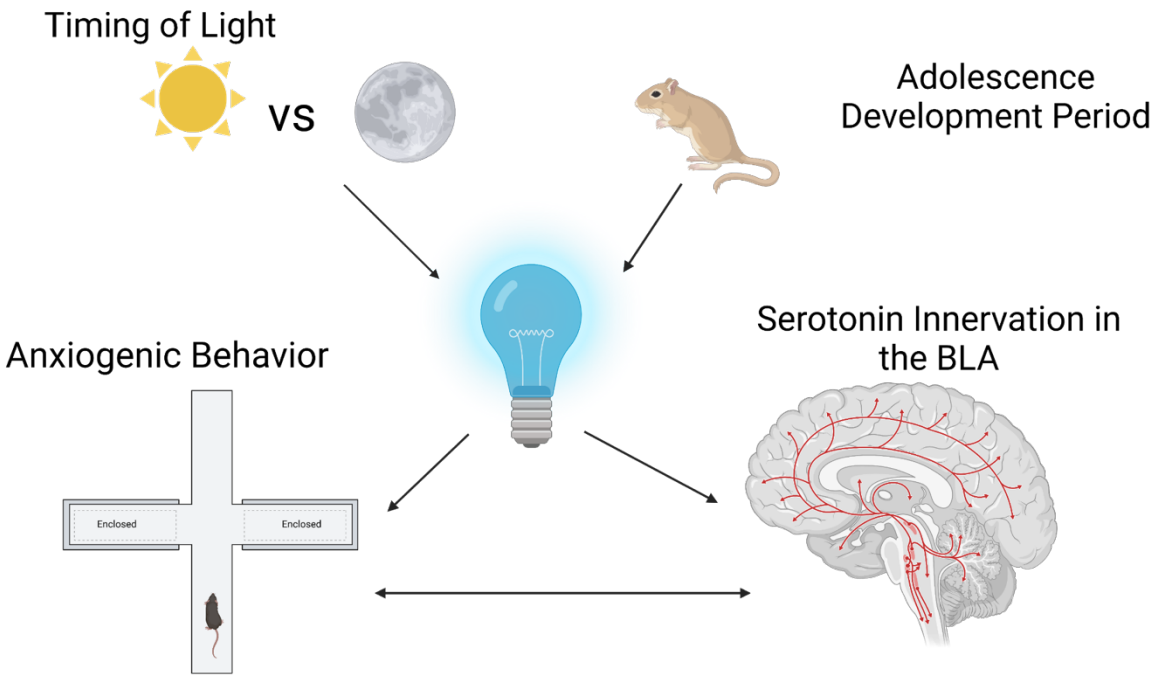

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Figure 4: Methods Timeline: This is an overview of the methodology used in the present study. Something to note is that further behavioral tests and immunohistology not included in this study were performed by P. Moon, H. R. Rodman, and M. Li.

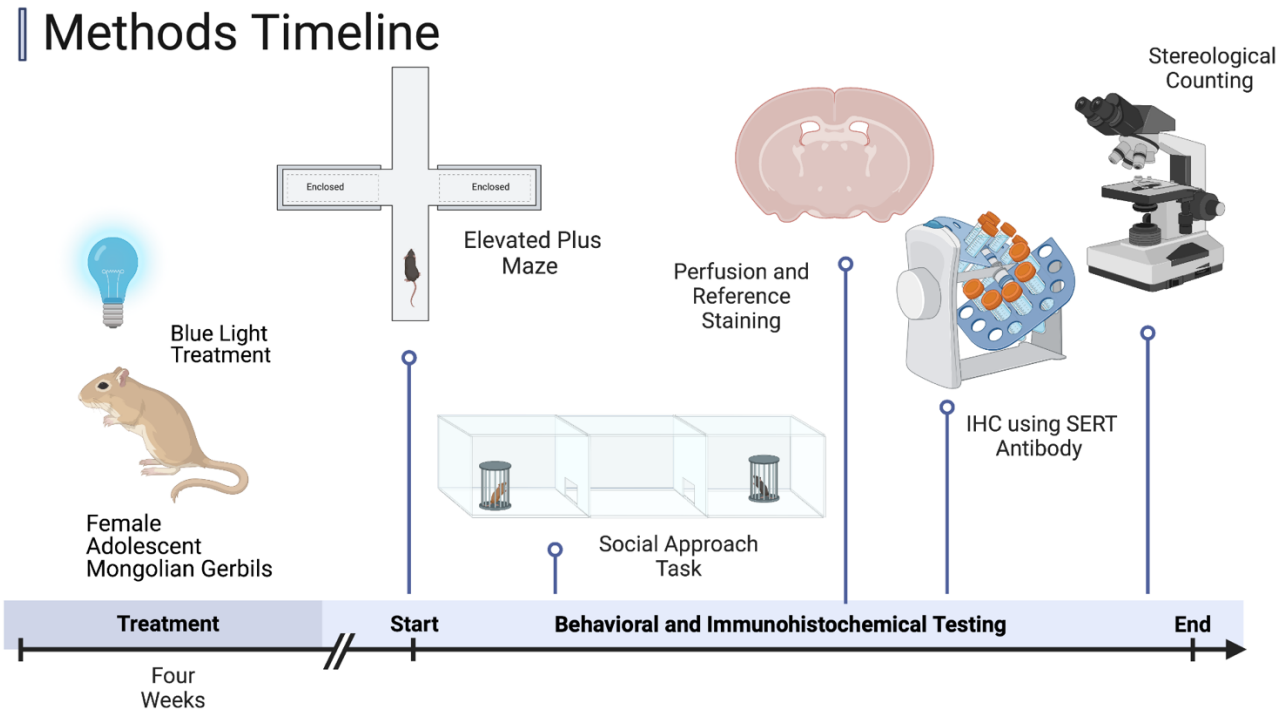

Figure 5: The Basolateral Amygdala identified in a Coronal Brain Section using the Radtke-Schuller et al (2016) atlas: The BLA has been identified in both hemispheres using the shape and size of the nucleus and cells.

