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The impact of blue light on emotion, behaviorally and neuroanatomically, in the immediate context and over the lifespan

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An abstract of a dissertation submitted to the Faculty of the

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The impact of blue light on mood and emotion

Abstract

Mistimed light, especially blue light, has been proposed to have depressogenic effects in both human and nonhuman subjects, possibly via projections of intrinsically photosensitive retinal ganglion cells (ipRGCs). In this dissertation, I provide evidence across two experiments that blue-enriched light has subtle impacts on mood- and emotion-related behaviors under ecologically informed experimental conditions, and present a pilot study of the neuroanatomical underpinnings of these impacts. In the first experiment, in a facial expression processing paradigm, human participants were more likely to make mistakes towards negative facial expressions while under a broad-spectrum light (i.e., a light that emits substantial blue light) versus a dimmer, warmer light (i.e., a light which does not emit much blue light). In other words, when participants misidentified emotional-social cues, they tended to mistake any expression for a negative expression in an environment that contained blue light. These effects were observed after only a short 20-minute exposure to our lighting paradigms. In the second experiment, a largely diurnal (day-active) species (the Mongolian gerbil, *Meriones unguiculatus*) was used to model the effects of extended mistimed blue light during the critical period of adolescence. Gerbils were exposed over several weeks to blue light either after "sunset" (beginning of the dark phase, *evening blue light*) or before "sunrise" (end of the dark phase, *morning blue light*) or kept in typical colony conditions. Gerbils in the evening blue light condition demonstrated subtle but meaningful behavioral shifts, such that they demonstrated more aggressive behaviors, increased social investigation, and decreases in exploration- or escape-related behaviors in a modified open field and forced swim task. These behaviors do not fully align with our prediction that depressive-like behaviors would increase following long-term exposure to blue light in the evening, but do suggest that atypically timed blue light can impact aggressive and social behaviors and behavioral strategies associated with environmental challenges. In a third, pilot study, we predicted that evening blue light would alter dopaminergic innervation of a structure that has been implicated in depressive shifts, namely the basolateral amygdaloid complex (BLA), but this hypothesis was not supported. The observed changes in social behavior and behavioral strategies in the other tasks, coupled with preliminary evidence for a lack of anatomical effects in the BLA, implicate other structures in the effects of mistimed blue light during adolescence. Behavioral strategies to use energy more efficiently in response to novel stimuli map onto the overall pattern of behavioral effects observed and suggest possible roles for the medial amygdala, hypothalamus, habenular region, and long-term changes in the HPA axis following exposure to atypically timed blue light. Together, the data presented here indicate that mistimed blue light has subtle impacts on the processing of some social stimuli in the short term and, when experienced in extended conditions in adolescence, may induce changes in both social behaviors and behavioral profiles on other tasks associated with mood in animal models. These subtle shifts may in turn be one of many factors that increase the likelihood of the development of mood disorders.

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Evolved visual systems in modern lighting environments

Humans evolved under very consistent lighting environments (i.e., day and night); however, modern cities and homes do not necessarily match their lighting schedules strictly to solar cues. The impact of vastly altered light conditions on mood and emotion regulation is not fully known. Many major cities have essentially zero hours of true, dark night (Bedrosian & Nelson, 2017), and homes increasingly incorporate technologies that emit light, such as screened devices (McClung, 2013). The mammalian visual system evolved to not only utilize light to provide maps and images of the environment, but also to receive environmental timing signals that likely do not provide much in terms of image formation (Legates, Fernandez, & Hattar, 2014). These non-image forming functions of the visual system govern appropriate timing of behavior. This latter function may cause mood or emotion disturbances when certain light cues, such as the color of a light source, are either mistimed or presented for unnaturally extended periods of time. While the association is not fully understood based on current data, rates of depression do appear to be increasing in modern societies and particularly in younger individuals (Hidaka, 2012). Constant artificial illumination, among many other factors (e.g., increased rates of obesity, increased competitiveness in social environments, etc.), may contribute to this association. Indeed, there may be unintended consequences of the enormous amount of artificial light present in modern society.

Recently, the field of vision research has acknowledged two sets of pathways from the so-called *intrinsically photosensitive retinal ganglion cells* (ipRGCs; cells in the retina that do not require rod and cone inputs to transmit information about light, discussed further below) to regions of the brain: i.) a circadian pathway (i.e., biological clocks), and ii.) pathways to numerous subcortical structures that bypass circadian circuitry (i.e., non-clock related; LeGates

et al., 2012; Vandewalle, Maquet, & Dijk, 2009). Both the circadian and non-clock pathways have the potential to impact mood and emotion. It can be difficult to differentiate between the effects of light that impact mood and emotion via circadian rhythms versus non-clock mood pathways, as both depend on the color (i.e., wavelength) and timing of light exposure. In addition, pathways originating in more conventional retinal circuits may influence mood and emotion circuits. Indeed, the impact of light on mood and emotion regulation is complex, and the two systems almost certainly interact in many respects.

This dissertation explores the impact of atypically timed light on mood- and emotion-like behaviors across two studies. In chapter 1, I explore short-term impacts of light containing blue light on the interpretation of emotional social cues in humans. In chapter 2, I explore the long-term impacts of atypically timed blue light exposure during critical developmental periods on mood- and emotion-like behaviors in a rodent species. In a final, pilot study, I explore how a specific brain structure relevant to mood and emotion was impacted by exposure to blue light at atypical times of the 24-cycle across critical developmental periods.

Impacts of mistimed light on mood regulation

Across the 24-hour rotation of the earth, there is a long period of light, or day, a long period of dark, or night, and critical transitionary periods in between them, known as twilight. Hours of twilight, which include both dusk and dawn, may be particularly meaningful for behavior as they signal transitionary periods between day and night during which both nocturnal and diurnal species may be active. Importantly, certain stages of twilight are saturated with blue light (Spitschan, Aguirre, Brainard, & Sweeney, 2016); many terrestrial species may have evolved to detect twilight conditions as a signal for danger, and presenting this signal at atypical times of the day or night may unnecessarily trigger a danger or fear response. Thus, even in

humans, twilight-like conditions may induce a state of preparedness to deal with environmental risks or stressors. When designing lighting environments of today's major cities, city planners may not adequately take into account the timing or spectral properties of city lighting. For example, in an effort to improve cost efficiency, many cities have moved to cheaper and brighter broad spectrum LED lights (Bedrosian & Nelson, 2017). Additionally, while household devices have started to be programed to take the spectral properties of light exposure into account with specific settings and applications (in fact, some work demonstrates positive aspects of reducing blue light output of smart phones; Heo et al, 2017), it is not clear how widely utilized these applications are. Thus, a signal that many species could have evolved to interpret as a danger cue may inadvertently be presented all throughout the day-night cycle with little attention to how it might be impacting mood and/or emotion.

There is a substantial literature suggesting that atypical lighting can impact not only behavior but also the structure and function of mood-related regions of the brain in non-human animals. For example, Green, Jackson, Iwamoto, Tackenberg, & McMahon (2015) discovered that, in a longer photoperiod (e.g., longer day lengths), levels of serotonin (5-HT) and norepinephrine (NE) were increased in the midbrain relative to a shorter photoperiod in mice and (5-HT) neuronal firing increased in response to longer photoperiods. Consistently, reduced 5-HT has been linked with depression. Behaviorally, animals in the long photoperiod condition spent less time immobile in the forced swim test (FST). The FST has been used as a probe of learned helplessness, a symptom of depression (i.e., more time spent immobile is a sign of learned helplessness; Li et al., 2011). Outside of photoperiod, light at night has also been shown to impact correlates of mood disorders in various rodent species. For example, increased body mass has also been associated with dim light at night (dLAN) in mice (Aubrecht, Jenkins, & Nelson,

2015), mice exposed to early life dLAN have been shown to demonstrate increased anxiety-like behaviors (Borniger, McHenry, Salloum, & Nelson, 2014), and dLAN leads to a depressive-like phenotype in mice and in the Nile grass rat (Walker et al., 2020; Fonken, Kitsmiller, Smale, & Nelson, 2012), to name a few examples. Thus, there is ample evidence that aberrant lighting conditions can impact behaviors associated with mood disorders.

There is less evidence of the impact of light on mood related behaviors and particularly the brain in humans, but the work that does exist suggests that humans are impacted by atypical light in a similar manner to other mammalian species, including rodents. Light in the early morning hours has long been used as an antidepressant for individuals who do not respond to typical antidepressants or therapy in humans (Li & Li, 2018) and has been shown to reduce depression-like behaviors in a diurnal rodent species (Zhu, Jia, & Zhou, 2021). It is thought that the antidepressant impacts of morning blue light are driven by both circadian retinal projections and non-circadian retinal projections (e.g., to the amygdala, lateral habenula, etc, discussed below). Importantly, the therapeutic impacts of light therapy are dependent on the color of the light; red lights have not been as associated with antidepressant effects as blue or broad-spectrum lights (Li & Li, 2018). Additionally, Chang, Aeschbach, Duffy, & Czeisler (2015) demonstrated that the use of electronic reading devices at night can have negative impacts on sleep as well as the timing of circadian rhythms and alertness the next day. Sleep deficits are often observed in individuals suffering from mood disorders (Thase 2006) and light at night likely inhibits sleep via alerting impacts of ipRGC projections (Maruani & Geoffroy, 2022). It is possible that any impacts of light at night on mood disorders are associated with above mentioned sleep disturbances, but it may also be the case that activation of the amygdala or other non-image forming targets later in the evening has the *opposite* effect of early morning blue light. Indeed,

nighttime blue light may be one of many factors involved in Hidaka's (2012) perspective on rates of depression in younger individuals in modern, industrialized cities.

Human visual system and retinal projections

The reason that mistimed light, and particularly mistimed blue light, may be problematic is evident when considering the neural structures that receive retinal inputs. The visual system uses light information in numerous ways. In the classic sense, light is used to create visual images and maps that are used to navigate and interact with the environment. Our eyes contain photoreceptors (cones and rods) within a thin layer of cells called the retina. Within photoreceptors, proteins known as photopigments (rhodopsin for low-light, non-color vision and photopsin for bright-light, high detail, color vision) detect the presence of light, at which point they change shape and generate an electrical impulse. Through stimulation of photoreceptors, information that will ultimately be used to derive contrast and color is sent through a series of intermediate cells before it reaches the retinal ganglion cell layer, where this information is collected into a bundle of axons called the optic nerve and sent back to visually oriented structures of the brain, such as the lateral geniculate nucleus (LGN) and the superior colliculus (SC). Eventually this information is carried to primary visual cortex (V1; Gray & Bjorklund, 2014).

For decades, rods and cones were thought to be the only primary source of phototransduction in mammals. More recently, it was discovered that a subset of retinal ganglion cells expresses a third type of photopigment called melanopsin (Hannibal & Fahrenkrug, 2004). Because these ganglion cells express a photopigment, they are sensitive to light on their own, and do not require signaling from classic photoreceptors (although these ganglion cells do receive some information from cones and rods; Legates et al., 2014). These cells were thusly

dubbed *intrinsically photosensitive retinal ganglion cells* (ipRGCs; Vandewalle et al., 2007), and ipRGCs project to many non-image forming regions of the brain, as well as to image-forming regions.

The ipRGC system may be particularly involved in the ways in which mistimed blue light might impact mood and emotion. The spectral sensitivities and temporal response properties of melanopsin positions ipRGCs to take in information about large shifts in ambient lighting, both from the sun and from artificial sources of illumination. As previously mentioned, solar illumination has been very consistent throughout the existence of terrestrial species. More specifically, hours of twilight are bathed in low intensity, blue saturated light. Melanopsin is maximally sensitive to short wavelength, blue light (roughly 480 nm. The human visual system is sensitive to roughly 400 nm to 700 nm with shorter wavelengths corresponding to more blue light and longer wavelengths corresponding to more orange or yellow light; Gray & Bjorklund, 2014). In addition, the ipRGC system seems primed to take in large shifts in lighting, as observed at twilight, which begins with very low-intensity, blue light, and slowly shifts to greater intensity, broad spectrum light (Graham & Wong, 2016; Spitschan et al., 2016).

Light sensitive structures relevant to mood disorders

One of the most well established roles of ipRGCs is to synchronize the timing of our internal biological rhythms, known as circadian rhythms, as well as seasonal biological rhythms, with the external world (McClung, 2013). Indeed, the *suprachiasmatic nucleus* (SCN) of the hypothalamus, the "master circadian clock," receives heavy innervations from ipRGCs, and blue light can have clear and profound impacts on circadian rhythms. However, ipRGCs also project to numerous other neural structures that are involved in a host of behavioral and psychological phenomena. Specifically, and while these projections have not all been confirmed in primates as

yet, in rodents, ipRGCs project to regions of the brain that play important roles in emotion such as the *medial amygdala* (MA) and the *lateral habenula* (LHb; Hannibal et al., 2014), as well as to subcortical visual structures. The amygdala is made up of several sub-nuclei such as the aforementioned MeA as well as the *basolateral amygdala* (BLA) and *central amygdala* (CeA). Each sub-nucleus of the amygdala processes related, but distinct information relevant to fear, risk, or danger within an individual's environment. The MeA is involved in aggressive or defensive responses to threats. The MeA feeds into the central amygdala, which works to generate fear conditioning, while the lateral and basal amygdalae are generally involved in the fear response and plasticity associated with fear conditioning (Keifer, Hurt, Ressler, & Marvar, 2015). Importantly, the amygdala in general and the BLA specifically have been shown to demonstrate morphological differences in those with depression (e.g., increased neurovascular cells in depressed individuals, increased resting blood flow in depressed individuals, increased reactivity to emotional expressions; Rubinow et al., 2016, Drevets et al., 1992, Sheline et al., 2001). Additionally, in adults and adolescents with depression, the amygdala has also been shown to be overactive (Yang et al., 2010). The BLA feeds into the MeA, and the lateral amygdala, which sends excitatory signals to the BLA, receives retinal projections via the pulvinar (Figure 1; McCue, LeDoux, & Cain, 2014; McFadyen, Mattingley, & Garrido, 2019) as well as highly processed visual inputs from visual association areas (Pessoa & Adolphs, 2010). Thus, there are multiple opportunities for visual information to impact the amygdala and subsequent emotional behaviors.

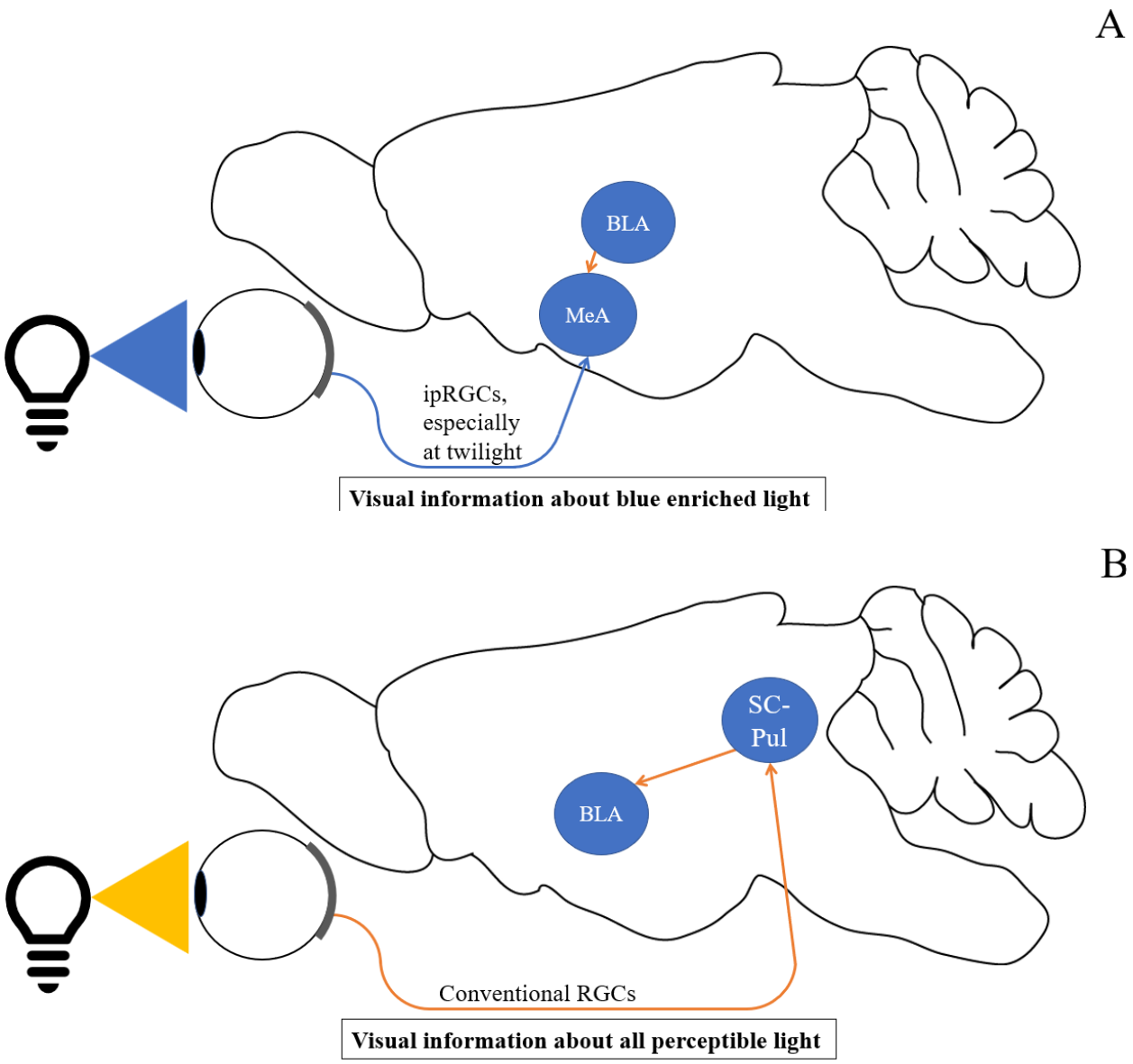

Figure 1: Non-image forming retinofugal pathways to relevant nuclei of the amygdala. A) represents non-image forming retinofugal pathways to the medial amygdala (not shown, there is an ipRGC pathway to the superior colliculus in primates), B) represents non-image forming retinofugal pathways to the basolateral amygdala via conventional RGCs. Image of rodent brain (note, this is a general depiction of a rodent brain, not of a gerbil brain specifically) taken from: https://scidraw.io/drawing/353.

While the amygdala plays a primary role, there is a broader network of neural structures that are dysfunctional in individuals with mood disorders such as depression. Essentially, depressed individuals show increased activation in interconnected regions such as the *anterior*

cingulate cortex (ACC) and the *insula* (Drevets, Price, & Furey, 2008; Kandilarova, Stoyanov, Kostianev, & Specht 2018). Normally, executive functioning structures, such as the *pre-frontal cortex* (PFC) dampen down negative thoughts and feelings generated by limbic (e.g., the amygdala) and sub-cortical regions via the thalamus (Drevets et al, 2008). However, this ability to dampen down negative thought patterns is compromised in mood disorders such that amygdala activity is not reduced as much by the PFC, and thus negative thought patterns persist. Retinal projections to the amygdala, either direct or indirect, may lead to subtle, but negative, biasing of mood and emotional states via activation by artificial light sources during atypical periods of the 24-hour light cycle.