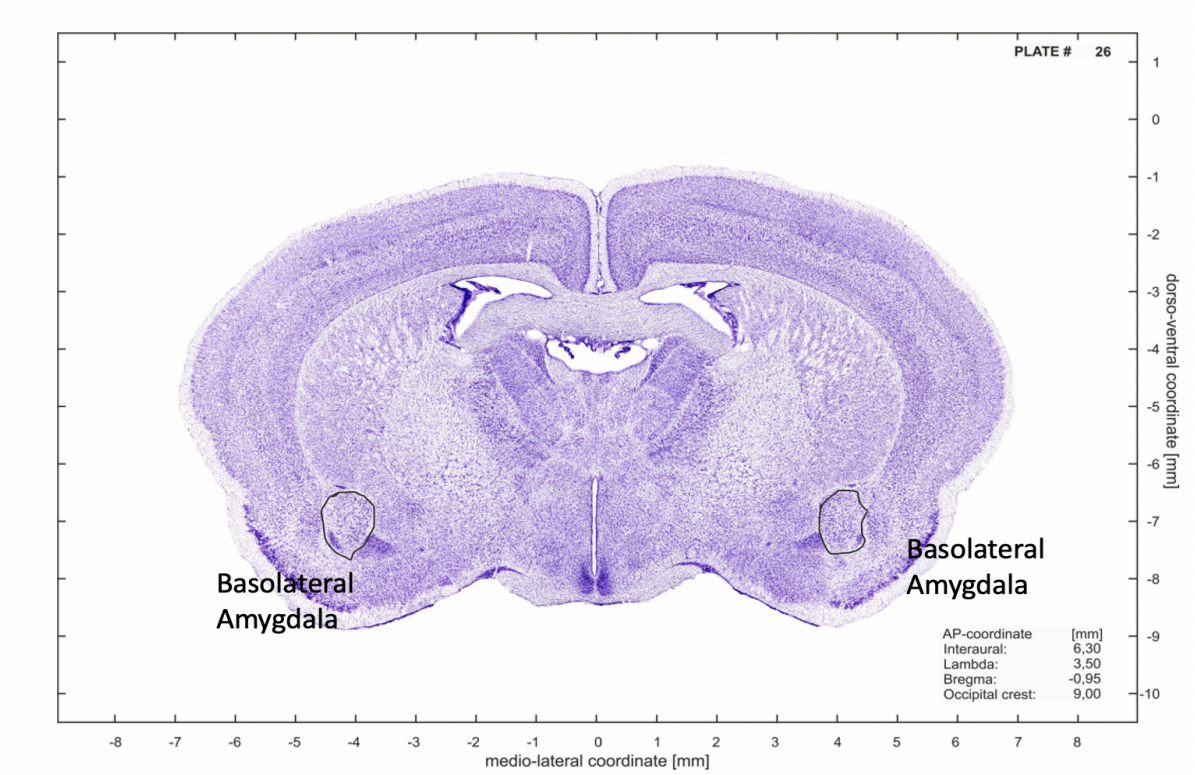

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Figure 6: SERT Coronal Brain Sections: These sections have undergone IHC using the SERT Antibody, DAB as Chromogen, and Giemsa Counterstain. They were cover slipped with Permount.

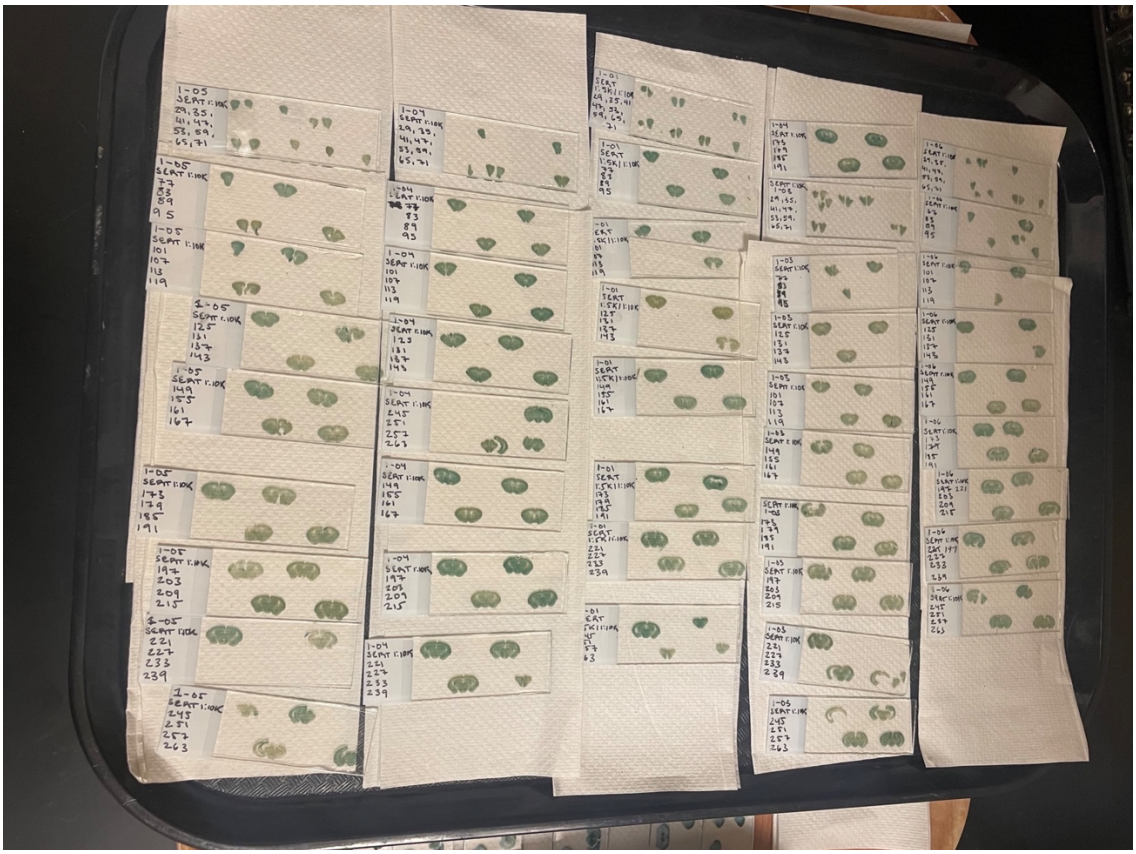


Figure 7: Photograph of SERT positive boutons in the BLA in an anterior image: The photographs were captured at 400x magnification and then uploaded to Microsoft PowerPoint. A transparent frame with 25 sampling boxes and counting frames within overlayed each image.

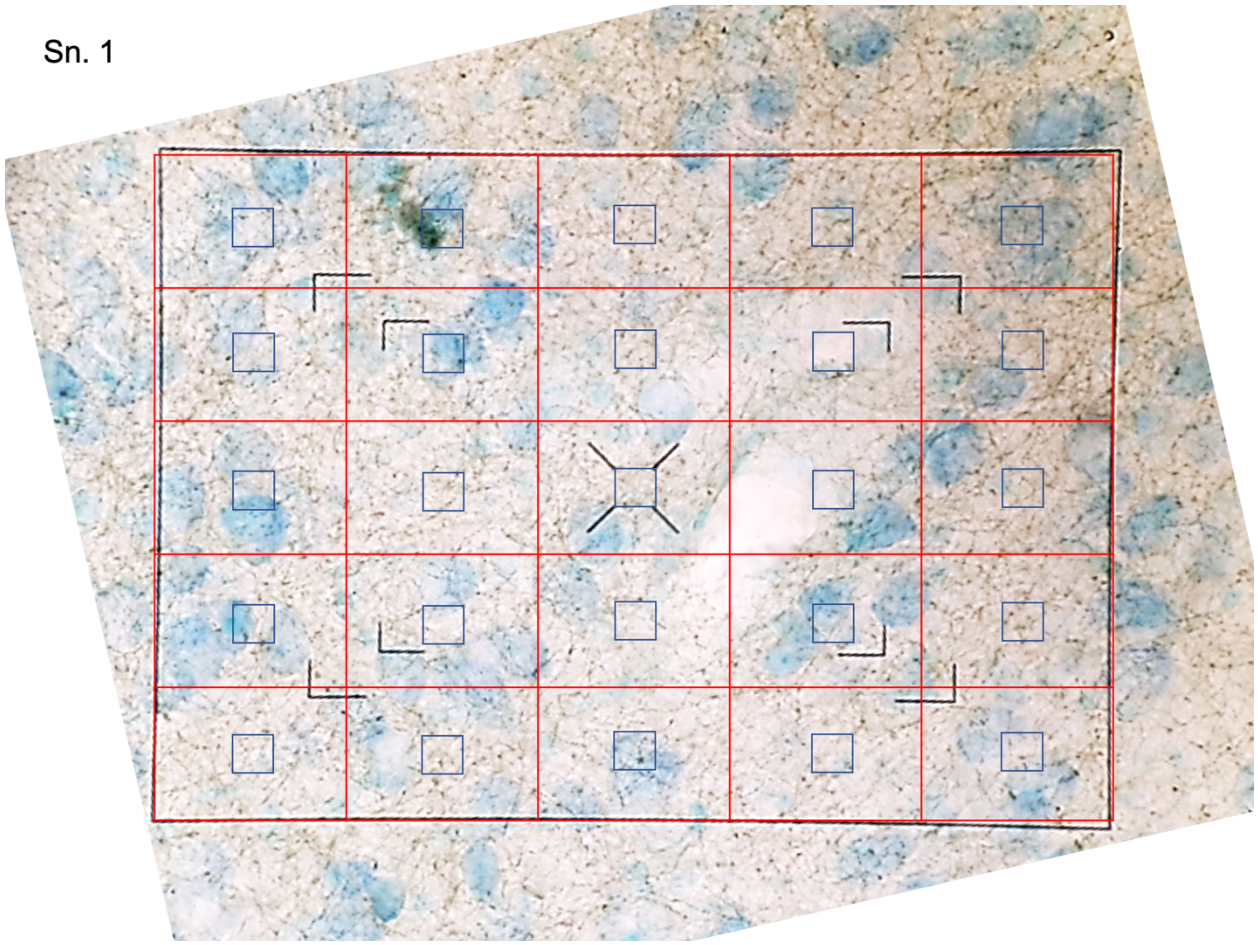


Figure 8: Photograph of SERT positive boutons in the BLA in an posterior image: The photographs were captured at 400x magnification and then uploaded to Microsoft PowerPoint. A transparent frame with 25 sampling boxes and counting frames within overlayed each image.