Even further, structures outside of the depression network mentioned above, such as the *lateral habenula* (LHb), *perihabenula* (PHb), and the *locus coeruleus* (LoC), may also be involved in how mistimed blue light impacts mood and emotion. The LHb plays a role in learned helplessness, which is a common symptom in those with depression (Li et al., 2011). The LHb is also involved in reward processing, and is sensitive to negative rewards (i.e., expecting a reward but not receiving one; Proulx, Hikosaka, & Malinow, 2014). The LHb does receive direct retinal projections from ipRGCs in rodents; however, it is unclear if primates demonstrate the same retinal projections in the very limited work addressing ipRGCs in primates to this point. The LoC is involved in arousal and shows increased activation in response to a stressor. In the same study that identified the right amygdala as a structure that is sensitive to blue light, the LoC also showed sensitivity to blue light (Vandewalle et al., 2007). The recently discovered perihabenular region has been implicated in direct impacts of light on mood and emotion via ipRGCs. Perihabenula neurons project to the *ventromedial prefrontal cortex* (vmPFC), which is generally involved in social and emotion processing (Fernandez et al., 2018).

It is possible that, in mammals, a pattern of retinal projections evolved to increase an individual's sense of arousal, fear, or anxiety in twilight-like conditions (i.e., in conditions in which the available lighting is highly blue saturated), as these conditions signaled the coming onset of dangerous periods of the 24-hour day-night cycle for non-predatory species. Thus, if these pathways, whether they be from conventional retinal ganglion cells or ipRGCS, are activated during periods of the 24-hour cycle that the rest of the circadian system has not evolved to receive these signals, then there may be a conflict between activation of mood and emotion regulating regions and the rest of the body's biological clocks such that there is subtle signaling of danger related neural networks. In other words, if light sensitive, mood and emotion regulating regions of the brain are activated by certain types of light at night while at the same time circadian cues signal to the rest of the brain and body that it is time for inactivity, then danger signals may be generated when the rest of the system had not evolved to deal with them and they are not necessary. In the short term, this may impact emotional states. If these lights are presented across important developmental periods (e.g., adolescence), networks involved in the development of mood disorders may become organized in such a way that individuals are put at greater risk for the development of said mood disorders.

Dissertation

My dissertation is aimed at understanding impacts of blue light exposure, and thus activation of a series of structures known to be associated with negative mood and emotional states, on behavioral and neuroanatomical changes associated with mood disorders. Through two experiments and a pilot study, I investigated both the immediate impact of blue light exposure on emotion and the more long-standing impact of blue light exposure on correlates of behaviors associated with depression, in addition to neuroanatomical changes in one of the key mood and

emotion regulating regions described above. As industrialized societies continue to move towards environments that are largely void of hours of true, dark night, and homes, both urban and rural, continue to become filled with light emitting, screened devices, it will be important to understand how these light cues impact psychological states. Experiment 1 investigated short-term impacts of blue light on a correlate of emotion in humans, while experiment 2 and the pilot study explored longer-term impacts of atypically timed blue light during a critical developmental period (roughly adolescence) in a rodent model.

Experiment 1: How does blue light impact the processing of emotional cues in human participants over the short term?

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Abstract

Blue light can impact mood, emotion, and cognition through retinal projections. These projections include those from intrinsically photosensitive retinal ganglion cells (ipRGCs) to neural structures such as the suprachiasmatic nucleus, limbic regions including the amygdala, and the lateral habenula, among others. Solar illumination has been consistent throughout human existence. However, modern societies expose us to blue-enriched lighting environments that do not necessarily mimic sunlight via light pollution and/or screened devices. In this study, we asked whether ambient illumination or chronotype (which likely influences an individual's exposure to light over the day), or an interaction between the two, impacted emotional processing using a facial expression processing (FEP) measure. Here, we provide evidence that there are be subtle, short-term impacts of blue-enriched light on emotion. Participants completed an FEP task in either an ipRGC driving (5,000^{o}K, 126 lux illuminance) or an ipRGC neutral (2,200^{o}K, 15 lux illuminance) lighting condition. Participants viewed happy, sad, angry, fearful, and neutral expressions from two models (one male, one female) for 100 ms and 1,500 ms at 40%, 75%, and 100% expressiveness, and identified the emotion presented on individual trials in a forced choice format. There were no mean differences between the lighting condition groups for accuracy of expression identification, either overall or for individual expressions. Chronotype of the participant showed only a minor interaction effect with one expression type (happy). However, the overall pattern of errors revealed that, in bluer compared to warmer light, when participants were wrong on any given trial, they tended to be wrong *towards* negative expressions, and *away* from positive or neutral expressions. Additionally, there was a similar trend specifically for neutral target expressions, such that participants tended to misidentify neutral targets as negative rather than happy expressions in bluer, brighter light. These data suggest that, when exposed to blue-enriched light, people are subtly biased towards mistaking any expression for a negative one.

The visual system evolved to process light information, both for image formation as well as many non-image forming functions. These non-image forming functions include taking in timing cues. Classically, vision research has focused on the former rather than the latter function. Indeed, the complex interactions between the numerous retinal pathways that lead to image formation have been well researched. Only more recently has the field of vision research begun to understand the vast range of non-image forming functions in which the retina and the rest of the visual system take part. Most notably, information about light is sent directly to the primary circadian pacemaker of the brain, the *suprachiasmatic nucleus of the hypothalamus* (SCN) via *intrinsically photosensitive retinal ganglion cells* (ipRGCs; Legates et al., 2012). This projection is heavily involved in the setting of our circadian rhythms (Bhadra, Thakkar, Das, & Bhadra, 2017). Additionally, ipRGCs project to mood regulating regions of the brain such as the *medial amygdala* (MeA) and the *lateral habenula* (LHb) in rodents (Vandewalle et al., 2007). It is likely the case that light, and particularly blue light which ipRGCs are highly sensitive to, impacts mood and emotion through non-image forming projections. The current study investigates the impact of light that is enhanced in blue wavelengths versus light that is not on a selected measure for emotion, facial expression processing (FEP).

Chronotype describes the relative timing of one's circadian rhythms (i.e., the timing of numerous internal biological clocks and outward behaviors; Horne & Östberg, 1976). Chronotype is typically measured using questionnaires such as the Morningness-Eveningness Questionnaire (MEQ; Horne & Östberg, 1976) or the Munich Chronotype Questionnaire (MCTQ; Roenneberg, Wirz-Justice, & Merrow, 2003). Individuals who choose to (or are biologically predisposed to) set their circadian rhythms later are exposed to more artificial light later into the dark phase than those who have set their circadian rhythms earlier. Consistently

activating the ipRGC system during atypical times of the day may have longer-lasting impacts on mood and emotion. Indeed, eveningness has been associated with numerous negative psychological outcomes, such as depression (McClung, 2013), anxiety, aggression, and substance use severity (Taylor & Hasler, 2018), but the mechanisms for these negative psychological outcomes are not known. Lighting exposure may be one contributing factor. Thus, the current study also investigated whether chronotype impacts mood and emotion via a proxy for emotion processing.

Facial expression processing

Facial expression processing (FEP) is a widely used measure of emotional processing in humans. As a highly social species, it is important for humans to quickly and correctly identify facial expressions of other humans. These expressions provide information about the other individual's intentions and emotional state. It is known that a person's emotional or mood state can influence FEP, such that if someone is in a negative mood state, they are negatively biased in their interpretation of facial expressions (Bourke, Douglas, & Porter, 2010). Here, we use an FEP paradigm to determine if lighting or chronotype influence facial expression processing.

Current study and predictions

We predicted that participants who are assigned to a lighting condition that drives the ipRGC system will be more accurate at identifying negatively valenced facial expressions. We also predicted that participants who are more evening oriented would be more accurate in identifying negatively valenced facial expressions. *The goal of this experiment* is to provide initial evidence for the impact of brief exposure of different types of light on short-term emotional states, as well as replicate findings that adhering to an evening-oriented timing of behavior has negative impacts on correlates of emotional states.

Methods

All experimental procedures were reviewed and approved by Emory University's Institutional Review Board before any participant took part in this study.

Demographics

We recruited 150 undergraduate students to take part in this experiment. One-hundred and five participants were recruited through the undergraduate research pool and 45 were recruited as paid participants (between the ages of 18 and 25 with normal or corrected to normal vision). All participants provided informed consent before taking part in the research study. Five participants were dropped from analysis because they left the experiment room before a 20-minute visual adaptation period. Data from 145 participants (43 male, 102 female) are reported here in the final analyses.

Experimental design

General procedure

Experimental sessions took place in either the morning (8:00 am through 11:00 am) or the evening (5:00 pm through 8:00 pm). To limit the impact of outside lighting, experimental sessions took place in a windowless room within a larger lab space. The lighting in the room was of a brightness of roughly 125 lux in the *bright-cool condition* and roughly 15 lux in the *dim-warm condition*.

Participants were seated in a computer chair in front of the computer monitor. Immediately behind participants were two lamps, each fitted with an LED bulb. The LED bulbs had three settings, two of which were used in the current study (described in more detail below). Behind the lamps, we hung a white curtain to reflect as much of the light back at the participants as possible (see Figure 2 for the layout of the experimental room).

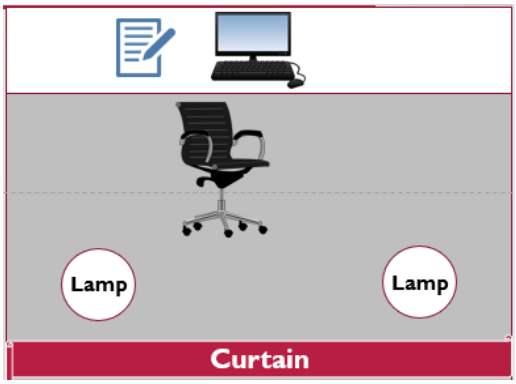

Figure 2: Layout of the experimental room.

Lighting

Two experimental conditions were chosen for this study: *ipRGC neutral* and *ipRGC driving*. Two Philips LED lamps with multiple light settings were used as our main experimental manipulation. The color temperature of the *ipRGC neutral* condition was 2,200 K, with a brightness output of 80 lumens. The color temperature of the *ipRGC driving* condition was 5,000 K, with a brightness output of 800 lumens. Participants were randomly assigned to either the *ipRGC driving* or the *ipRGC neutral* condition before arriving at the study session. Using a light meter, study room illuminances for the two experimental lighting conditions positioned where participants were seated were 126 lux for *ipRGC driving* versus 15 lux for *ipRGC neutral*.

Questionnaires

As the visual system takes several minutes to adjust to a new lighting environment, participants were given twenty minutes to complete paper questionnaires in the experimental conditions to ensure visual adjustment before completing a facial expression processing task on a desktop computer. To measure chronotype, we used two established chronotype questionnaires, the reduced Morningness-Eveningness Questionnaire (rMEQ; Horne & Östberg, 1976) and the

Munich Chronotype Questionnaire (MCTQ; Roenneberg et al., 2003). In addition, a number of other questionnaires were completed as "fillers" by the participants as they visually acclimated to the lighting environment (i.e., these questionnaires were presented to give participants something to work on as they visually adapted for the 20 minutes period). These were the Three-Factor Eating Questionnaire - reduced 18 (TFEQ R18) to assess eating behaviors that may be associated with chronotype, the Beck Depression Inventory with the suicidality question removed to assess sub-clinical depressive symptoms that may be associated with chronotype or our lighting manipulations, the Stanford Sleepiness Scale (SSS) to assess the alertness of the participants pre- and post-FEP as well as to determine if sleepiness impacted FEP, as finally a modified version of the Artistic Creativity Domains Compendium (ACDC) questionnaire to assess any potential relationships between creativity and chronotype. The SSS was presented twice, once before the FEP paradigm, and once after (Figure 3).

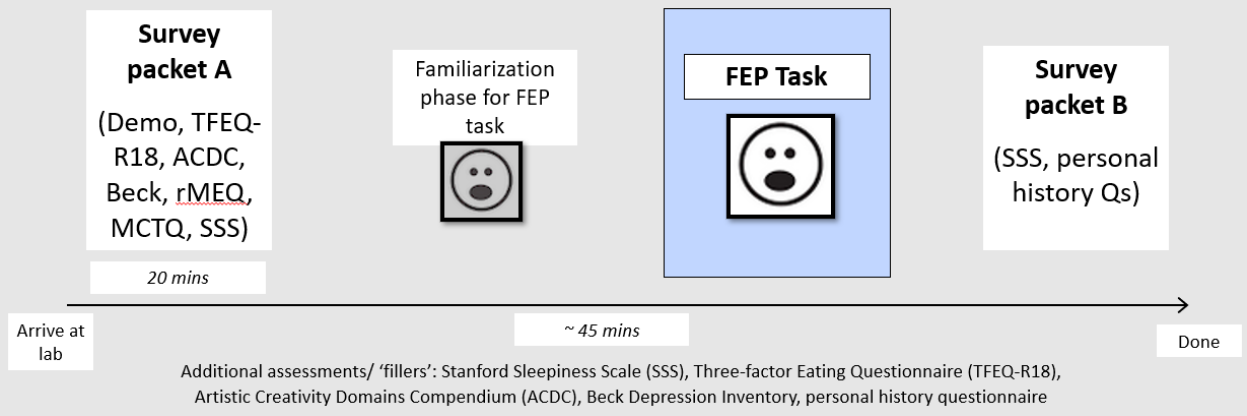

Figure 3: Timeline of events for participants during the study.

FEP paradigm

The facial expression processing task utilized a subset of the NimStim facial expression database. Two models were selected, one male and one female, both Caucasian adults. Each face was cropped and gray scaled to reduce the impact of any distractors such as skin color or hairline

and ears (Sinha, Balas, Ostrovsky, & Russel, 2006). The program (EPrime) was used to present stimuli and collect data. The EPrime display was set to have a black background with white text to reduce light emitted from the screen.

The expressions selected for the experiment were happy, sad, angry, fearful, and neutral. Each expression was morphed with the neutral expression to create steps of 40% expressiveness, 75% expressiveness, and 100% expressiveness. Participants were also shown true neutral expressions. Before each expression was presented, participants were shown a fixation point. Each expression was displayed twice, once for 100 ms and once for 1,500 ms, in a random order. Following the presentation of each expression, participants selected on the keyboard which expression had just been presented in a forced choice paradigm. Participants were not given a timeline to respond but were requested to respond quickly. Responses options were taped to number keys at the top of the keyboard. Neutral, happy, sad, fearful, and angry were mapped to 6, 7, 8, 9, and 0 respectively. Participants were then asked to rate how confident they were in their response on a 1 to 4 scale.

Results

Analyses were run using the statistics program SPSS. Data are presented with the standard error of the mean.

Primary analyses

The primary analyses of the impact of lighting and chronotype on a proxy for emotion were completed in two steps. First, we investigated whether there were mean differences in accuracy on either expression type or intensity across the two lighting conditions and between chronotype. Second, we analyzed patterns or errors across lighting conditions and across chronotypes.

It should be noted that our sample was heavily skewed towards eveningness, as expected for a college student population (Randler, Schredl, & Goritz, 2017). To take this factor into account, we split our data into thirds on the morningness-eveningness measure and used the third of the distribution with the highest (N = 49) and the and third with the lowest (N = 49) scores for our analysis. From here on, the two groups will be referred to as evening types and non-evening types, respectively.

Mean difference testing

A three-way, mixed-model ANOVA was used to investigate between and within subject factors related to accuracy of identifying facial expressions with respect to different lighting conditions. The within subject variables were accuracy across different facial expressions and accuracy as a function of expression intensity. The between subject variable was lighting condition (i.e., *ipRGC-driving* vs. *ipRGC-neutral*). Analyses were collapsed across the male and female models. There were no mean differences between the lighting conditions, nor were there significant interactions between the lighting groups and the different individual facial expressions, for either the 100 ms presentation time or the 1,500 ms presentation time, $F(1, 143) = 3.404$, $p > 0.05$ and $F(1, 143) = 0.057$, $p > 0.05$, respectively (Figure 4).

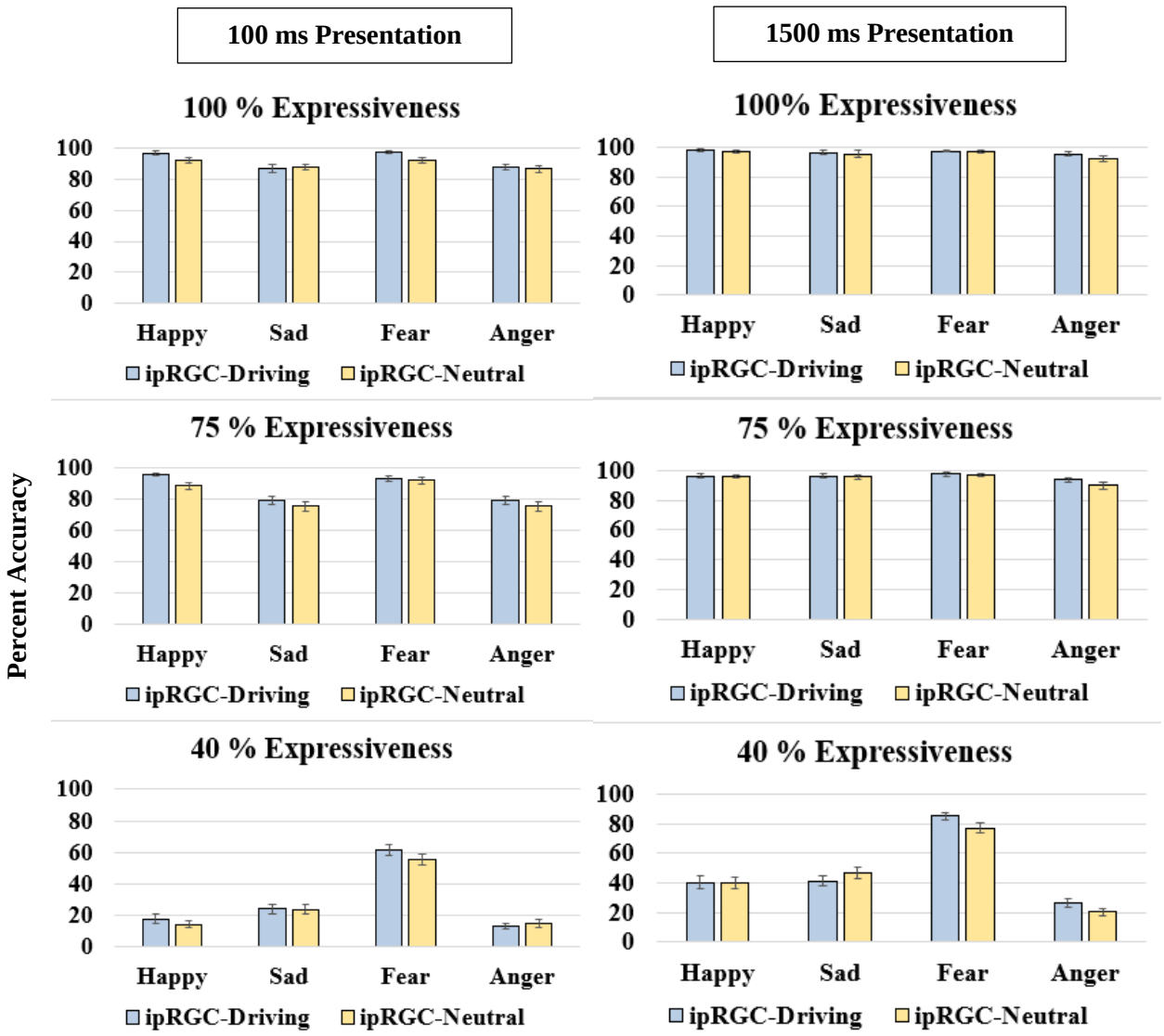

Figure 4: Mean differences of lighting condition. Top frame is FEP accuracy at 100% intensity, second frame is FEP accuracy at 75% intensity, and the third frame is FEP accuracy at 40% intensity. Left hand charts are 100 ms presentation time, right hand charts are 1,500 ms presentation time. Error bars are one-standard area of the mean (SEM).

A three-way, mixed model ANOVA was similarly used to investigate between and within subject factors related to accuracy of identifying facial expressions with respect to measures of chronotype. The within subject variables were accuracy for different facial expressions and accuracy as a function of expression intensity. The between subject variable was chronotype, comparing, as described above, the most and least evening-oriented thirds of the distribution of the morningness-eveningness measure. There were no mean differences between the chronotype

groups, nor were there significant interactions between the chronotype groups and the different individual facial expressions, for either the 100 ms presentation time or the 1,500 ms presentation time, $F(1, 78) = 0.002$, $p > 0.05$ and $F(1, 78) = 0.122$, $p > 0.05$ (Figure 5).

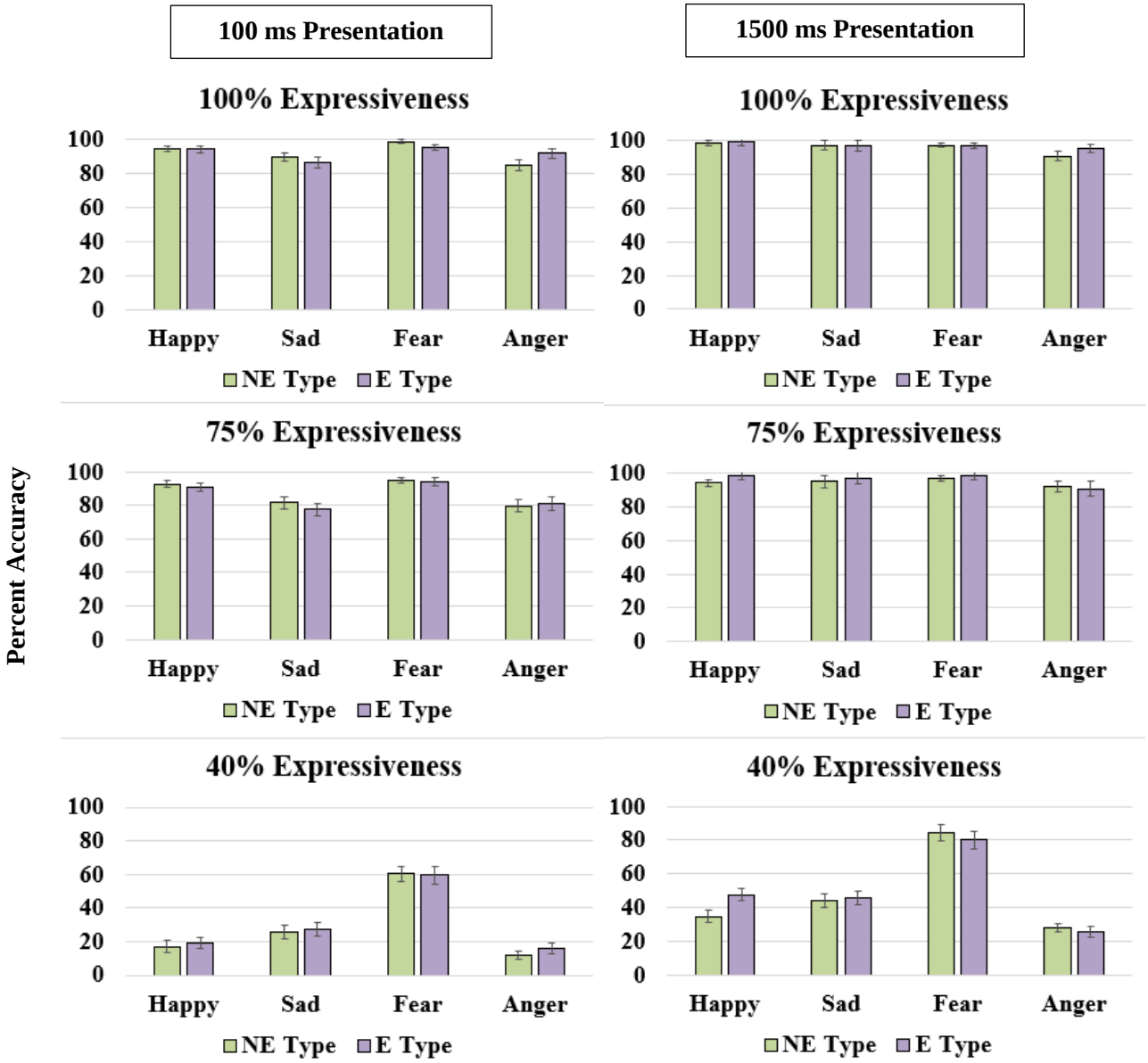

Figure 5: Mean differences of most extreme evening and morning type participants. Top frame is FEP accuracy at 100% intensity, second frame is FEP accuracy at 75% intensity, and the third frame is FEP accuracy at 40% intensity. Error bars are one SEM.

There was one significant interaction in accuracy scores across lighting and chronotype such that, at the 100 ms presentation time, in the *ipRGC-neutral* condition, non-evening types were more accurate at identifying happy expressions while in the *ipRGC-driving* condition; evening types were more accurate at identifying happy facial expressions (see table 1).

Interactions - Chronotype and Lighting - 100 ms				**Interactions - Chronotype and Lighting – 1,500 ms**			
Expression and intensity	*Mean Square*	*F*	*p-value*	*Expression and intensity*	*Mean Square*	*F*	*p-value*
Anger at 40%	14.07	0.044	0.835	Anger at 40%	1377.115	2.071	0.154
Fear at 40%	206.507	0.212	0.647	Fear at 40%	1610.171	3.165	0.079
Happy at 40%	6.938	0.013	0.911	Happy at 40%	2185.123	2.047	0.157
Sad at 40%	57.284	0.09	0.765	Sad at 40%	1641.09	1.647	0.203
Anger at 75%	46.947	0.082	0.775	Anger at 75%	47.175	0.201	0.655
Fear at 75%	261.495	1.531	0.22	Fear at 75%	17.093	0.558	0.457
Happy at 75%	43.362	0.236	0.629	Happy at 75%	52.831	0.558	0.457
Sad at 75%	108.563	0.239	0.626	Sad at 75%	46.718	0.451	0.504
Anger at 100%	58.808	0.197	0.658	Anger at 100%	34.828	0.176	0.676
Fear at 100%	1.869	0.029	0.866	Fear at 100%	7.294	0.126	0.724
Happy at 100%	910.494	6.725	.011*	Happy at 100%	1.823	0.078	0.781
Sad at 100%	26.881	0.079	0.78	Sad at 100%	9.512	0.212	0.646

Table 1: Interactions effects between lighting conditions and chronotypes. Significant effects denoted with *

Confidence

Independent samples t-tests revealed no mean differences in average confidence across lighting conditions at 100 ms, $t(143) = 0.174$, $p > 0.05$, nor at 1500 ms, $t(143) = 1.138$, $p > 0.05$.

Independent samples t-tests revealed no mean differences in average confidence scores for non-evening versus evening types at 100 ms, $t(111) = 0.046$, $p > 0.05$, nor at 1500 ms, $t(111) = 0.357$, $p > 0.05$.

Pattern of errors

To investigate patterns of errors, we utilized a chi-square analysis to test whether the observed differences in error patterns on individual trials were different from what would be expected just from chance. This analysis revealed that participants who were tested in the *ipRGC driving* condition compared to the *ipRGC neutral* condition were more likely, when they were wrong on any given trial, to incorrectly select a negative expression (χ^2 (1, N=145) = 39.22, $p < 0.05$) (Figure 6). For example, in blue enriched lighting, on trials in which the participant selected the wrong expression type, they were more likely to incorrectly identify the expression as either a sad, angry, or fearful expression, and less likely to incorrectly identify it as a happy or a neutral face compared to a lighting condition that did not include proportionately as much blue-enriched light across all expression types.

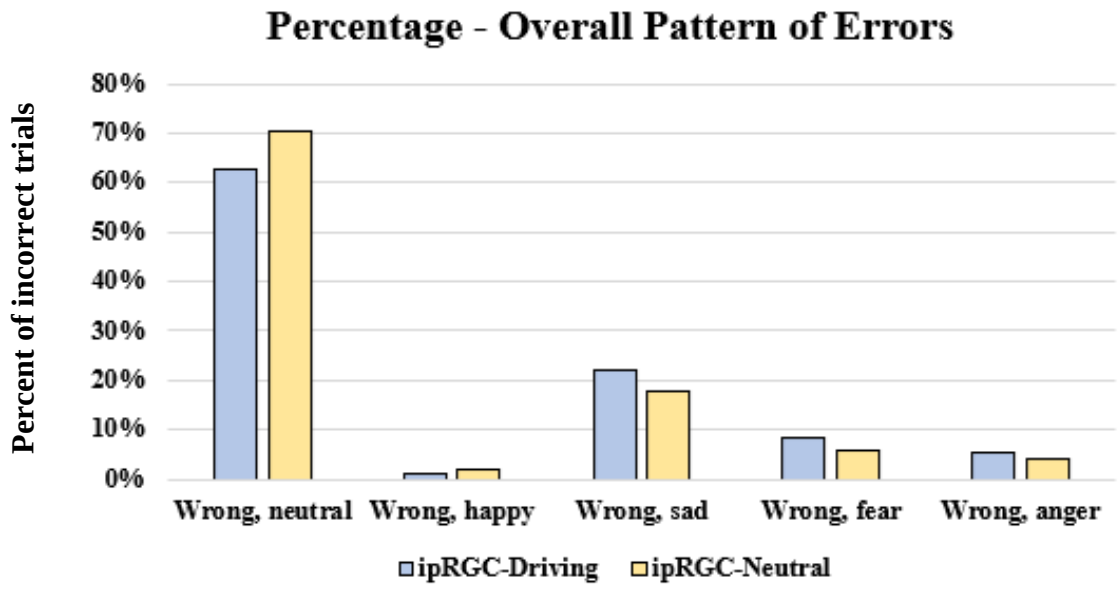

Figure 6: Percentage of patterns of errors between lighting conditions across all facial expressions. Percentages presented here represent the pooled percentage of all trials in which participants made a mistake across all facial expression types.

Additionally, in the *ipRGC driving* condition compared to the *ipRGC neutral* condition, when participants incorrectly identified a neutral expression, they were marginally more likely to select a negative expression (sad, angry, or fearful) than incorrectly identifying it as a happy expression, χ^2 (1, N=145) = 7.66, p = 0.053) (Figure 7).

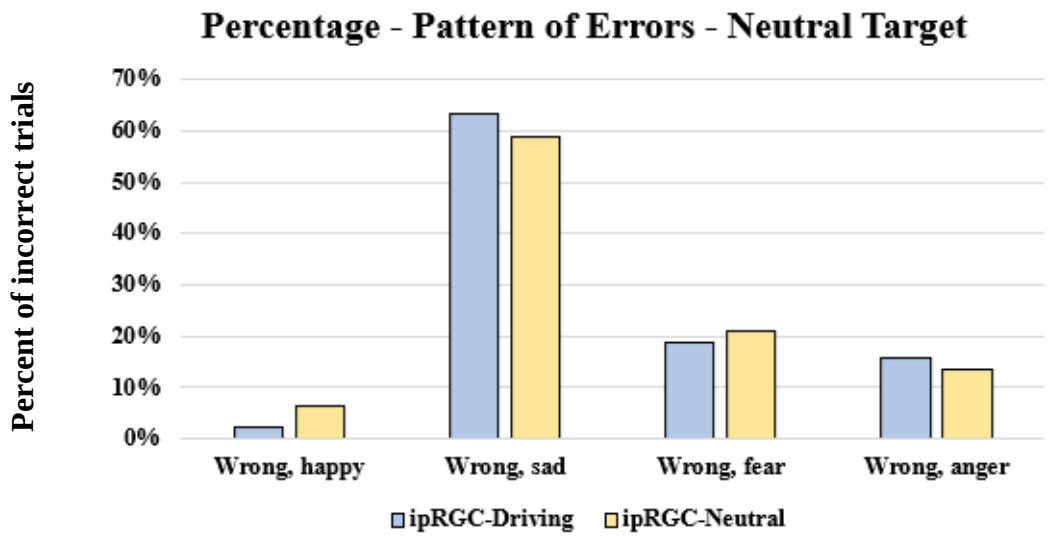

Figure 7: Percentage of patterns of errors between lighting conditions when participants were viewing a neutral facial expression. Percentages presented here represent the pooled percentage of all trials in which participants made a mistake across all facial expression types except neutral.

We also applied a chi-square analysis to our measure of chronotype. Non-evening types were more likely to select negative expressions when they were wrong compared to evening types ($\chi 2 = 12.67$, $p < 0.05$) (Figure 8).

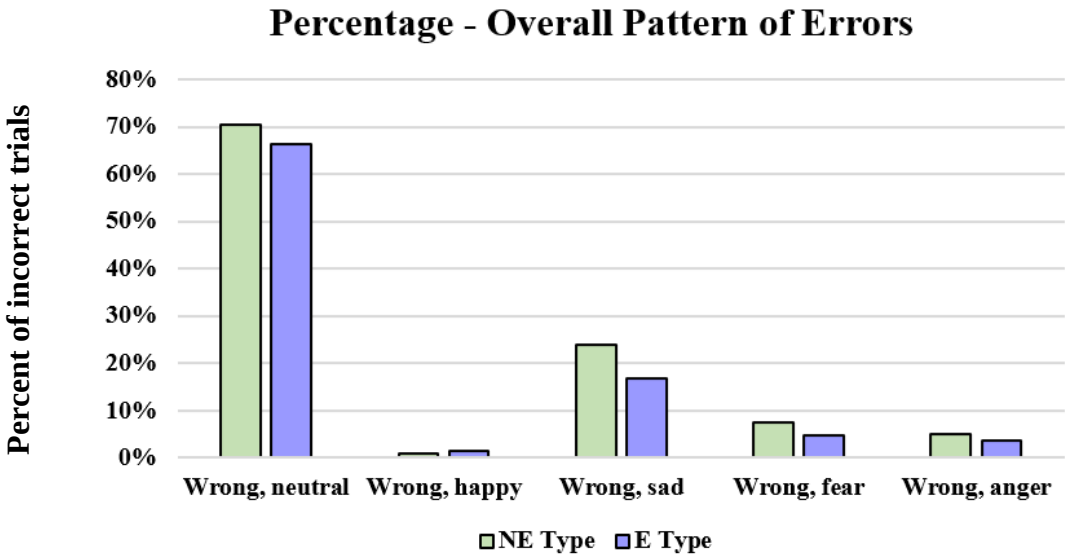

Figure 8: Percentage of patterns of errors between chronotype across all facial expressions. Percentages presented here represent the pooled percentage of all trials in which participants made a mistake across all facial expression types.

Signal Detection Analysis

It may have been the case that either (or both) lighting and chronotype impacted the decision rule that participants utilized when identifying expressions. Signal detection theory (SDT) posits that in situations in which there is some signal as well as some unrelated, or related, noise (i.e., uncertainty between the target and some other external or internal distractor[s]), a decision rule must be generated to distinguish the signal from the noise (Corwin 1994). In the current experiment there were several more distractors than noise on any given trial (e.g., for a trial in which the target was a happy face, there are four sources of noise: neutral, sad, angry, and fearful expressions). The two-high threshold (2HT) theory is a commonly used alternative to SDT when the number of distractors and targets are not equal (Surguladze et al., 2004).

The 2HT approach calculates a measure of percent recognized correct, Pr, as well as a bias score, Br. The data are transformed into Pr and Br scores via transformation formulas provided in Corwin, 1994 (Pr = [number of hits + 0.5/number of targets + 1] – [number of false alarms + 0.5/number of distractors + 1] and Br = [number of false alarms + 0.5/number of distractors +1]/[1 – Pr]). Because our experiment has unequal signals and distractors, we utilized the 2HT variant of signal-detection theory in these exploratory analyses.

Two-High Threshold (2HT)

We posited that environments which drive the ipRGC system may have led to reduced accuracy via Pr scores on neutral and happy expressions and increased Pr on negative expressions compared to the warmer light conditions. Additionally, bias scores, Br, may be

increased for negative expressions in the cooler lighting condition but not for neutral or happy expressions.

Before statistical tests were applied to Pr and Br scores, a test of skewness revealed a strong negative skew. Various Log and root transformations were applied, but the data remained skewed. Therefore, we utilized a non-parametric analysis, the Mann-Whitney *U* test, to test for differences in Pr and Br between our two lighting conditions.

There were no significant differences in Br across the lighting groups for any of the expressions. For Pr, Mann-Whitney *U* tests revealed a significant difference for participant's response accuracy for the happy condition such that, in ipRGC-driving lighting, participants' accuracy score was higher (Mdn = 0.72) than in the ipRGC-neutral lighting (Mdn = 0.69), U ($N_{ipRGC\text{-}Driving} = 72$, $N_{ipRGC\text{-}Neutral} = 73$) = 1835.00, $z = -3.15$, $p < 0.05$.

Comparison of Br and Pr for Chronotype

There were no significant differences for Br or Pr across chronotype classification for the entire dataset altogether, nor for each individual expression.