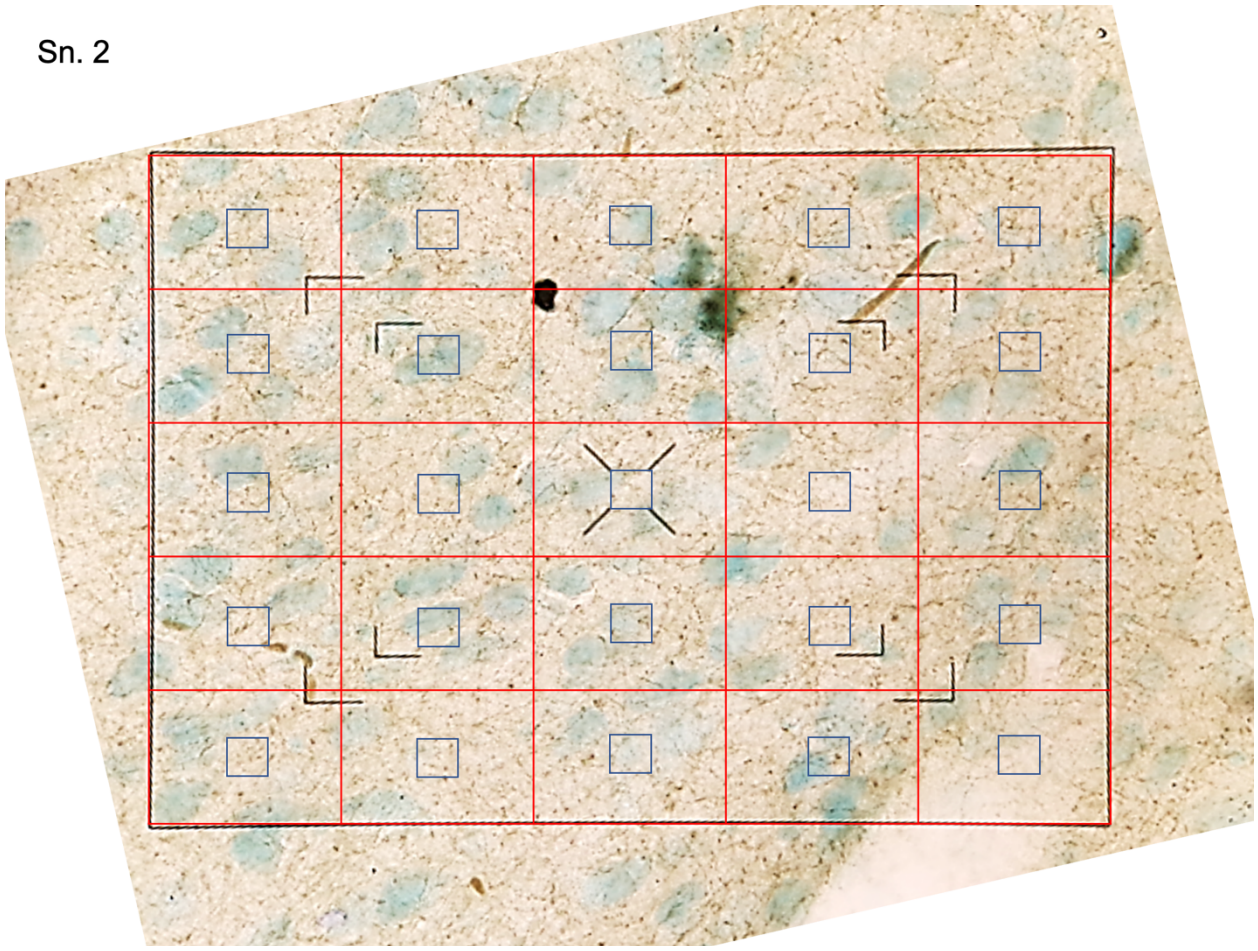


Figure 9: Analysis of SERT density in Left Hemisphere by Lighting Condition: There was not a significant difference between the conditions: $F(2,15) = 1.20$, $p = .328$, $\omega^2 = .011$. The error bars for the subsequent graphs represents one standard deviation of the mean. See Appendix A for further information about SERT density levels.