Exploratory – Reaction Time

Data on reaction time was also collected by default in EPrime. We had no *a priori* hypotheses regarding reaction time for either lighting or chronotype. However, the ipRGC-driving condition, which was not only more blue-saturated but also brighter, may have led to faster reaction time as more of the cone system would be active in the brighter lighting, thus making identification of the facial expressions quicker. Additionally, the ipRGC-driving condition presumably led to increased alertness as this is a hallmark of ipRGC system activation, which may also lead to shorter reaction times (LeGates et al., 2014). It is not clear how

chronotype might impact reaction time. Independent samples t-tests revealed no significant differences in reaction time across lighting conditions at 100 ms, $t(143) = 0.017$, $p > 0.05$, nor at 1,500 ms, $t(143) = -0.803$, $p > 0.05$. Independent samples t-tests also revealed no significant differences in reaction time across non-evening and evening chronotypes at 100 ms ($t(111) = -0.520$, $p > 0.05$), nor at 1,500 ms ($t(111) = 0.206$, $p > 0.05$).

Discussion, limitations, and future directions

Our data presented here indicate that blue light subtly but negatively impacts the way in which college-aged students interpret facial expressions. While our data do not support our *a priori* hypotheses regarding mean differences between the groups on accuracy of identifying facial expressions as a function of lighting or chronotype, we did find group-related patterns of errors that point to shifts in the assessment of facial expressions under conditions of uncertainty., Discussion of the data is broken down below for both lighting and chronotype.

Lighting

When investigating patterns of errors, we discovered that participants in the *ipRGC-driving* condition were more likely to misidentify any given expression as a negative one rather than a positive or neutral one. Additionally, participants in the *ipRGC-driving* condition were marginally more likely to misidentify neutral expressions as negative ones rather than happy expressions. Light at atypical times of the light-dark cycle, particularly at night, has been shown to negatively impact psychological and physical health through circadian pathways in various ways across numerous species (Fonken et al., 2019; Cissé, Peng, & Nelson, 2016; Bedrosian, et al., 2013). However, this is the first report, to our knowledge, to demonstrate that short-term exposure to a light source which differentially drives the ipRGC system can lead to rapid, negative biasing in the way in which college-aged students respond to emotional-social cues.

This finding may be important for understanding human behavior in the modern world, which continues to be filled with sources of artificial light both through light pollution in cities and through screened devices in homes. Moreover, college-aged individuals are more likely to display later chronotypes (Fischer, Lombardi, Marucci-Wellman, & Roenneberg, 2017). Thus, these students could be exposed to artificial lighting that likely contains blue light later into the evening, which may expose them to lighting environments similar to those reported here. Additionally, while the experiment took place in the morning and the evening, light exposure did not occur during particularly extreme portions of the light-dark cycle. It may be the case that a similar paradigm, presented at more extreme portions of the light-dark cycle (i.e., well into the dark phase) might be even more impactful on emotional and social phenomena. Lastly, and even more striking, is that our effects presented here were observed using common, household lighting, demonstrating that, while subtle, even the household lighting environment may lead to negative impacts on underlying emotional states if blue-enriched lighting is used indiscriminately.

The interaction effect between lighting and chronotype for accuracy of 100% happy expressions is more difficult to interpret. Accuracy for 100% happy expressions at 100 ms across lighting was dependent on chronotype such that, evening types were more accurate in *ipRGC-driving* lighting compared to non-evening types, who were more accurate at identifying 100% happy expressions in the *ipRGC-neutral* condition. It is possible that chronic eveningness exposes one to more artificial lighting later into the evening, and is thus less impactful in altering our ability to identify positive expressions when compared to the non-evening types. However, the rest of our data do not support this conclusion. Further investigation should be done to better understand this relationship.

Analyses on confidence ratings revealed no significant mean differences in confidence for identifying facial expressions across lighting conditions. We had no *a priori* prediction for this variable as it was not clear from the literature how confidence might be impacted by lighting. These data suggest that there is no relationship between these variables.

We determined that reaction time does not differ across lighting conditions utilized here. This result runs counter to a study by Zhu et al.'s (2019), also using a facial expression identification paradigm, which found that brighter, cooler lights lead to faster reaction times. This may be due to differences in study design, discussed in more detail below. It is reasonable to assume, and evidence would suggest (Zhu et al., 2019), that brighter lighting might lead to shorter reaction time, as it will activate more of the cone system and lead to potentially faster judgments of the facial expressions. It is also reasonable to assume that lighting which differentially activates the ipRGC system might lead to faster reaction time as activation of this system leads to higher levels of alertness (LeGates et al., 2014). Neither of these assumptions were supported here. However, it may be that the lighting environments need to be either bluer, brighter, or both in order to observe statistically significant differences. Further research may help to better determine any underlying effects here.

The 2HT analysis revealed that participants in the *ipRGC-driving* condition had higher accuracy scores compared to participants in the *ipRGC-neutral* condition for happy expressions. This result follows the interaction effect but goes somewhat counter to our hypothesis. We predicted that participants in cooler lighting would show higher accuracy for negative faces. This effect may be due to differences in lighting intensity rather than light color. In bright lighting, the cone system is more active and is able to pick up on more fine-grained spatial frequencies. In addition, negative facial expressions, such as those associated with deception and specifically

anger, have been associated with *low* spatial frequency, magnocellular processing of the visual system (Kihara & Takeda, 2019). Thus, brighter light may have biased participants in the brighter *ipRGC-driving* condition away from negative expressions to show higher accuracy scores for happy expressions in the 2HT analysis. Our data do not support the prediction that cooler light will lead to increased accuracy or bias scores on negative expressions.

Some of the data presented here also run counter to a recent publication that demonstrated an effect of correlated color temperature (CCT) on mood and cognition (Zhu et al., 2019). Zhu et al. (2019) present data showing lowest mood (as measured using the Positive Affect Negative Affect Schedule [PANAS]) in a dim cool light condition (200 lux, 6,500 K, eye level) relative to a bright cool condition (1,200 lux, 6,500 K), a dim warm condition (200 lux, 3,000K), and a bright warm condition (1,200 lux, 3,000 K). We did not find a main effect of CCT on mood based on a different measure of mood, the Beck Depression Inventory (BDI). On the other hand, they presented data showing a null main effect of accuracy on FEP across CCT, which does fit with our overall findings on average FEP accuracy across lighting. Moreover, our pattern of errors data fall in line with Zhu et al. (2019). Participants were more likely to mistake any given expression as a negative expression, which falls in line with mood and depression literature and falls in line with our overall predictions that short wavelength light may negatively impact measures of mood and emotion.

There are a number of differences between Zhu et al.'s (2019) work and the present study which may explain the lack of an effect of CCT on a measure of mood presented here. Here we used the BDI which measures for depression rather than more general shifts in affect, as the PANAS does. The BDI may have been too specific to depression to detect more subtle changes in general mood. Further, our *ipRGC-driving* condition had a much lower color temperature than

what was used in Zhu et al. (2019) at 5,000 K versus 6,500 K, respectively. While 5,000 K CCT likely emits relatively more short wavelength light compared to our *ipRGC-neutral* condition at 2,200 K, it may not emit proportionately enough short wavelength light to elicit changes in mood as measured by mood questionnaires. Additionally, Zhu et al. (2019) mounted nine luminaires directly in front of participants. Our lights were two free-standing floor lamps. It is possible that both the increased number of lights and more direct exposure (i.e., eye level in Zhu et al. [2019] versus pointed towards the ceiling in the current study) led to overall more ipRGC activation relative to our design. Further, Zhu et al.'s (2019) low cool condition was at a brightness of 200 lux. The lighting presented here were at 15 lux and 126 lux. Our lighting manipulations may not have been not sufficiently bright to activate the ipRGC system to observe the predicted effects on our mood questionnaire. Finally, Zhu et al. used a different facial expression set and Chinese participants in a Chinese setting, all of which may have contributed to the differences on the mood measure.

One limitation of the current study was the use of our chosen commercially available, ecologically informed, lighting as our lighting manipulations. While the color temperatures used in the two conditions were fairly different from one another, in order to observe changes in accuracy, the *ipRGC-driving* condition may have needed to be a more extreme color temperature. While a 5,000 K light bulb emits substantially more short wavelength light than a 2,200 K light bulb, there are artificial lights available with much higher color temperatures (e.g., 10,000 K). A light with a higher color temperature may be necessary to fully drive the ipRGC system and lead to more pronounced changes in FEP. Additionally, the intensities of the two lighting conditions were not particularly different from one another (126 lux for *ipRGC-driving* versus 15 lux *ipRGC-neutral*). A brighter light source may be necessary to drive the ipRGC

system enough to alter how accurate participants are when completing the FEP task. The ipRGC system tends to react to light more slowly than other photoreceptors and stay active longer (up to 18 minutes). Importantly, they also appear to be well positioned to react to large changes in illumination, such as those observed during the shift between night and day (Graham & Wong, 2016; Vandewalle et al., 2007).

Chronotype

Our initial predictions for chronotype were also not supported by our data, as there were no mean differences between evening oriented and non-evening-oriented participants in accuracy of identifying facial expressions. In particular, evening-oriented participants were not more accurate for negative expressions relative to less evening-oriented participants. These null effects may be due to inherent limitations in sampling from a college-aged population. Specifically, our study was limited in the spread of chronotype. There were very few morning types, and no definite morning types at all. The majority of participants were either intermediate or evening oriented. This may have led to an underlying biased sample, as in a large study of 500 participants ranging between six and 65 years old, chronotype was evenly distributed across the sample (Roenneberg et al., 2003). However, this skew towards eveningness is not unexpected. Chronotype demonstrates a clear developmental trajectory, with adolescents being decidedly more evening oriented than children, adults, and the elderly. It is possible that a questionnaire specifically targeted towards adolescents will help to alleviate the skew, but at present there is no such questionnaire that is widely used in the literature (although see Tonetti, 2007). Future studies may develop a chronotype questionnaire specifically targeted at understanding the phenomenon in an adolescent population.

The chronotype data for patterns of errors are counter intuitive. In general, non-evening types were more likely to select a negative expression when making a mistake. Extant literature suggests that individuals who are more evening oriented relative to the non-evening types would be more likely to more accurately identify negative facial expressions (Horne et al., 2016). This may have again been due to sampling in our study, as our participants were highly skewed towards eveningness.

Analyses of confidence ratings revealed no significant mean differences in confidence for identifying facial expressions across chronotype. We had no a priori prediction for this variable as it was not clear from the literature how confidence might be impacted by chronotype. These data suggest that there is no relationship between these variables.

Lastly, the 2HT analyses revealed no differences between evening-types and non-evening-types in accuracy nor bias. This result follows our main set of mean difference tests, but goes counter to the literature (McClung 2013, Jonason et al., 2013). This may, again, be due to the limited number of true morning types within our sample.

Reaction time also did not differ across chronotypes. It is less clear how one would predict a difference in reaction time between chronotypes. It may be the case that morning types (or, non-evening types, in the current data) are generally quicker to respond to stimuli as they tend to demonstrate fewer mood and health issues overall (McClung 2013). Indeed, individuals with psychiatric disorders tend to show greater variability in reaction time tasks, such as Go/No Go (Kaiser et al., 2008).

Conclusion – experiment 1

Humans are an immensely social species for whom accurately identifying facial expressions is critical. Misidentifying a facial expression can have negative impacts on how any one individual interacts with another, as correctly identifying facial expressions provides information about the other person's intentions and psychological states (e.g., mood). Critically, social deficits are a hallmark of depression, and may precede or co-occur with its onset (Segrin 2000). In addition, our twilight and evening environments continue to be flooded with artificial light without adequate consideration of the spectral properties of these lights. Here, we provide evidence that brighter, blue-enhanced light can, relative to warmer, dimmer light, subtly and negatively alter how people interpret social cues, such that participants are more likely to mistake any given expression for a negative one. As major cities continue to become bathed in essentially 24-hours of artificial lighting and as handheld, light emitting devices continue to become more prevalent in people's nighttime routines, these data demonstrate that it is critical that we better understand how lighting might impact the psychological condition outside of classic image-formation.

Experiment 2: Behavioral impacts of blue lighting on mood and emotion over critical developmental periods

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Abstract

Blue-enriched lighting, which characterizes many modern human lighting environments, can impact mood and emotional processing. Moreover, mistimed lighting during development has been shown to have depressogenic effects in animal models. This study investigated the long-term impacts of exposure to blue light during atypical times of the day-night cycle during adolescence in a mainly diurnal rodent species, the Mongolian gerbil (*Meriones unguiculatus*). Gerbils were randomly assigned to a morning blue light condition, an evening blue light condition, or a control condition with no targeted blue light exposure. Animals were raised under their assigned lighting condition from the equivalent of adolescence to the end of puberty (roughly two months of age). Gerbils then completed the sucrose preference test, elevated plus maze, social approach test, intruder test, open-field test, and forced swim test. Behaviorally, gerbils raised under blue light at night demonstrated increased aggressive behaviors in the intruder task as well as marginally significant increases in social investigatory behaviors in the social approach test. Depression-like behaviors were not observed. However, gerbils raised in atypical lighting also showed reduced jumping and rearing and increased grooming in the open-field test. These data suggest that long-term exposure to blue light at night may have more meaningful impacts on behaviors critical for aggression and social interactions than on depression-like behaviors *per se*. Alternatively, and in addition, extended experience with mistimed blue light may shift behavioral strategies to more energetically efficient approaches in response to novel stimuli.

Previous work has provided evidence that brief exposure (20 minutes) to a light that contains some short-wavelength, blue light can have subtle, but negative impacts on a correlate of emotion in humans (facial expression processing; Moon, Diplas, Kheraj, & Rodman, *in prep.*) and that judging facial expressions in the evening may cause one to exaggerate the expressiveness of negative expressions (Recht, Moon, & Rodman, *in prep.*). It is important to note that artificial lights in industrialized cities are essentially ubiquitous, and individuals may be exposed to blue light across multiple days, not simply one instance of blue light. This exposure may be particularly important for developing children and adolescents. Indeed, it is well established that numerous life experiences can have impacts on development across many domains during these critical periods, which, in turn, may put individuals at greater risk for a host of psychological or behavioral disorders (Chen & Baram, 2016). Here, we hypothesize that if atypically timed blue light is presented across critical developmental periods (e.g., adolescence), then it may have longer-term impacts on mood and emotion related behaviors. This study was designed to use ecologically valid lighting paradigms (i.e., lighting that roughly mimics conditions that one might encounter in their daily lives and not unrealistically intense or long light exposure) to investigate the possibility that blue light might impact correlates of mood and emotion.

Issues within animal studies of light on mood and emotion

The literature on the impact of light on mood and emotion in animal models is vast. However, often, animal models investigating the impact of light on affect have focused on species with a nocturnal (night active) rather than a diurnal (day active) timing of activity. Indeed, in a literature review, only four of 33 articles investigating the impact of light on affective behaviors utilized a diurnal rodent species. All four utilized the Nile grass rat

(Ashkenazy-Frolinger, Kronfeld-Schor, Juetten, & Einat, 2010; Fonken, Haim, & Nelson, 2012; Fonken, Kitsmiller, Smale, & Nelson, 2012; Soler, Robison, Nunez, & Ya, 2017). It is well established that most primates, including humans, are not nocturnal by nature and tend to stick to a diurnal or somewhat flexible pattern of daily activity (chronotype; Horne & Östberg, 1976). Thus, much of the work on light and its impact on affective behaviors in rodent species has been on species that do not share similar exposure to light as humans. This difference may be critical considering diurnal and nocturnal species evolved to take advantage of different ecological niches. The same environmental cue (i.e., blue light at certain times of the day or night) may be processed slightly differently depending on when a species is active across day-night cycle (e.g., melatonin is released in the dark for both nocturnal and diurnal species. However, melatonin is processed as a sleep promoter in diurnal species but not in nocturnal species; Kumar 1996).

The Mongolian gerbil

The Mongolian gerbil (*Meriones unguiculatus*) is a rodent species that typically lives in arid, desert environments and has become an increasingly desirable species used in animal models of psychological phenomena. Mongolian gerbils are social, form mate pairs, and, importantly, do not usually demonstrate a nocturnal pattern of activity and are somewhat flexible in them (Batchelder, Keller, Sauer, & West, 2012; Refinetti et al., 2016). In fact, Mongolian gerbils mapped on quite well to human patterns of activity as nearly all members of both Mongolian gerbils and humans begin their active phases within a roughly six-hour time window; in other words, in both species, “morning” oriented individuals begin their active phase at the beginning of the six-hour window and “evening” oriented individuals begin their active phase near the end of the six-hour window (Refinetti et al., 2016). Similarly, humans show a wide range in the timing of individual activity patterns, a phenomenon known as chronotype (Horne &

Östberg, 1976). This variability in activity patterns in both species make the Mongolian gerbil well suited for animal models of human psychological phenomena. Importantly, many behavioral tests relevant to depressive endophenotypes have been validated in gerbils as a species (Varty, Morgan, Cohen-Williams, Coffin, & Carey, 2002; Wallace-Boone, Newton, Wright, Lodge, & McElroy, 2008; Rico, Penagos-Gil, Castañeda, & Corredor, 2016). For these reasons, we decided to use Mongolian gerbils for this work.

Gerbil visual pathways relevant to mood and emotion regulating regions

Like humans and all mammalian species studied to date, gerbils have intrinsically photosensitive retinal ganglion cells (ipRGCs; Jeong & Jeon, 2015). Conventional retinal ganglion cells (RGCs) involved in classic image formation require input from rods and cones; ipRGCs express the photopigment melanopsin, allowing them sensitivity to light cues without rod-cone input (Vandewalle et al., 2007). Research has confirmed that ipRGCs project to the MeA in gerbils (it should be noted that the majority of retinofugal projections to the MeA were from conventional RGCs, but the blue light sensitive ipRGCs do project to the MeA; Luan et al., 2018). Conventional RGCs may be involved in amygdala sensitivity to light as well. Roughly 20% of conventional RGCs project to the superior colliculus (SC), where visual information is integrated in head and eye movements. Retinal information from the SC is then relayed to the BLA via the pulvinar. This pathway has been thought to be responsible for the “low-road” of amygdala activation to emotionally salient stimuli before conscious perception of the stimulus (Carr 2015). Regardless of the visual pathway, it is clear that information about light can impact the primary mood and emotion regulator, the amygdala.

Gerbil developmental periods

There are several critical periods for the development of long-term mood and emotion in Mongolian gerbils. Gerbils reach sexual maturity around post-natal day (PND) 42, which may be similar to a critical period for mood and emotional development in humans known as puberty (Pinto-Fochi, Negrin, Scarano, Taboga, & Goes, 2016). In addition, there is a sensitive period at which gerbils develop long-term reactivity to a stressful light stimulus between PND 30 and 60 (Clark & Galef, 1979). Lastly, DA system inputs to the basolateral and central amygdala, which are thought to be dysfunctional in individuals with depression, show significant development between PND 14 and 20 (Brummelte & Teuchert-Noodt, 2006). All three of these developmental periods may be critical for the developmental of long-term mood and emotion; in other words, these may be periods during which environmental perturbations, including atypically timed blue light, may be particularly impactful on the development of mood and emotion.

Types of lighting manipulations used in studies of light's impact on behavior

Studies of animal models of the impact of lighting on neurodevelopmental or cognitive functioning often manipulate the length of the day, or photoperiod, under which the animal is housed. Consistently, research has shown that light-dark cycles lengths that mirror the natural day of 12 hours of light and 12 hours of dark (12:12 LD) or provide longer light exposure are the most beneficial, both physically and mentally. Shorter photoperiods have, likewise, consistently shown to lead to deleterious effects on physical and psychological phenomena. For a few examples of the impact of photoperiod on psychological phenomena: 12:12 LD or 16:8 LD led to fewer behavioral correlates of depression and increased serotonin (5-HT) in mice relative to 8:16 LD cycles (Green, Jackson, Iwamoto, Tackenberg, & McMahon, 2015), chipmunks respond similarly as mice to longer photoperiods behaviorally, but dopamine effects are species specific

(Goda et al., 2015), and retinal dopamine as well as visual function also appear sensitive to the photoperiod around an animal's birth in mice (Jackson, Capozzi, Dai, & McMahon, 2014).

The impact of photoperiod on brain and behavior is well established, particularly as it relates to circadian functioning; however, less well understood is the impact of specific spectral properties (color) of light at specific times of the day on affective states or traits. There has been work demonstrating that blue light early in the morning can be used as an antidepressant for certain patients suffering from depression. It is thought that these antidepressant effects of light therapy are primarily manifested through circadian circuitry, but it is likely that limbic structures are involved as well (Li & Li, 2018). Blue light later into the evening, or after sunset, may potentially have an *opposite* effect on affective states through direct ipRGC projections to limbic structures or indirect projections of conventional RGCs. In other words, if blue light in the morning-as the rest of the circadian systems are ramping up-has an anti-depressive effect, blue light in the evening, as the rest of circadian systems are ramping down, may have a depressive effect via subtle activation of limbic structures. It is already known that blue light, relative to other light colors (e.g., red), has powerful impacts on circadian rhythms via the SCN (Lockley, Brainbard, & Czeisler, 2003), However, it is unclear how activation of these pathways impacts mood and emotion circuitry during developmental periods.

Current study and predictions

The goal of this experiment is to understand how blue light in the early evening across a critical developmental period impacts mood and emotion relevant behaviors in a rodent model relative to blue light in the morning or no blue light in a species whose patterns of activity are similar to human patterns of activity. This experiment also attempts to manipulate lighting in an ecologically valid way (i.e., lighting manipulations that are not overly intense or long in

exposure). This study will use an array of behavioral tests that have been validated in gerbils to probe behavioral correlated of mood disorders such as depression. These data are critical as it is not fully clear how retinofugal projections to limbic structures might directly impact mood and emotion outside of circadian disruption, behaviorally. We predicted that animals raised in blue light at night during adolescence, a critical developmental stage, will demonstrate increased depressive-like behaviors.

Methods

All experimental procedures were reviewed and approved by Emory University's Institutional Care and Use Committee (IACUC) before any animals took part in this study.

Animals

A total of 40 gerbils were ordered from Charles River to be included in the current study. Thirty-six gerbils were included as experimental animals and four stimulus gerbils used in social behavioral tests. The sample size used here was determined following a power analysis (using the G*Power program) after review of relevant extant literature.

All gerbils were housed in cages with water and food available *ad libitum*, except for a brief food and water deprivation period during sucrose preference (described below). DAR-provided cages are made of clear plastic to allow light to penetrate the walls of the cage. Semi-opaque filter paper was placed over the top of each cage, ensuring even distribution of light within the cage. Materials for burrowing were provided. Gerbils were all female and were ordered in batches of six. Animals were pair-housed upon arrival until midway through the sucrose preference test (described below). Pairs were randomly assigned to one of three experimental lighting conditions (described below) just before arrival. All experimental gerbils were roughly 28 to 32 days old upon arrival, were acclimated to the colony for 10 days, and were

placed in experimental lighting between PND 38-42. Animals then spent the next three and ½ weeks in experimental lighting before being returned to colony lighting at PND 63-68 at which point they were singly-housed. Animals then began behavioral testing at PND 67-72 and completed all portions of the experiment at PND 71-77. Euthanasia occurred over the next week.

Stimulus gerbils were also purchased through Charles River. These animals were all female and were roughly 54 to 57 days old upon arrival. Stimulus gerbils were ordered at an older age to roughly match the age of the experimental gerbils at the time of behavioral testing. Stimulus animals were not sibling pairs and were singly-housed for the entirety of the experiment. These animals were not euthanized and were handed over to Emory's Division of Animal Resources following the completion of the experiment.

Light manipulations

There were three experimental lighting conditions: morning blue light exposure, no blue light exposure (colony lighting or control), and evening blue light exposure. Colony lighting of a 12:12 DL cycle (lights turn on at 7:00 am and turn off at 7:00 pm) was used for the typical "day" lighting for all three conditions and was the only lighting to which the control animals were exposed.

The light emitting diode (LED) light strips that were used here were purchased from Waveform Lighting. The decision to purchase light strips from Waveform was made after an extensive review of commercially available LED lights, as well as a consultation from the Aguirre lab at the University of Pennsylvania. The SimpleColor Blue LED Strip Lights were purchased to create our light rigs. These strips were selected because their peak spectral output was similar to that of the peak sensitivity of ipRGCs at roughly 460 nm (Figure 9).

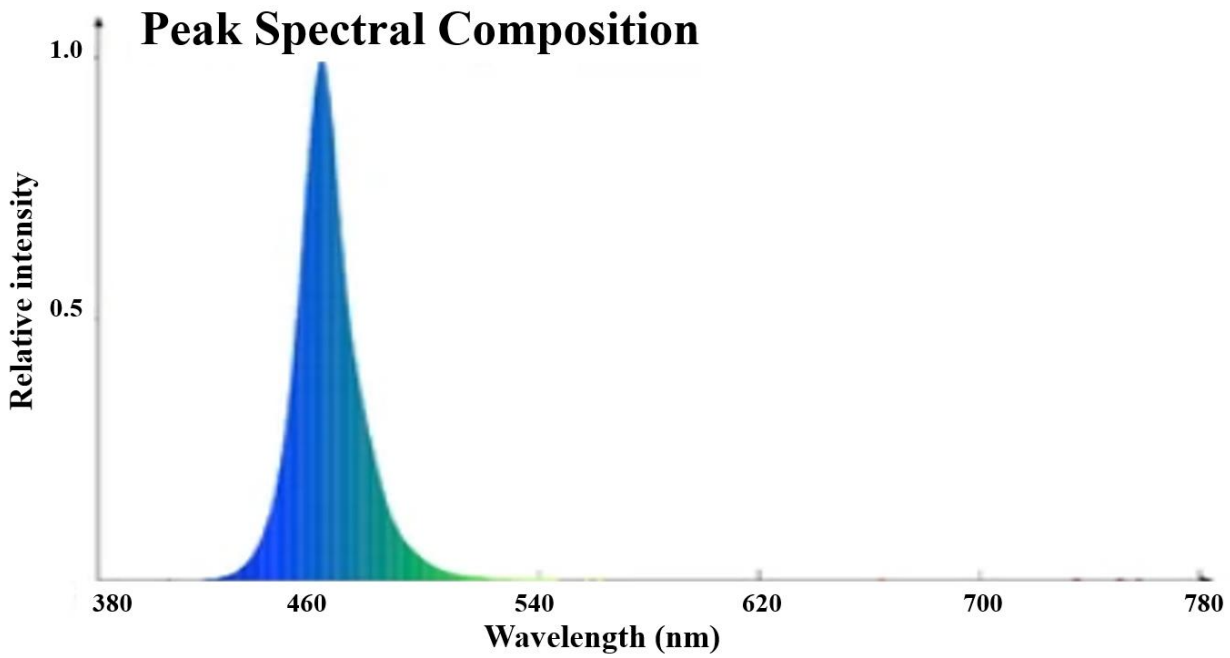

Figure 9: Spectral properties of the LED light strips from Waveform with a peak output at 460 nm. Taken from Waveform documentation.

Light rigging

The experimental lighting consisted of two light "rigs," one each for the two experimental conditions (e.g., pre-sunrise and post-sunset, or "Netflix" light exposure; there was no rig for control lighting). The light rigs were made by connecting three commercially available oven racks together via zip ties and weaving light strips through the racks. Each rig contained three 18" LED light strips per oven rack (so nine total per rig) and each oven rack was positioned over a gerbil cage on the shelf above (Figures 10 and 11, respectively).

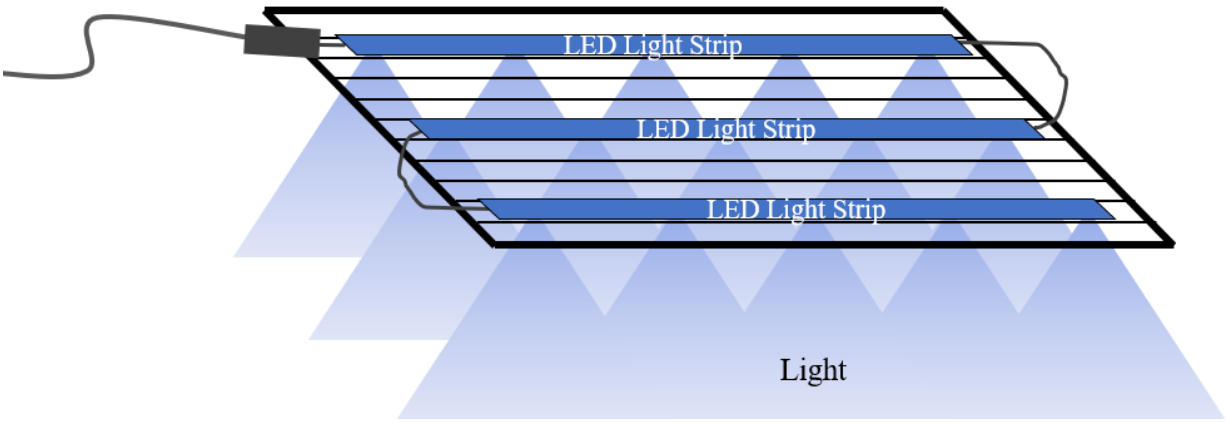

Figure 10: Light rigging with LED lights pointed downward.

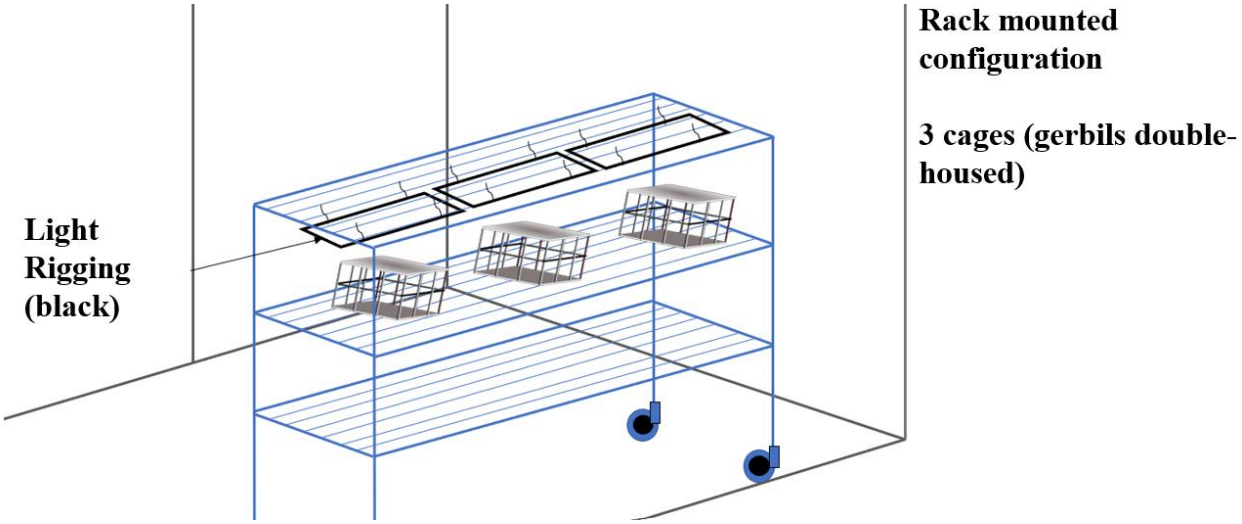

Figure 11: Light rigging as it was configured during the light exposure phase of the experiment.

The timing of both the morning and the evening lighting was set to a timer that would supply power only during specific times of the day. A battery was built into the timer to ensure that lights-on and -off were saved, even if brief power outages occurred. These timers were set to have lights turn on at 5:00 am and turn off at 7:05 am for morning light exposure, and have lights turn on at 6:55 pm and turn off at 9:00 pm for the evening light exposure. There was a short,

five-minute overlap for both experimental lighting conditions between the colony lighting and the experimental lighting as we did not want to startle the rodents with our light rigs.

A researcher would check that the lights turned on as programmed. Researchers also ensured that no gerbil was demonstrating increased signs of distress from the lighting once a new batch of animals was moved to experimental lighting. Researchers also ensured that the heat emitted from the light rigs was not high enough to cause discomfort or harm the experimental animals.

The number of light strips used in each light rig was adjusted to ensure that the light strips alone would be sufficiently bright, but not so bright that they would startle the gerbils and would not exceed what one might reasonably experience on an evening-to-evening basis. Using a lightmeter, the intensities of the colony lighting, the colony lighting with the experimental lighting, and the experimental lighting were measured. Colony lighting on its own was roughly 146 lx, colony lighting with the experimental lighting was roughly 454 lx, and experimental lighting on its own was roughly 309 lx. These measurements were taken inside of an empty gerbil cage with the wire rack, water bottle, and plastic top with filter paper in place to simulate the gerbil's experience of the lighting as closely as possible.

Light exposure timeline

Animals acclimated to the colony space for 10 days following their arrival. Following acclimation, animals were moved from a holding cubicle to one of the three experimental lighting conditions. Animals assigned to the control condition remained in the colony lighting cubicle.

Animals assigned to the morning (pre-sunrise) light condition or the evening (post-sunset, or "Netflix") light condition were moved to the appropriate cubicle following acclimation. The morning light condition consisted of two hours of blue light before the onset of colony lighting, and the evening light condition (or the "Netflix" condition) consisted of two hours of blue light after the offset of colony lighting (Figure 12). Control lighting consisted of 12:12 colony lighting, with light onset at 7:00 am and light offset at 7:00 pm. Across all three conditions, animals were placed on the same shelf within their respective cubicles (the second from the top shelf, directly under the light rigs) to ensure roughly equivalent lighting during the "day" and roughly equal environmental conditions within the cubicles.

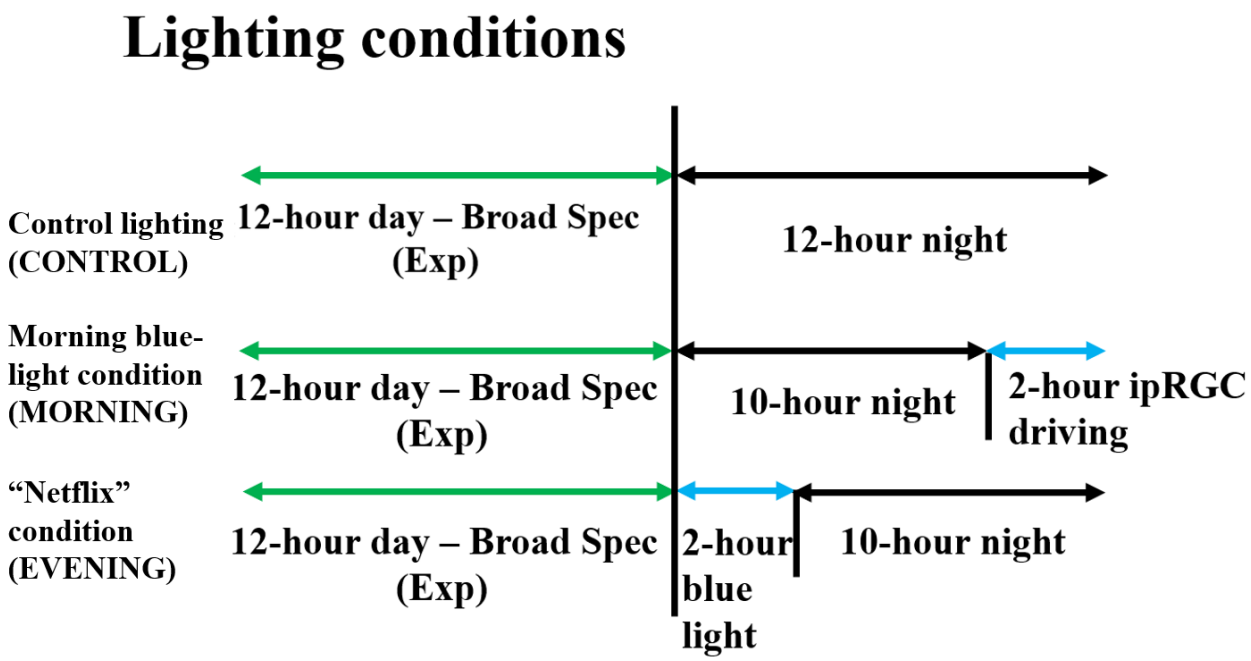

Figure 12: Timing of experimental lighting during the 24-hour period for the three lighting conditions. 12 hours of broad spectrum lighting was consistent across all lighting conditions (i.e., this was colony lighting).

Animals remained in experimental lighting for nearly three and ½ weeks (Figure 13). Following their four-week period of light exposure, gerbils were returned to the holding cubicle at one of the lower shelves as they completed behavioral testing.

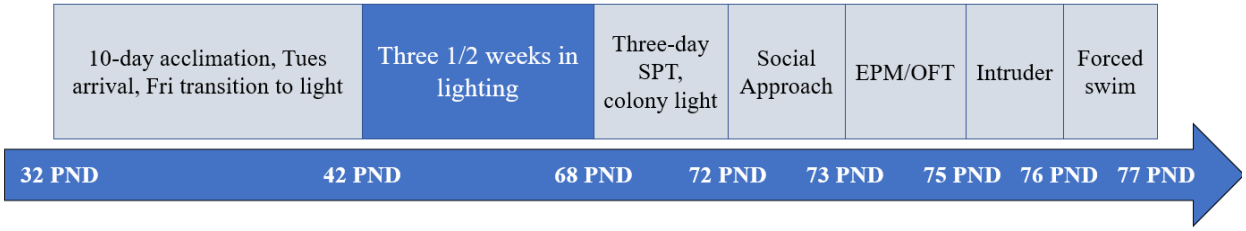

Figure 13: Timeline of an individual animal's participation in the experiment. SPT = sucrose preference, OFT = open field test, and EPM = elevated plus maze. Ages at which gerbils began each test are displayed below.