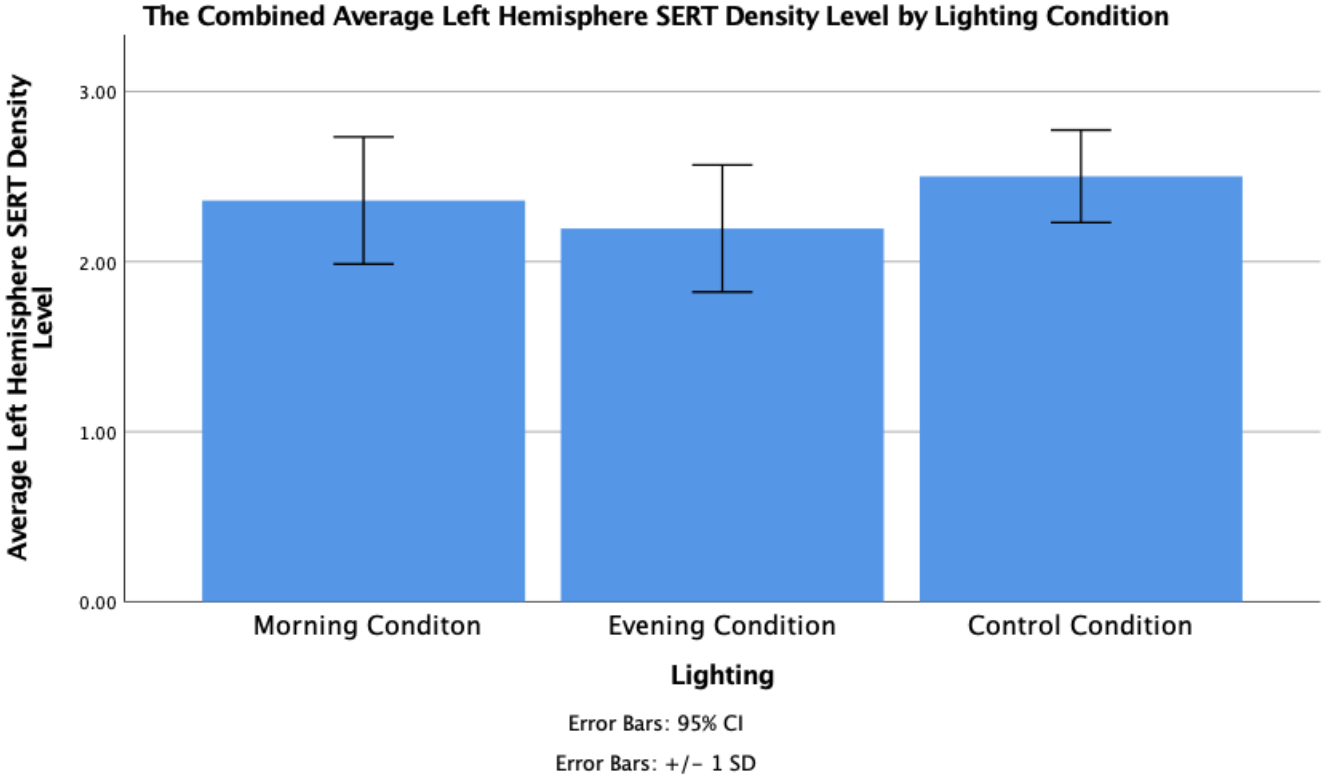


Figure 10: Analysis of SERT density in Right Hemisphere by Lighting Condition: There was not a significant difference between the conditions: $F(2,15) = 1.87$, $p = .832$, $\omega^2 = -.047$.
See Appendix A for further information about SERT density levels.

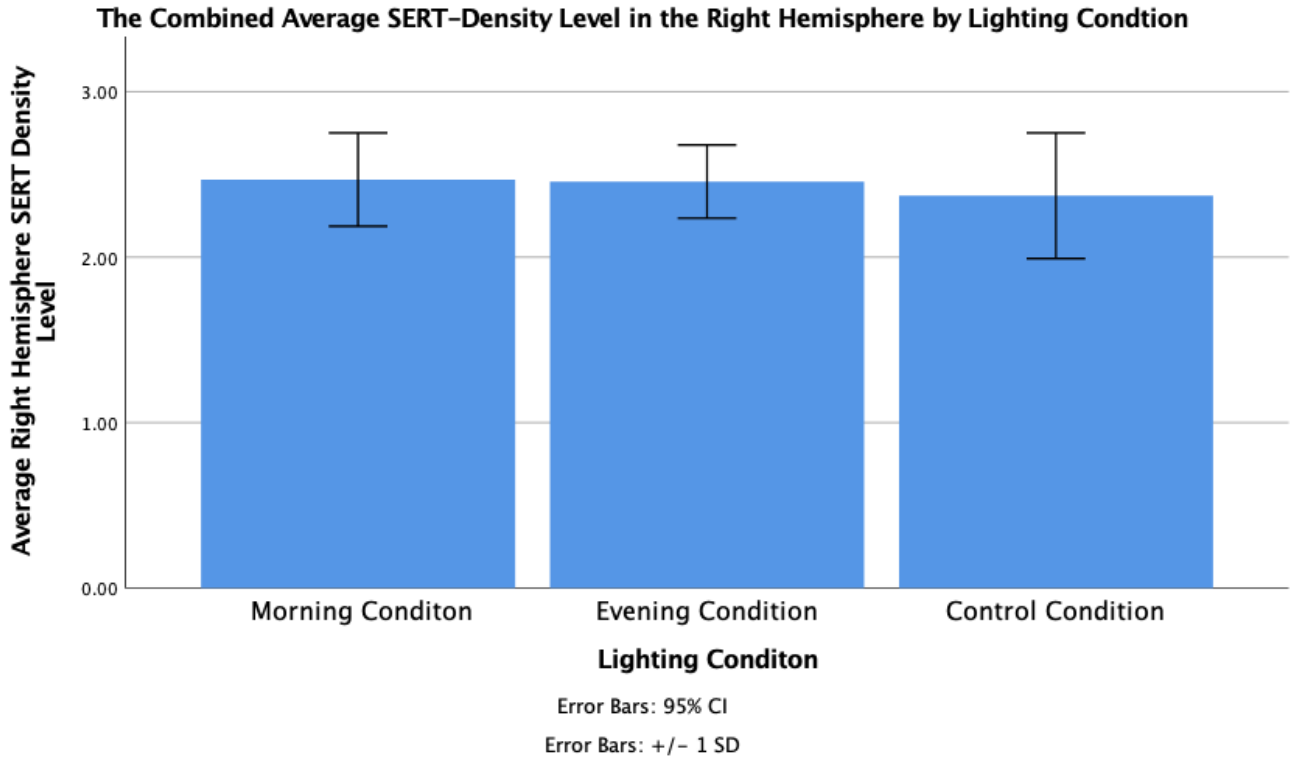


Figure 11: Analysis of Time Spent in the Open Arms of EPM by Lighting Condition: There was not a significant difference between the conditions: $F(2,33) = .850$, $p = .437$, $\omega^2 = .081$.