Behavioral tests

Several behavioral tests were used to understand the impact of atypically timed blue light on mood and emotion relevant behaviors during adolescence. The behavioral tests used in this study were: social approach, elevated plus maze, open field test, modified intruder test, forced swim test, and sucrose preference test. All coding was performed by members of the research team who were blinded to the animal's experimental condition to prevent knowledge of which experimental condition each animal had been assigned.

Sucrose preference

Gustation, or the sense of taste, provides information about the contents of food resources. Sweetness is an approach taste as it provides energy through sugars. Thus, once an animal has learned that a given food or water source is sweet, they should approach it, barring dysfunction. It is thought that lack of approach to sucrose (sugar) water in rodent species is a correlate of anhedonia in depressed humans (He et al., 2020).

During the final week of experimental light, gerbils acclimated to the sucrose preference test. Water was typically provided by a single bottle in the wire rack to the right-hand side of the cage. On the Friday before the final week of lighting exposure while gerbils were still pair-housed, a second water bottle was added to acclimate animals to two sources of water. Both bottles contained regular water provided by DAR. The following Monday, two bottles of a 1% sucrose water solution were provided to acclimate the animals to sucrose water. The Wednesday following acclimation to sucrose, animals were provided one bottle of regular water and one bottle of the 1% sucrose solution. The position of the sucrose bottle was randomized across animals. Animals were singly-housed at this stage to acclimate them to single housing before the sucrose preference data collection portion. The following Thursday, the positions of the sucrose and the regular water bottle were switched to avoid location preference. The following Friday, each animal was food and water deprived at 6:00 pm for 15 hours to ensure they were motivated to approach the water bottles. On Saturday at 9:00 am, food was returned and one smaller bottle (50 ml) of the 1% sucrose solution was provided on one side of the cage and one smaller bottle of regular water is provided on the other side of the cage. Animals were then allowed to consume from either water bottle freely for 33 hours before a final weight measurement on Sunday at 6:00 pm; this was the data collection portion of the procedure. Once the procedure was completed, a single, regular water bottle was placed in each cage.

Water used to mix the 1% sucrose solution was taken from standard, DAR provided water bottles. A batch of sucrose water would be mixed for each group of animals as they entered the sucrose preference stage.

Social approach

As gerbils are generally a highly social species, and reduced sociability is observed in individuals with depression, this test was used to determine if blunted sociability was observed in our animals. The social approach test was adapted from Fricker, Seifert, and Kelly (2022). This test involved placing an experimental animal in a clear plexiglass arena (32” in length, 12” in width, and 16” in height) for three minutes to acclimate to the arena. Once the three minutes have passed, a small, clear plastic “gate” is closed, keeping the experimental animal separated on one side of the arena. A second, stimulus gerbil is placed in the social arena on the other side of the clear plastic “gate” and is then trapped under a metal cage and a weight is placed on top to prevent its escape. The plastic gate is then opened, and the test begins. The test phase of the social approach lasted six minutes.

Behaviors coded were latency to approach the stimulus gerbil, time spent on the stimulus gerbil’s side of the arena versus the original empty side, time spent within one body length of the stimulus gerbil, time spent investigating the stimulus animal, grooming, and number of crossings of the midline of the arena. Additional behaviors observed included rearings (lifting up onto the animal’s rear paws), seizures, and freezing.

Elevated plus maze

Extended time spent in an open area is thought to be a sign of lack of anxiety in rodents. The elevated plus maze tests for anxiety (often found to be comorbid with depression) by comparing the time a rodent spends in either an open arm of a plus-shaped maze, or a closed arm (arms are 43 ¼” long, 3 ¾” wide, and ¼” thick, closed arm walls are 15 ¾” tall). The maze is elevated off the ground at a height that the gerbils can perceive but not high enough that it would cause physical damage to the animal (18 1/2” tall). The gerbil is placed in the center of the maze

facing towards one of the open arms. Each session lasted five minutes and the sessions were recorded on a digital video camera. It is thought that time spent in the closed arm indicates anxiety (Varty et al., 2002).

To quantify exploration of the maze, each arm of the maze was divided into three equally size rectangles by the coder. Behaviors coded were time spent in open arms versus the closed arms, time spent in the extreme end of the arms, time spent in the center square, and the number of times the animal crossed the various sections of the maze. Entries were defined as when the animal's rear paws enter the arm or section (i.e., all four paws in the area). Additional behaviors observed included seizures and freezing.

Modified open field test

The open field test is another measure that is thought to test for anxiety-like behaviors in rodents based on exploration (Bridges & Starkey, 2004). This test consists of an open, clear plexiglass box which the animal can freely explore. The center of the open box is conceived of as representing a particularly risky portion of the environment, as ecologically, a rodent is more at risk for predation if it is in the middle of an open space. Thus, more time spent in the center region is thought to demonstrate a lack of anxiety-like behaviors. The open field test included here is considered "modified" because the arena used was considerably smaller than those used in a number of relevant works (Choleris, Thomas, Kavaliers, & Prato, 2001; Oldham & Morlock 1970; Nauman 1968; Fiore & Ratti, 2007). It was decided to use this arena as it has previously been used for open field tests, was similar in size to that used in Oldham and Morlock (1970), and it allowed for the use of a Digiscan device to track overall ambulation.

To begin, gerbils were placed in the center of a 16" x 16" x 18" open field apparatus and allowed to explore the open field for 10 minutes. The floor of the apparatus was demarcated into

equal sized squares in a 5 x 5 configuration and subsequently into an outer-middle-inner configuration (based on Choleris et al., 2001, configuration). Movements of the animal were also captured using a Digiscan Activity Monitor. The Digiscan Activity Monitor tracks movements within a square open space using an 8 x 8 grid of infrared sensors. The sensors track the number of times an animal crosses a sensor (ambulation), as well as the number of times the animal moved within a sensor. In the current study, total ambulation was used as the primary indicator of total movement within the open field apparatus.

Behaviors coded were time spent in the center square, time spent in the middle portion of the grid, time spent in the outer portion of the grid, and overall ambulation as recorded from the Digiscan recording device. Entries were defined as when the animal's rear paws enter the arm or section (i.e., all four paws in the area). Additional behaviors observed included rearings (lifting up onto the animal's rear paws), seizures, freezing, and jumping within the open field apparatus.

Modified intruder test

When an unknown conspecific is introduced to a resident animal's home cage environment, the resident animal typically acts with aggression to defend its territory. Lack of said aggression, or passivity may be associated with anhedonia or general depression (Henn & Vollmayr, 2005). In the current study, we used the same social arena as the social approach test to examine whether our lighting conditions impacts territorial behaviors through a modified version of the intruder test. Importantly, the same stimulus gerbil was never used in both of the social approach and intruder tests to ensure the animal was a novel conspecific in each test.

The typical intruder test involves introducing an unknown conspecific animal, the intruder, into the test animal's home cage. Any potential harm to either animal was minimized here by having the gerbils complete this test in a previously neutral social arena rather than the

experimental gerbils home cage. To ensure that the social arena was perceived as the resident gerbil's home cage, the social arena was populated with items from the resident gerbil's home cage (i.e., the resident animal's nestlet, chewing block, manzanita stick, and food bowl were moved into the social arena). The resident gerbil was then allowed to acclimate to the social arena with their home cage items for 10 minutes. Observationally, following the 10-minute acclimation phase, the "resident" gerbil frequently returned to its nest and home-cage objects throughout the test, suggesting that it did have a sense of familiarity with or ownership of this portion of the social arena.

Following the acclimation phase for the resident gerbil, the clear plastic "gate" was shut within the social arena, separating the "home" side of the arena from the "intruder" side. The intruder gerbil was then placed in the closed, empty portion of the social arena. The gate was then opened, and the gerbils were allowed to interact for 10 minutes during the test phase of the intruder test.

Behaviors coded for were latency to interact with intruder gerbil, time spent on "home" side of the arena versus the intruder side, bouts of fighting through locked fighting or grappling, sidling, self-grooming, mutual-grooming, biting, chasing, and following. Additional behaviors observed included rearings (lifting up onto the animal's rear paws), seizures, and freezing.

Forced swim test

The forced swim test is thought to test for signs of learned helplessness (Li et al., 2011). During the test, gerbils were dropped into a relatively cool tank of water (roughly 72 degrees F, 13 ¼" x 15 ¾") and allowed to swim freely for six minutes. The size of the FST tank was determined based on Wallace-Boone et al. (2008) to avoid a tank too small to be viable in the FST. Water was filled deep enough so that the animals could not touch the bottom of the bucket.

It is important that the water be somewhat cool or cold to ensure the animal is motivated to exit the water. The amount of time spent attempting to escape is thought to represent learned helplessness; less time swimming or climbing may demonstrate learned helplessness in the animal. If gerbils demonstrated excessive signs of distress which could lead to potential harm to the animal, they were removed from the test. Once animals finished the test, either following the six minutes or from being removed early, they were placed in a warming and drying cage. Temperatures in the drying cage were kept at roughly 82 to 85 degrees F.

Behaviors coded for were latency to first bout of immobility, total bouts of immobility, time spent swimming, time spent floating, and overall time spent within the forced swim test.

Results

Analyses were run using the statistics program SPSS. Data are presented with the standard error of the mean.

Strategy for behavioral analyses

Analysis of behavior tests were broken up into three primary sets of analyses. First, we ran ANOVAs to determine if lighting had an impact on individual behaviors overall. Second, we ran t-tests to determine if morning light versus evening light, excluding the control condition, had differential impacts on behavior (these are captured in the omnibus ANOVA via contrasts). Third, we ran t-tests to determine if both experimental lighting conditions combined had an impact on behavior relative to no experimental lighting (controls).

Sucrose preference

Results from a one-way ANOVA revealed no significant differences in sucrose preference across lighting conditions. Results of t-tests revealed no significant differences in

sucrose preference between morning and evening gerbils. Results of t-tests also revealed no significant differences between morning and evening gerbils combined and control gerbils.

Social approach test

Results of one-way ANOVAs revealed no significant differences across lighting conditions for body-length distance from stimulus gerbil, crossings over the center line of the social arena, investigatory behaviors of the stimulus gerbil, latency to approach the stimulus gerbil, or side preference of the social arena.

Results of t-tests revealed no significant differences between morning- and evening-light conditions for body-length distance from stimulus gerbil, latency to approach the stimulus gerbil, or side preference of the social arena.

T-tests between morning- and evening-light conditions revealed significant differences in number of crossings of the center line (gerbils in the morning-light condition crossed the center more), $t(22) = 2.083$, $p < 0.05$, and a marginally significant effect of time spent investigating the stimulus gerbil (gerbils in the evening-light condition spent more time investigating the stimulus gerbil, the opposite direction predicted), $t(22) = 1.798$, $p = 0.086$ (Figure 14).

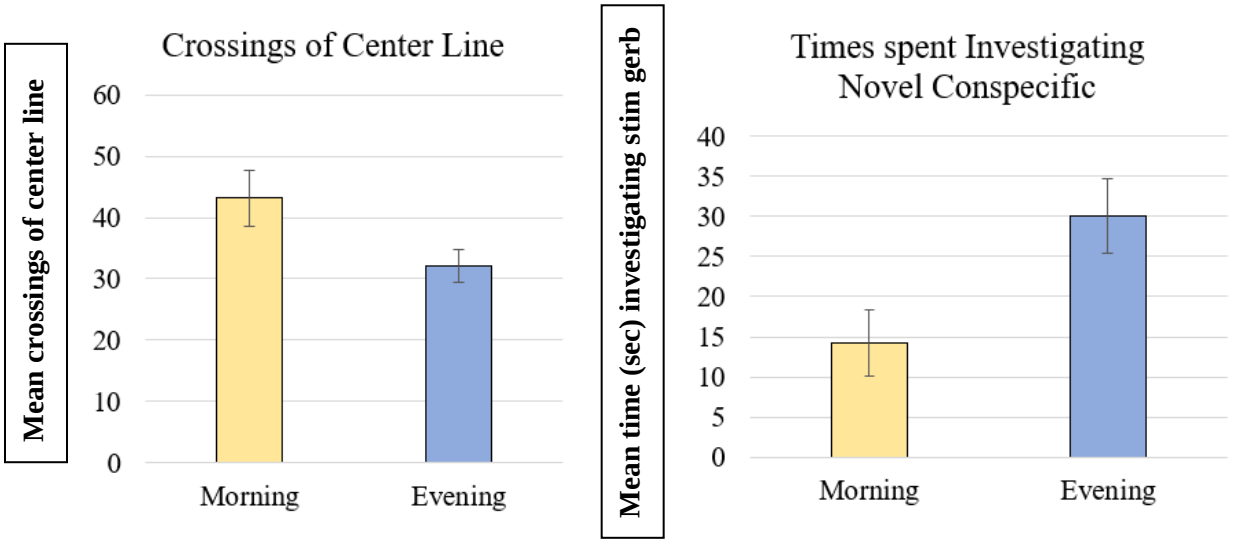

Figure 14: Mean of total number of crossings of the center line (left) and overall time spent interacting with a novel conspecific (right) in the social approach test. Error bars are standard error of the mean (SEM).

Elevated plus maze

Results from a one-way ANOVA revealed no significant differences in the percentages of time spent in the open, closed, or center of the maze across lighting conditions either.

Results of t-tests revealed no significant differences in control lighting and experimental lighting conditions in the percentage of time spent in the open versus closed arms of the maze, nor differences in the time spent in the extreme portions of the open or closed arms.

Results of t-tests revealed no significant differences between morning- and evening-light conditions in the percentage of time spent in open versus closed arms of the maze, nor differences in time spent in the extreme portions of the open or closed arms.

Modified open-field test

Results of a one-way ANOVAs revealed no significant differences in time spent in the center, middle, or outer portions of the open-field arena, instances of grooming, or ambulation, across the three lighting conditions. A one-way ANOVA revealed a significant difference across our lighting conditions for instances of rearing, $F(2, 26) = 4.881$, $p < 0.05$ and contrasts revealed that control animals reared more than evening light animals, $t(26) = 3.315$, $p < 0.05$ and marginally significantly more than morning light animals, $t(26) = 1.863$, $p = 0.074$. A one-way ANOVA revealed a marginally significant effect of jumping, $F(3, 33) = 2.830$, $p = 0.073$ and contrasts revealed that controls jumped significantly more than evening gerbils, $t(33) = 2.265$, $p < 0.05$.

To test whether the addition of higher walls impacted jumping behaviors, the first six animals were removed from the analysis. The omnibus ANOVA dropped below our threshold for

marginal significance, $F(2, 27) = 1.727$, $p = 0.197$, but the contrast between evening blue light gerbils and controls remained marginally significant, $t(27) = 1.653$, $p = 0.055$ (Figure 15).

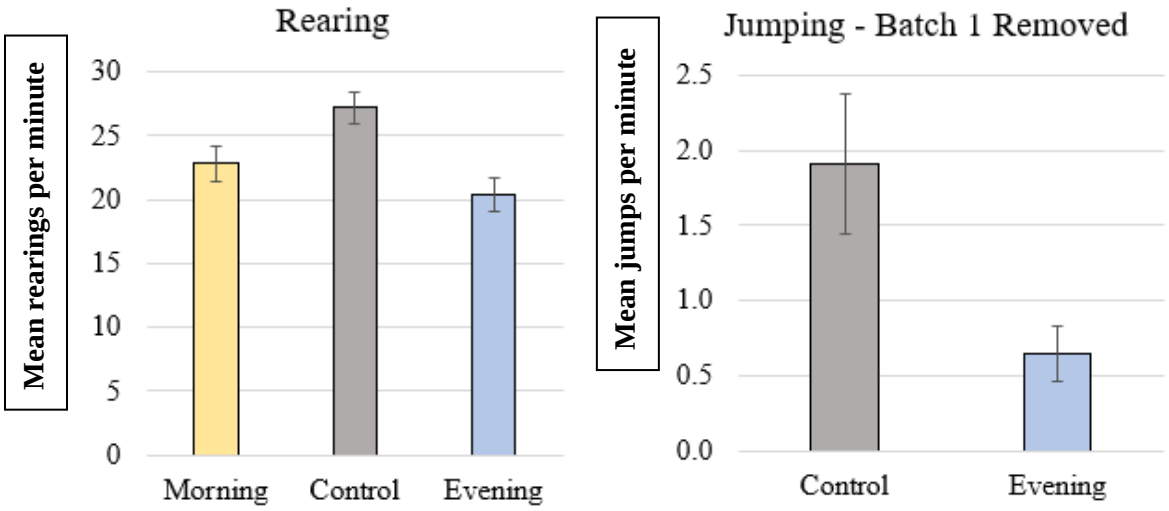

Figure 15: Rearing (left) and jumping (right) per minute across in open field test. Error bars are standard error of the mean (SEM).

T-tests revealed that gerbils in experimental lighting spent more time grooming than control gerbils, $t(33) = 1.783$, $p < 0.05$ (Figure 16). T-tests revealed no significant differences in time spent in the center, middle, or outer portions of the open-field arena, jumping, ambulation, or instances of rearing between gerbils in experimental lighting and control gerbils.

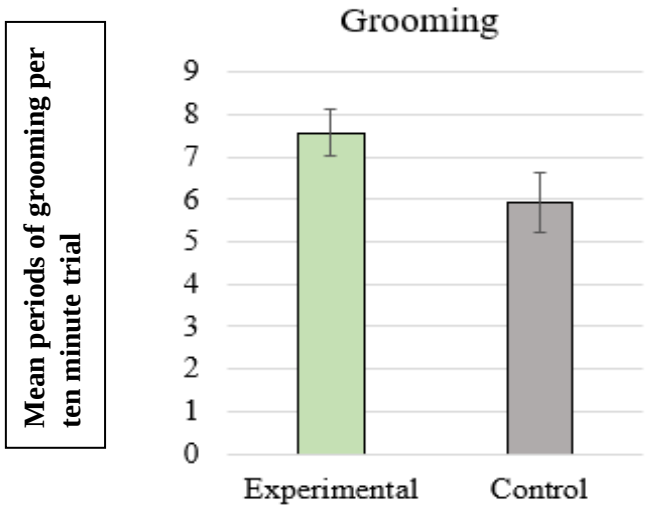

Figure 16: Mean instances of grooming in the ten minutes trial period between experimental lighting conditions combined versus control lighting. Error bars are standard error or the mean (SEM).

Results of t-tests between morning and evening lighting revealed no significant differences in time spent in the center, middle, or outer portions of the open-field arena, instances of grooming, jumping, ambulation, or instances of rearing across the two lighting conditions.

Modified intruder test

Results of one-way ANOVAs revealed no significant differences in biting, chasing, groin sniffing, intruder "takeover" of resident nest, jumping, latency to aggression, latency to approach, mutual grooming, note touching, rearing, self-grooming, sidling, time spent grappling, or time on home side of the arena. A one-way ANOVA did reveal an overall different in number of instances of grappling, $F(2, 33) = 4.191$, $p < 0.05$, and post-hoc comparisons revealed that rodents reared in the evening-light were involved in significantly more instances of grapplings relative to morning- and control-light, $t(33) = 2.389$, $p < 0.05$ and $t(33) = 2.611$, $p < 0.05$, respectively (Figure 17).