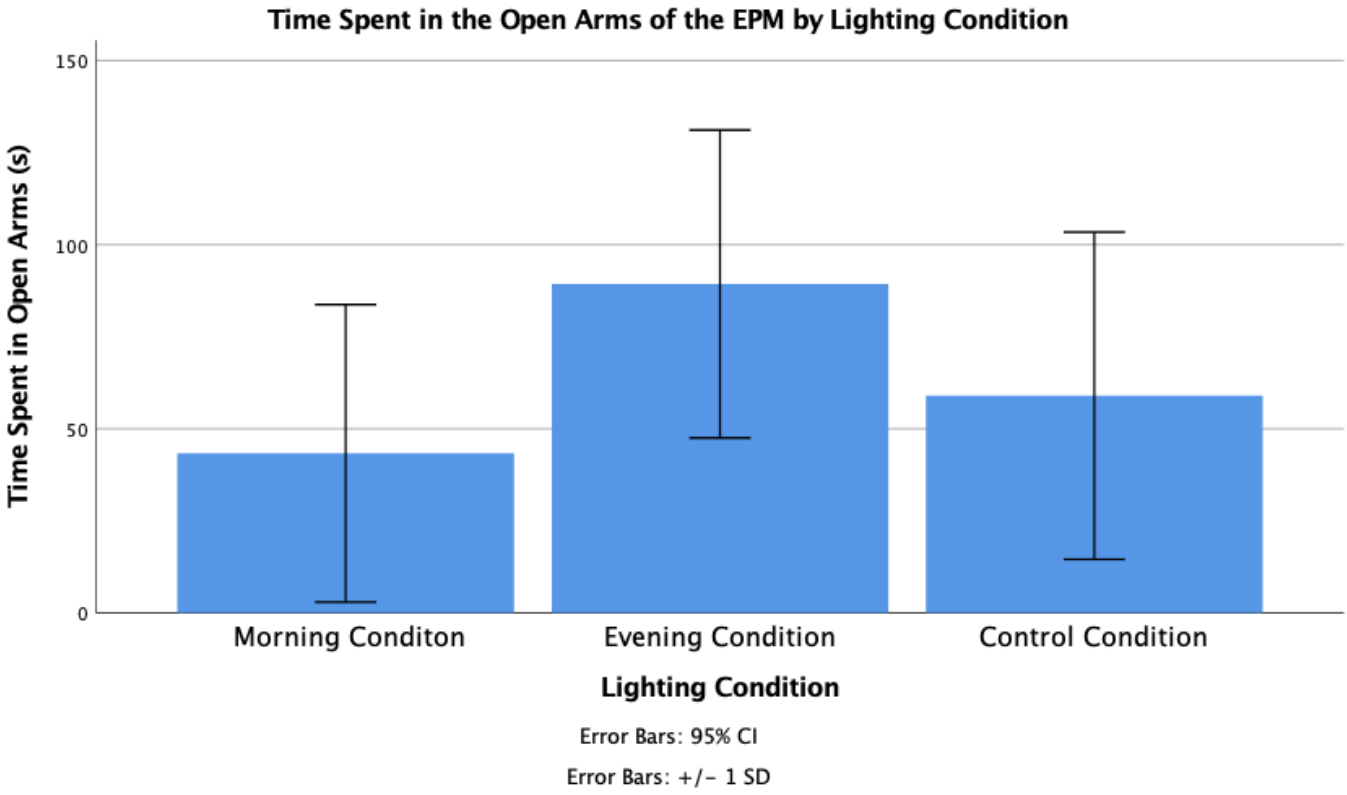


Figure 12: Analysis of Time Spent in the Closed Arms of EPM by Lighting Condition: There was not a significant difference between the conditions: $F(2,33) = .169$, $p = .846$, $\omega^2 = .022$.

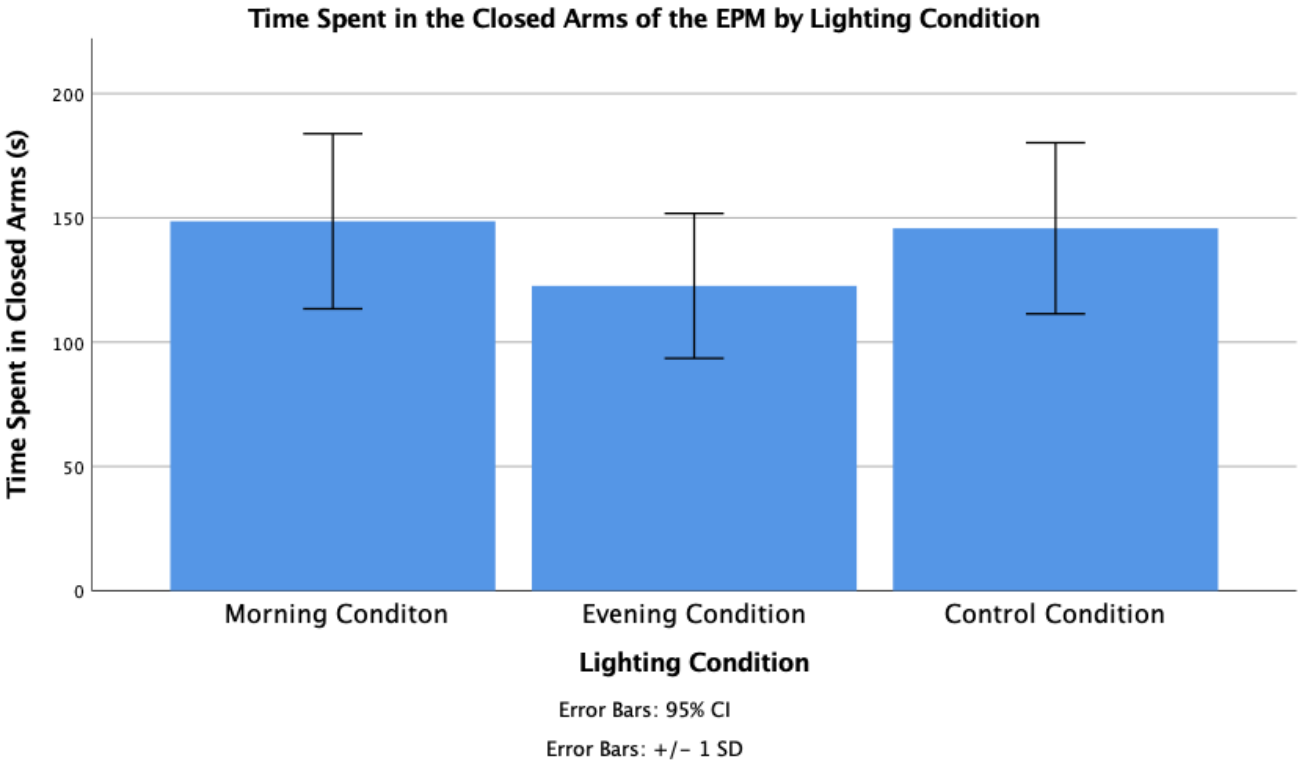


Figure 13: The Time Spent in the Different Sides of the Sociability Chamber by Lighting Condition: There was not a significant difference between the time spent in the experimental gerbil side, $F(2,33) = .410$, $p = .667$, $\omega^2 = -.017$ and the stimulus gerbil side $F(2,33) = .361$, $p = .699$, $\omega^2 = -.018$

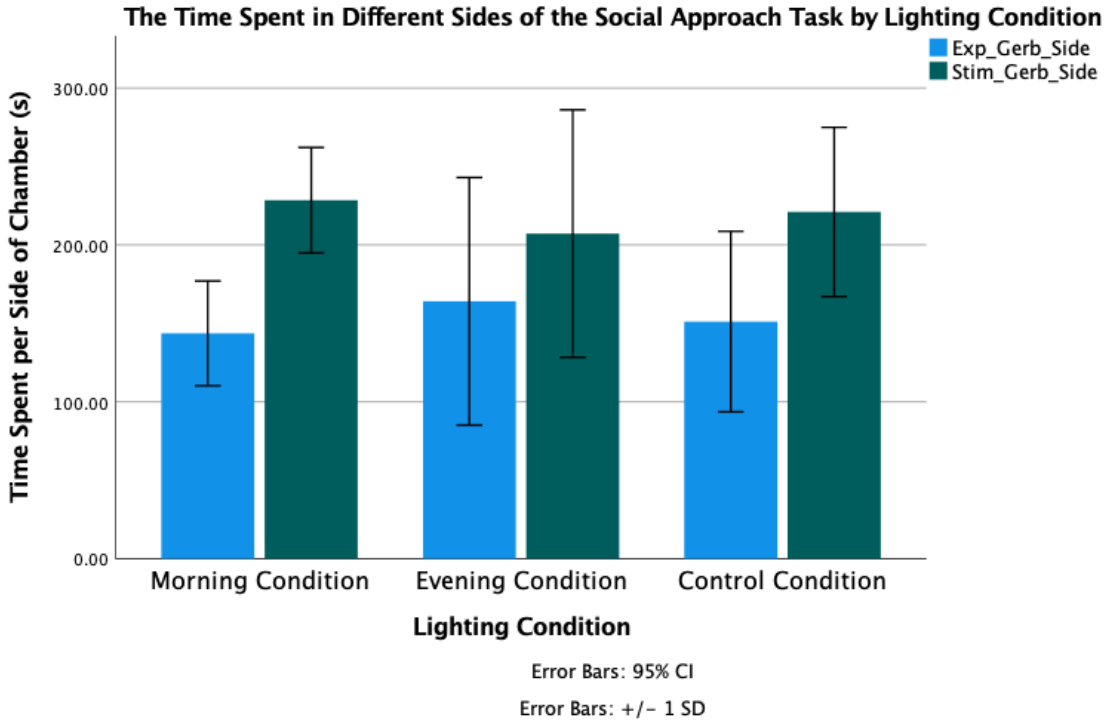


Figure 14: The Time Spent in the Open Arms of the EPM by SERT Density in the right hemisphere: There was not a significant correlation between the two variables: r (18) = .051, $p > 0.01$ See Appendix A for further information about SERT density levels.