A one-way ANOVA also revealed a marginally significant difference in instances of locked fighting, $F(2, 35) = 3.003$, $p = 0.063$, such that animals reared in the evening-light were involved in more instances of locked fighting relative to animals reared in control-light, $t(33) = 2.334$, $p < 0.05$, and animals reared in the morning-light were involved in marginally more likely to be involved in instances of locked fighting relative to animals reared in control-light, $t(33) = 1.815$, $p = 0.079$ (Figure 17).

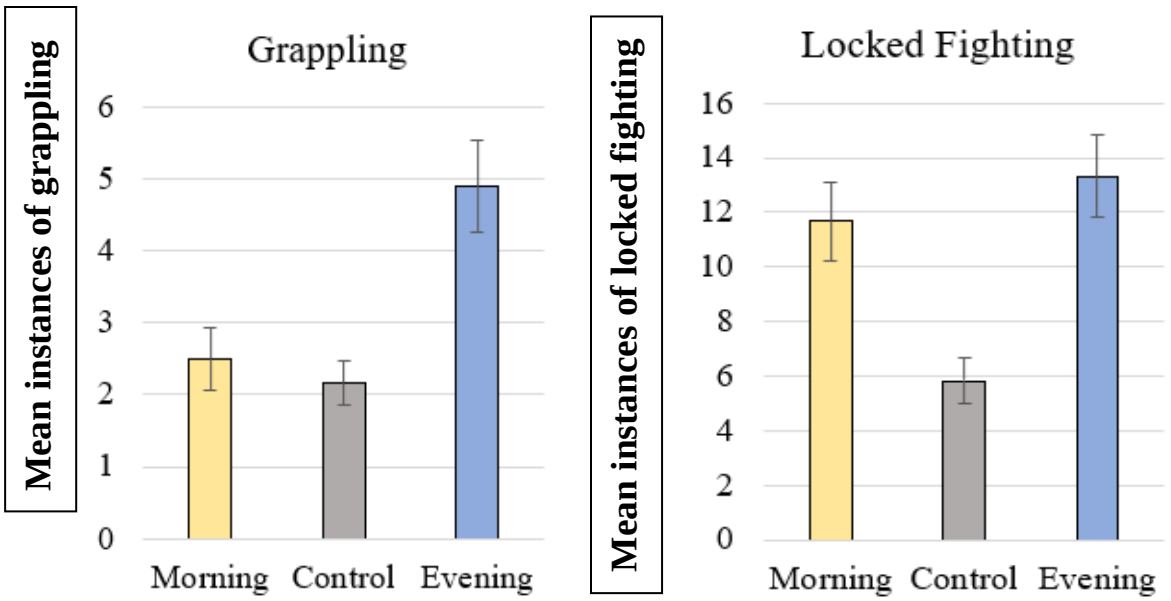

Figure 17: Mean instances of grappling (left) and locked fighting (right) during the ten minute trial period. Animals reared in the evening light demonstrated more instances of aggressive grappling while animals raised in both morning and evening light demonstrated more instances of locked fighting. Error bars are standard error of the mean (SEM).

T-tests revealed a marginally significant difference between the control group and the experimental lighting conditions in latency to approach (experimental gerbils were faster to approach the intruder animal; $t(34) = 1.722$, $p = 0.09$) and locked fighting (experimental gerbils spent more time in locked fights; $t(34) = 2.421$, $p < 0.05$) (Figure 18).

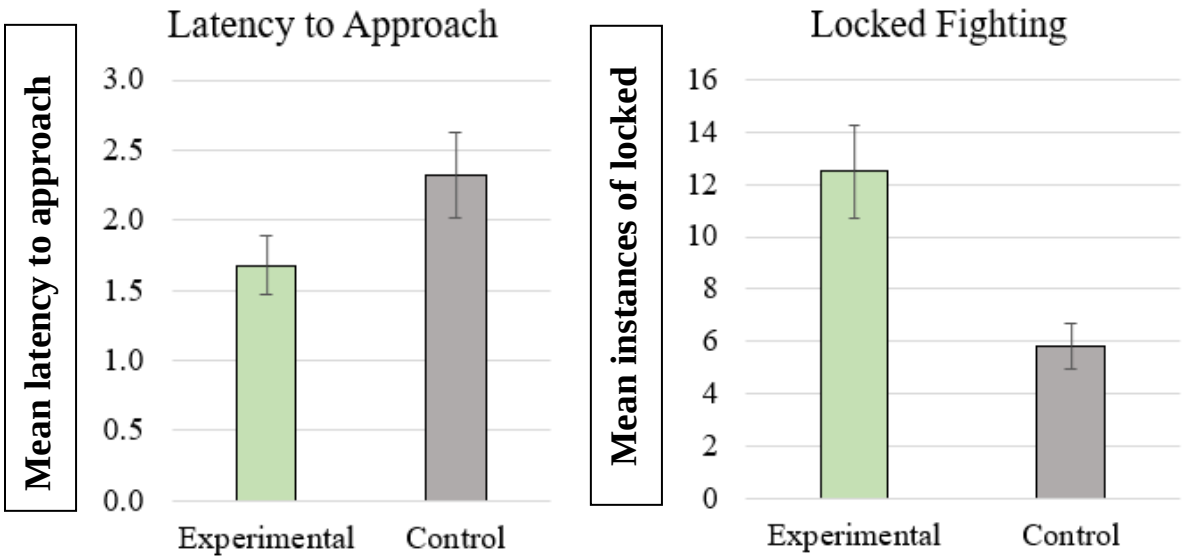

Figure 18: Mean differences for latency to approach (left) and locked fighting (right). Error bars are standard error of the mean (SEM).

T-tests revealed significant differences between the morning light condition and the evening light condition and grapplings such that animals reared in the evening light were involved in more grappling behavior, $t(22) = 2.038$, $p < 0.05$ (Figure 17).

Modified forced swim test

Results of one-way ANOVAs revealed no significant differences in time to first immobility, time spent climbing, time spent immobile, time spent spinning, or time spent swimming. A one-way ANOVA revealed a significant difference in bouts of immobility, $F(2, 27) = 3.613$, $p < 0.05$, and t-tests revealed that animals reared in control-lighting demonstrated greater bouts of immobility relative to animals reared in evening-light, $t(27) = 2.583$, $p < 0.05$. T-tests revealed a significant difference between morning and evening gerbils in bouts of immobility such that morning gerbils demonstrated more bouts of immobility, $t(18) = 2.371$, $p < 0.05$ (Figure 19).

T-tests revealed that gerbils raised in control lighting spent significantly more time climbing (e.g., attempting to escape the arena by scratching upwards on the walls of the tub) than morning and evening light gerbils combined, $t(27) = 1.871$, $p < 0.05$ (Figure 19).

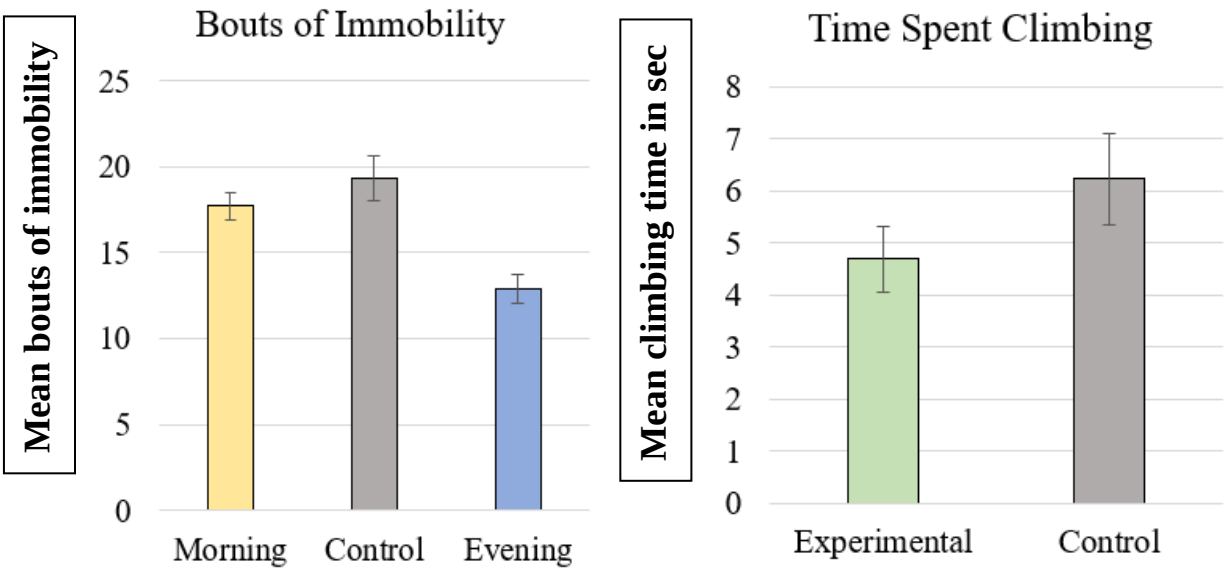

Figure 19: Mean bouts of immobility across lighting conditions (left) and time spent climbing in seconds between experimental groups combined versus control animals(right). Error bars are standard error of the mean (SEM).

T-tests revealed that there was no difference in the latency to the first bout of immobility across our lighting conditions.

Discussion, limitations, and future directions

Overall, our data suggest a subtle, but important suite of behavioral modifications associated with exposure to blue light into the evening and/or the morning. We predicted that a depressive phenotype would be observed here. This hypothesis was not fully supported. Behavioral modifications here suggest subtle shifting of aggressive and social behaviors, as well as some possible correlates of and anxiety. A possible overall profile of a general shift in behaviors relevant to energy expenditure is also described here as, generally, experimental lighting gerbils (e.g., either morning or evening light) demonstrated a pattern of behavior of conservation of energy in novel environments.

Aggressive and social behaviors

Here we found evidence of increased aggressive behaviors in the intruder test. Animals in the evening blue lighting condition demonstrated significantly more bouts of grappling relative to animals in the morning or control conditions. Likewise, locked fighting also showed a marginally-significant effect such that there were more instances of locked fighting in animals reared in evening and morning blue light relative to controls. Together, the current data suggest that exposure to atypically timed blue light, and particularly evening blue light, during adolescence may prime the animals to act more aggressively to an unknown conspecific. These effects may be associated with activation of the amygdala via retinofugal pathways.

Our data also suggest a slight impact of lighting on social behaviors. A t-test revealed a marginally-significant increase in social investigation in the social approach task for evening gerbils relative to morning gerbils. It seems counterintuitive that the same experimental manipulation (i.e., blue light at night or after sunset) could instigate both aggressive and social behaviors. The nature of the tests performed here may explain why both types of behaviors were elicited. The intruder test allows for free interaction with the intruder in an open arena, while the social approach test does not. Thus, the constraints of the test may dictate how these behaviors manifest. Given a social environment void of potential aggression, animals raised in the evening blue light condition may default to a more social or investigatory pattern of behaviors rather than aggressive behaviors. Indeed, it is possible that these effects may also be associated with increased amygdala activity as the amygdala plays an important role in social behaviors (Adolphs, 2010).

It should be noted that not all behaviors relevant to aggressive or social behaviors were associated with exposure to blue light at night or in the morning. This may highlight the subtlety

through which our more ecologically valid manipulations of blue light might impact behavior. Indeed, many if not most people are exposed to blue light well into the evening for years throughout their lifespan, and yet we do not observe widespread clinical cases of increased aggression or atypical social behaviors. What may occur is subtle shifting of these behaviors following long-term exposure but not to the disordered level. These subtle shifts may not be captured in psychological diagnostics but may impact individuals on a day-to-day basis in more understated ways. When compounded with other life stressors and/or genetic predispositions, aberrant lighting may push one closer to the manifestation of a mood disorder.

It should be noted that housing conditions, and exposure to unrelated conspecifics, were dependent on assignment to experimental lighting. Our experiment required that two cubicles be dedicated to our morning and evening lighting conditions (one cubicle per condition) while a third was dedicated to control animals as well as housing for the stimulus gerbils and to acclimate new gerbils to the lab space. Thus, gerbils assigned to the control condition were exposed to other, unrelated gerbils throughout the experiment while gerbils assigned to morning and evening conditions were only exposed to this environment during acclimation. After acclimation, evening and morning gerbils would have been exposed to two additional gerbil cages, at most. It is possible that a lack of exposure to unknown conspecifics confounded aggressive and social behaviors observed in experimental animals in the current work.

Depressive-like behaviors

Our initial hypothesis, that blue light at night (or after sunset) would result in depressive-like behaviors relative to controls and morning blue light, was not fully supported by these data. This may be due to several factors. First, the amygdala is not a unitary structure and is made up of multiple sub-nuclei. These sub-nuclei are involved in similar, but unique psychological and

affective phenomena. As mentioned above, it is generally accepted that in one manifestation of depression, the amygdala is hyperactive, preventing or overriding medial-frontal regions of the brain from dampening down negative thought patterns. However, this pattern of activity is more often associated with reduced dopamine (DA) in the basolateral amygdala (BLA). DA regulation is complex, and can be regulated by the infralimbic structures via BLA projections to the VTA. This may in turn lead to less DA release in the BLA via bidirectional connectivity with the VTA, and thus depressive symptoms such as anhedonia (Belujon & Grace, 2017), not activation in the medial amygdala (MeA). Critically, the primary retinofugal ipRGC projections to the amygdala, which we attempted to primarily target here, are to the medial amygdala (MeA). The BLA and the MeA are connected sub-structures of the amygdala, but given the extant literature, this connectivity seems to be unidirectional with the BLA innervating the MeA (Carr 2015). Thus, long-term increased activation of the MeA during adolescence may not necessarily lead to long-term increased activation of the BLA and may not lead to increased depressive-like behaviors one would expect from a hyperactive BLA.

A second factor relevant to null effects related to depressive-like symptoms may be the intensity of the light stimuli used in our experiment. This is discussed in more detail in limitations, but most experiments investigating the impact of lighting on some cognitive or affective outcome uses either much more intense lighting or much longer exposure (e.g., 24-hour light exposure). We chose not to exposure our animals to more intense lighting to increase ecological validity of our experiment.

Significant effects of the forced swim test also suggest subtle, if counterintuitive, anxiety- or depressive-like effects. We expected that gerbils reared in evening blue light would demonstrate greater learned helplessness-like behaviors, such as increased time spent immobile

or overall bouts of immobility due to ipRGC projections to the lateral habenula. This was not supported by our data. Control gerbils showed more instances of immobility relative to evening blue light gerbils. This finding may have been due to the apparatus used for our forced swim paradigm. It has been reported that the size of the tank selected for forced swim tests may itself have an impact on an animal's performance in the test (Wallace-Boone et al., 2008). These authors found that animals in smaller forced swim arenas were less likely to complete the entire trial (six minutes in the tank, the same time as the current study). Here, we chose a bucket that was larger than the arenas indicated by Wallace-Boone et al. (2008) to be too small for the animals to successfully complete the trial (30 cm in diameter or smaller). However, our arena did not match the size of the tank recommended by the authors (50 cm in diameter) as ours was a roughly 33 cm by 38 cm. While all animals were subjected to the same forced swim tank, and thus any impacts of a smaller tank should average across all conditions, it may be the case that the smaller tank used here had some effect on the outcome of our results.

Another possible explanation for our forced swim test effects is, as some argue, that data from the forced-swim test are often misinterpreted or misunderstood. Indeed, Molendijk and Kloet (2015) suggest that immobility may in fact be adaptive in the forced swim test. Once the animal has exhausted all available exit routes via swimming, a state of immobility reduces energy consumption and allows the animal to float, increasing survival likelihood. The lack of an effect of increased bouts of immobility in the evening blue light gerbils here may reflect an increased anxiety or "panicked" state in the animal, not a lack of learned helplessness. However, control gerbils spent more time climbing the walls of the FST tub relative to morning or evening light animals combined, which may suggest learned helplessness type behaviors in the experimental lighting animals. Overall, these data may highlight the complexity of the forced

swim test; further study is necessary to fully understand how forced swim test data should be interpreted.

Gerbils reared in the evening blue light also jumped and reared less than control animals in the modified open field test. Observationally, many gerbils used jumping as a way to escape the open field arena (one animal successfully escaped the arena before the plexiglass extension was added) and rearing as a means to estimate the height of the arena before jumping. It is possible that a reduction of jumping and rearing is another indicator of learned helplessness. However, the same argument that increased bouts of immobility equated to adaptive behaviors in the forced swim test could apply to jumping in the open field arena, although our results for the open field test were in the opposite direction as the forced swim (i.e., there were fewer "attempts to escape," or increased learned helplessness, in the evening blue light gerbils, which could map on to fewer bouts of immobility in the control gerbils observed in the forced swim test). Overall, our data on jumping is difficult to interpret, and jumping behaviors are often not included in overall analyses of depressive-like behaviors.

Potential HPA involvement – energy consumption

While several of our findings presented here point to an aggressive or social phenotype following exposure to our lighting manipulations, the overall pattern of effects does not fit neatly into this narrative, nor into our predicted depressive phenotype. We argue that blue saturated hours of the day-night cycle represent hours of increased risk and that many species evolved to detect light cues associated with twilight as risk signals; however, these hours of the day-night cycle may also represent a period of increased opportunity. Thus, individuals who act in an energetically efficient way, either to conserve energy and/or be prepared to use energy stores if necessary, may be more likely to benefit from these periods. It is possible that our experimental

gerbils developed long-term coping strategies from exposure to twilight-like conditions (or novel stimuli in general) that our control gerbils were not afforded.

The hypothalamus receives direction ipRGC innervations to the SCN as well as to the lateral (involved in motivation related to feeding behaviors; Petrovich 2018) and ventromedial hypothalamus (associated with many functions, including feeding and aggressive behaviors as well as circadian energy expenditure; Nisbett, 1972; Orozco-Solis et al., 2016; Olivier, 1977; Hattar et al., 2006). The amygdala and the hypothalamus are also interconnected and appear to be involved in the regulation of aggressive behaviors (Gouveia et al., 2019). The hypothalamus in general works to regulate homeostasis via the hypothalamus-pituitary-adrenal (HPA) axis, which in turn regulates hormone release and the usage of energy in response to threats. Particularly, cortisol (a hormone that works to divert glucose stores in the presence of a threat, amongst many other functions) release is regulated by the HPA axis (Gary and Bjorklund, 2014). It is possible that animals reared in our experimental lighting conditions have been primed to react to subtly stressful environments following long-term exposure to atypically timed blue light in such a way that they do not waste energy on behaviors that are not beneficial (e.g., swimming, jumping) and instead shift energy sources to beneficial behaviors such as grooming.