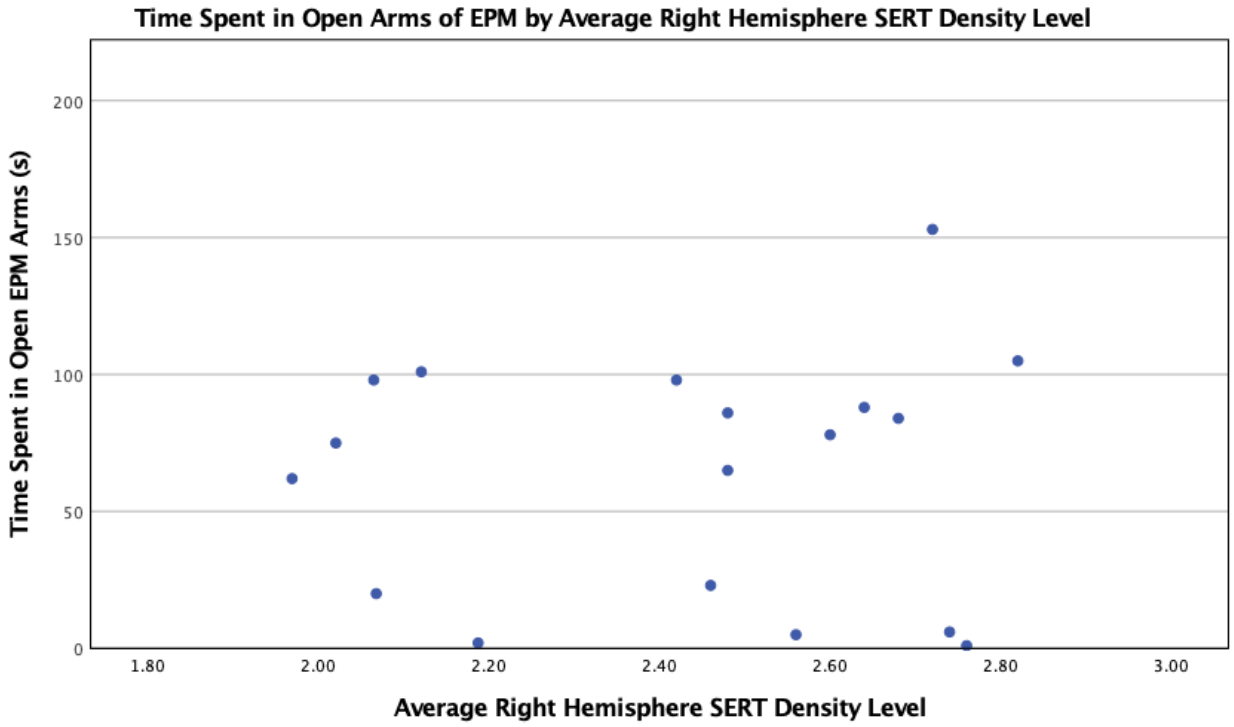
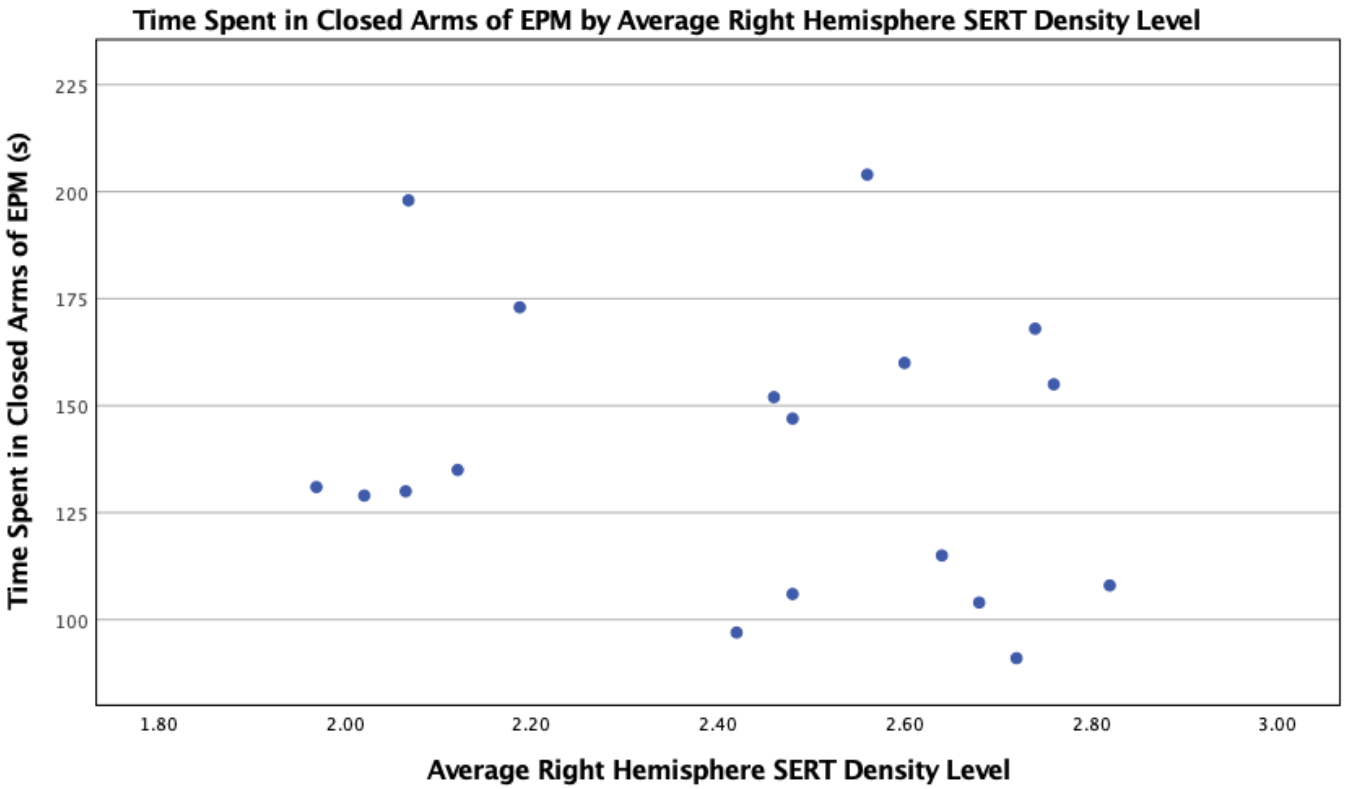


Figure 15: The Time Spent in the Closed Arms of the EPM by SERT Density in the right hemisphere: There was not a significant correlation between the two variables: r (18) = -.193, p > 0.01. See Appendix A for further information about SERT density levels.

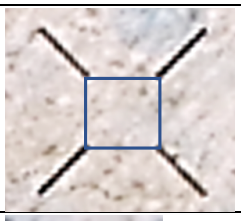
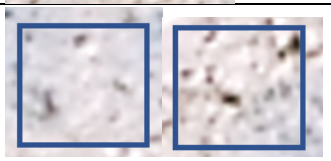
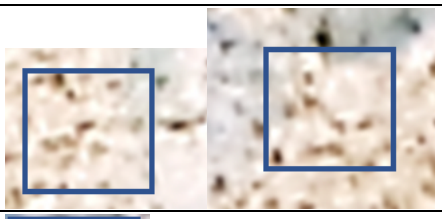


Appendix A

SERT Fiber Quantification
Visual and Comparative Neuroscience Lab

General Guidelines:

- Boutons out of frame/out of focus on are NOT accounted for
- Frames with blood vessels/significant artifacts present are OMITTED
- Overlapping boutons can only accurately be counted as 1 bouton
- Used 200X magnification on Microsoft Power-point

Density Level (1-5)	Written Description	Image Examples
1	- 0-1 boutons	
2	- 2-5 boutons	
3	- 6-9 boutons	
4	- 9-12 boutons	
5	- 12+ boutons