With regards to our data, we found that animals reared in evening blue light demonstrated fewer bouts of immobility and reduced climbing relative to controls in the forced swim test, reduced jumping relative to controls in the open-field test, and both evening and morning gerbils showed reduced rearing in the open-field test. If one is primed to use available endogenous resources as efficiently as possible, once the possibility of escape from either of these tests has been exhausted, they may switch to other types of behaviors that are in some other way productive. Indeed, in the open-field test, experimental light gerbils (both morning and evening

light) demonstrated more grooming behaviors, which may suggest that they were either more comfortable in this environment or had switched their behavioral profile towards a more useful one.

This shift in behavioral approach may also have manifested in both the intruder test and the social approach test. When afforded the opportunity to investigate a conspecific in the social approach test, evening light gerbils investigated the stimulus gerbil more than morning light gerbils. Given that gerbils are an inherently social species, it may be adaptive for them to orient and approach a conspecific when there is no risk to do so. In the intruder test, evening gerbils grappled more than controls, and both evening and morning gerbils were involved in more instances of locked fighting. Together, these behaviors may have represented a switch to prioritize territorial behaviors when a conspecific was allowed to freely interact with the resident gerbil's home nestlet versus when the conspecific was not in a familiar setting and was not able to directly interact with the resident gerbil. Neither cortisol nor its metabolites were measured here, thus it is not possible to know whether differences in HPA activity played a role. Future work may explore this as a potential contributor to observed behaviors.

Possible impacts of sleep on observed behavioral effects

Blue light has clear and strong impacts on sleep onset through ipRGC pathways (Dumont & Beaulieu, 2007). In the current study, we did not directly measure sleep, nor how our lighting impacted sleeping behaviors or circadian timing in our experimental animals. Sleep disturbances and deprivation have well-established impacts on many psychological phenomena, including emotion, mood, social, and aggressive behaviors relevant here (Touitou, Touitou, & Reinberg, 2016). In addition, atypical sleeping patterns almost always coexist with mood and emotion disorders (Benca et al., 1997); even further, shifts in ambient light from the sun across seasons,

which can impact sleep timing and circadian rhythms, can lead to the onset of seasonal affective disorder (SAD; Dumont & Beaulieu, 2007). There is little doubt that our lighting conditions, and specifically blue light in the evening, impacted overall sleep.

When considering regions of the brain impacted by sleep, it becomes clear that sleep disturbances may have played a role in the observed effects reported here. Sleep deprivation particularly impacts frontal regions of the brain, reducing their inhibitory control over limbic structures. In turn, this impact on frontal regions can lead to depressive or anxiety-like behaviors that mimics what one might expect from an overactive amygdala (Feng, Becker, Zheng, & Feng, 2018). For example, in an fMRI paradigm, participants who were sleep deprived demonstrated significantly more amygdala activity in response to "emotionally evocative" images compared to well rested controls. Critically, the authors argued that a reduction in functional connectivity between the PFC and amygdala in sleep deprived individuals may have played a critical role in the observed effects (Killgore 2010). It is possible that any behavioral effects observed here, whether they be social or emotional, are, in part or whole, due to changes in gerbil sleeping patterns.

It is important to note that our light conditions would not have impacted sleep in the same way. On the one hand, blue light in the morning has been shown to have an anti-depressive effect in some individuals and likely works to set circadian rhythms with some involvement from limbic structures (Li & Li, 2018). Thus, morning light may not impact sleep *per se*, but may shift the animals' overall circadian rhythms earlier. On the other hand, blue light at night would likely not only push circadian rhythms further into the evening, but would also negatively impact sleep onset once the dark cycle began. Blue light has specifically been shown to have an attentional and awareness boosting effect, which would negatively impact sleep onset outside of circadian

effects on sleep (Bedrosian & Nelson, 2017). Thus, any effects of blue light exposure at night may be secondary to its impact on sleep onset and/or quality.

Limitations and future directions

The current study was limited in a number of ways. First, the lighting conditions that animals were exposed to were of fairly low intensity. Often, experiments that expose non-human animals to atypical light either use very intense lighting conditions (i.e., very bright), or expose animals to extremely long light-dark cycles (e.g., 24-hours of light). We chose not to expose animals to more extreme lighting conditions here as these lighting conditions do not mimic lighting observed in by people daily. Light rigs for this protocol were of about 300 lx at the level where the gerbils lived and was only presented for two hours before or after light onset/offset, respectively. While it is impossible to mimic every permutation of lighting that people may expose themselves to, our lighting conditions may mimic (to a degree) the light that one may expose themselves to while preparing for morning exercise (morning blue light) or watching streaming television into the evening (evening blue light) more closely than very intense lights or light across the entire night.

Second, we were restricted in the ages under which we could obtain and experimentally manipulate weanling gerbils. Animals arrived at lab between 28 and 32 days. Younger animals may have allowed us to manipulate other relevant developmental stages. For example, peak brain growth, synaptogenesis, and pruning all occur at PND ~10 in rats (Zeiss 2021) and myelination begins at roughly PND 10 and ends PND 90 with peak myelination around PND 20 to 30 (Zeiss 2021). It would not have been possible to obtain younger gerbils without the development of our own lab gerbil colony.

Third, both of our experimental lighting conditions exposed animals to an extended photoperiod which confounds conclusions about blue light at specific times of the 24-hour cycle. As mentioned above, the study of the impact of long- versus short-photoperiod is extensive and, in general, suggests that longer photoperiods have many positive impacts on psychological states and traits relative to shorter ones. Further work may control for day length while manipulating blue light exposure. For example, researchers may reconstruct the 14:10 LD cycle used in our experimental conditions here while extending the control LD cycle to match the 14:10 LD cycle but with no blue light. This type of study may allow for conclusions about late-night, blue light exposure while controlling for day length across experimental conditions.

Fourth, exposure to unknown conspecifics was not controlled for across all our lighting conditions. Control animals were placed in a cubicle with rotating batches of new animals as new animals acclimated to our housing space. Stimulus gerbils were housed in this cubicle for the entirety of their time in the experiment as well. Experimental lighting gerbils were removed from the holding cubicle after the 10-day acclimation period and were placed in cubicles with no more than two other gerbil cages at any given time. While all attempts were made to reduce the impact of a high volume of animal turnover in the control cubicle (e.g., placing stimulus gerbils on the bottom rack and moving control gerbils to the top rack after the acclimation stage, placing acclimating gerbils on the second to bottom rack), it was not possible to fully remove auditory, olfactory, and potentially visual cues. Thus, it is possible that any effects in social tests were due to exposure to other gerbils throughout the experiment in the control animals. Indeed, control animals demonstrated fewer aggressive behaviors and social approach behaviors in general in the intruder and social approach tests, respectively. Future work should eliminate this confound altogether by having a dedicated holding cubicle for acclimating animals and stimulus gerbils.

Future work may try to elucidate any potential HPA involvement in the observed behavioral effects. The HPA axis has wide ranging impacts on both the brain and behavior via hormone release. Given that both the lateral and ventromedial hypothalamus receive direct ipRGC innervations, are involved in eating behaviors, and may have been associated with aggressive and energy consumption strategies observed here, more closely following the eating behaviors and/or weight of the gerbils may provide further evidence of the hypothalamus's involvement. Taking serum hormone levels may also provide more definitive evidence of the HPA axis's involvement.

Conclusion – experiment 2

Evidence presented here suggests that long-term exposure to atypically timed blue light, and particularly blue light at night, subtly biases animals towards aggressive and social behaviors. Overall, depressive behaviors were not observed (e.g., anhedonia), while some subtle, but counterintuitive anxiety-like behaviors were. These results may implicate the amygdala or other mood regulating, light sensitive regions such as the habenula. The hypothalamus may also play heavily in our results as its involvement in behavior is vast and may help to explain some of the more perplexing findings here. Indeed, experimental gerbils may have been better equipped to respond to novel behavioral tests relative to control animals. Regardless of the visual pathway, inappropriate aggressive or social behaviors following exposure to blue light at night may have significant impacts on one's wellbeing. Even subtle acts of aggression may negatively impact one's ability to make or keep social connections. Considering how ubiquitous devices which emit blue light are in modern industrialized cities, these data provide critical evidence that exposure to them later into the evening during adolescence may have unintended consequences.

Pilot study: Organizational impacts of blue lighting on a structure critical for mood and emotion regulation over critical developmental periods

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Abstract

Blue light can have many effects on mood, emotion, and cognition. Previous work has shown that exposure to blue light at night during adolescence impacts behavior in a rodent model. The current pilot study investigated potential neuroanatomical changes associated with exposure to blue light during adolescence in a rodent species (the Mongolian gerbil, *Meriones unguiculatus*) in a paradigm that has previously been shown to elicit behavioral shifts that may be relevant to mood, emotion, or a stress response (i.e., HPA activity) (Moon et al., in prep). If atypically timed blue light (e.g., at night or in the morning) is presented consistently during an adolescence stage, we predicted that critical brain structures relevant to mood regulation will develop to resemble a depressive phenotype. We chose to focus on the basolateral amygdaloid complex (BLA), a structure known to be dysfunctional in depression in human studies, with accompanying alterations in dopamine function. Specifically, we asked whether changes in innervation of the BLA by catecholamine pathways were associated with exposure to atypically timed blue light during adolescence. Gerbils were randomly assigned to one of three lighting conditions upon arrival to the lab: morning blue light, evening blue light, or no blue light. Animals were raised under their assigned lighting condition from the equivalent of adolescence to the end of puberty (roughly two months of age). Animals were then euthanized and the brains processed using tyrosine hydroxylase immunohistochemistry to reveal patterns of innervation of catecholaminergic fibers in the BLA. Neither average fiber densities nor ratios of BLA to BLP fiber densities were dependent on our lighting conditions. Thus, our data do not support the prediction that long-term exposure to blue light at atypical times of the 24-hour cycle during adolescence alters dopaminergic innervation of the BLA. Considering that behavioral changes have been observed after similar light exposure, it will be important to consider which other brain structures, modulatory systems, or other features of the catecholaminergic modulation of the BLA contribute to these changes.

In a previous study, we asked whether blue saturated light presented at atypical times of the day-night cycle (i.e., before sunrise and after sunset) impacts mood related behaviors. This previous work has provided evidence that exposure to blue light at night can have subtle impacts on aggressive and social behaviors or alternatively a potentially broader suite of behavior relevant to efficient use of available energy stores in Mongolian gerbils (*Mongolian gerbils;* Moon, Li, & Rodman, *in prep*.). Here, we investigated a potential neural source, the amygdala, for observed behavioral impacts of blue light at night during adolescence. Drawing from additional previous literature (described below), we specifically investigated possible changes in catecholaminergic innervation of the basolateral amygdala which have been shown to be dysfunctional in depression.

The amygdala in depression

While not the only structure involved, the amygdala is an important node in a network of structures that are dysfunctional in mood disorders (Disner, Beevers, Haigh, & Beck, 2011). It has been hypothesized that, in healthy individuals, cortical regions exert executive control over limbic structures to reduce maladaptive thinking patterns associated with limbic overactivity, and that this pattern of activity is impaired in depressed individuals (Wang et al., 2008). A bidirectional relationship via the thalamus between the amygdala and the medial PFC (mPFC) is likely involved in reduced executive functioning and attentional control in patients with major depressive disorder (MDD). Importantly for the current study, dopamine (DA) is generally downregulated in depression, and it is thought that BLA activity and the downregulation of DA are inherently tied to one another (the BLA essentially inhibits VTA release of DA in some depression models; Belujon & Grace, 2017). Thus, chronic amygdala activation may lead to

reduced DA activity and dysregulation of the amygdala itself following a general reduction in overall DA release from the VTA (i.e., a positive feedback loop).

Neurotransmitter (NT) systems affected by depression

Neurotransmitter systems are also dysfunctional in mood disorders. Retinofugal pathways may, and likely do, impact multiple NT systems, including those involved in mood and emotion (Green et al., 2015). Dysfunction of monoamine systems have often been associated with psychological disorders, including depression. Specifically, and most commonly, deficits in the serotonin (5-HT) system have been associated with depression. Indeed, some of the most frequently prescribed medications to treat depression are those that specifically target the serotonin system. The retina does project to the 5-HT producing dorsal raphe nucleus in rats (Shen & Semba, 1994), and while their efficacy is somewhat questionable, rapid effects of SSRIs are thought to work by reducing amygdala overactivity (Murphy, Norbury, O'Sullivan, Cowen, & Harmer, 2009); thus, it is possible that the visual system could impact mood and emotion via projections to the DRN, and in turn, amygdala modulation. However, these medications can take weeks to take effect, and do not work in all patients; thus, 5-HT deficits cannot be the only way in which depression manifests (Belujon & Grace, 2017).

Dopamine (DA), another monoamine neurotransmitter, has also been associated with numerous psychological disorders including schizophrenia, attention deficit disorder, and depression (Gray & Bjorklund, 2014; del Campo, Chamberlain, Sahakian, & Robbins, 2011) to name a few. Primarily released by the ventral tegmental area (VTA) in the midbrain, DA producing neurons project widely throughout the brain and are often involved in reward seeking behaviors (Gray & Bjorklund, 2014). Relevant here, VTA cells project to and receive inputs from limbic structures, including the amygdala. Specifically, in depression, it is thought that DA

cells may be inhibited by cells in the BLA, reducing overall DA. In addition, the central amygdala (CeA) sends GABAergic projections to the VTA (Zhang, Zhang, Holmes, & Pan, 2021), increasing the amygdala's inhibitory control over the VTA. Indeed, reduced DA system activity has also been associated with depression (Diehl & Gershon, 1992). This reduction in DA release in reward structures may be associated with one of the hallmarks of depression, anhedonia (Belujon & Grace, 2017). It may be that, through retinofugal projections to the amygdala, and particularly to the BLA via the LA, atypically timed blue light amps-up BLA activity and dampens-down DA release through either conventional RGCs or a possible pathway from ipRGCs to the MeA and eventually the BLA (however, it is unclear if the MeA projects to the BLA; Figure 20). Importantly, the BLA has specifically been shown to be sensitive to early-life perturbations following hippocampal lesions (Barokas, Moon, Bachevalier, & Rodman, *in prep.*). Thus, the BLA is well set-up to be a particularly vulnerable structure to early-life stressors, such as atypical lighting.

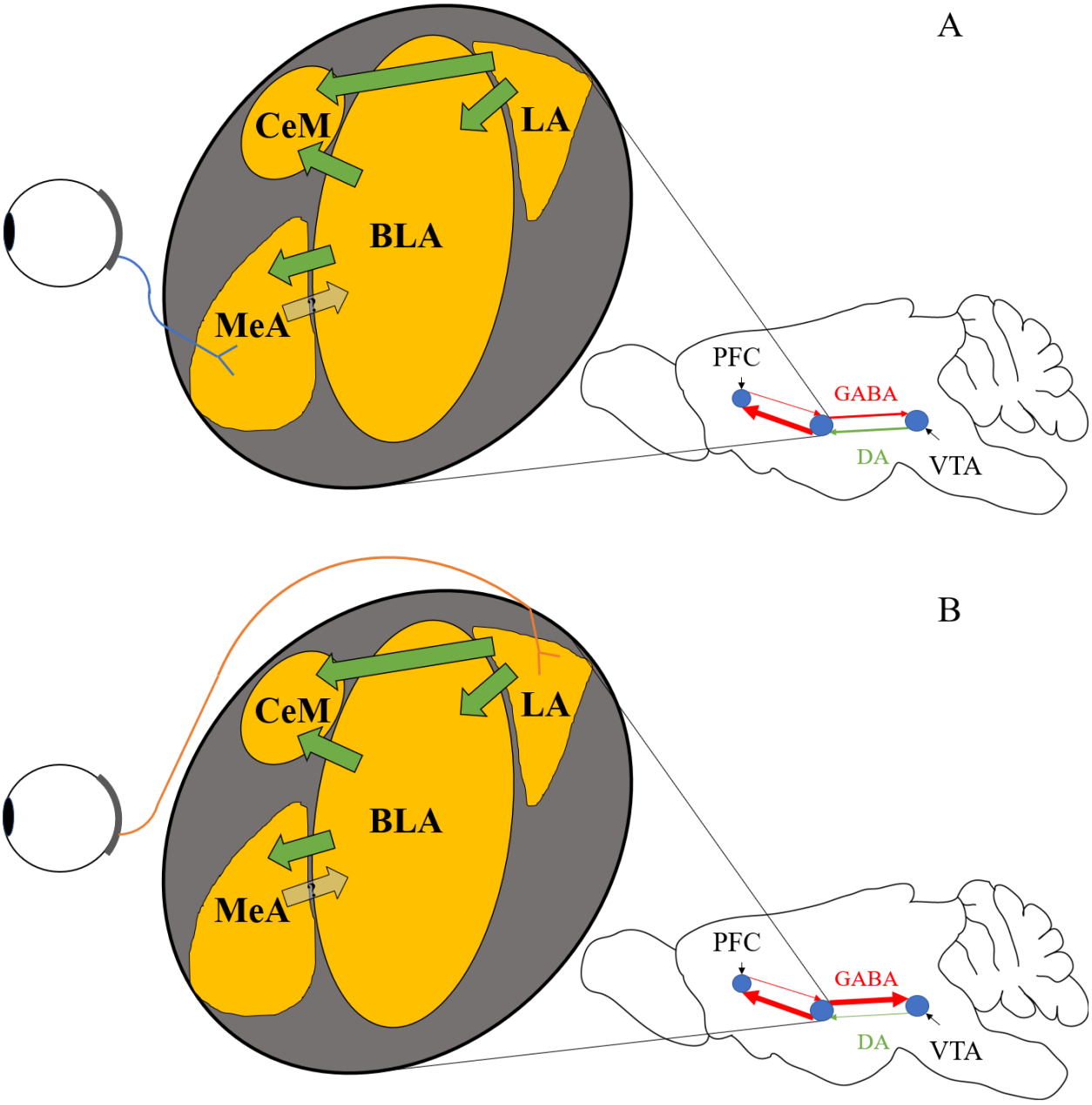

Figure 20: Simplified model of intra-amygdala circuitry and possible interconnectivity between sub-nuclei and other structures relevant to mood and emotion. Green arrows between sub-nuclei of the amygdala depict glutamatergic pathways. ipRGC projections of the MeA (A) suggest isolated MeA activation, while conventional RGC pathways (B) to the BLA suggest widespread activation of amygdala subnuclei. Potential impacts on PFC and VTA are also depicted.

For the above-mentioned reasons, the neuroanatomical structure of interest related to depression investigated here was the basolateral amygdaloid nucleus (BLA). More specifically, the anterior and posterior portions of the basolateral amygdala were targeted in the present work

(BLA and BLP, respectively; Figure 21). The BLA and BLP are generally involved in similar tasks, but the BLA has been more so associated with social deficits while the BLP has been associated with spatial memory via projections to the CA1 region of the hippocampus (Yang & Wang, 2017). Moreover, the BLA and BLP are differentially innervated by catecholamine pathways in rodents, with the BLP having a somewhat denser input (McDonald 2020).

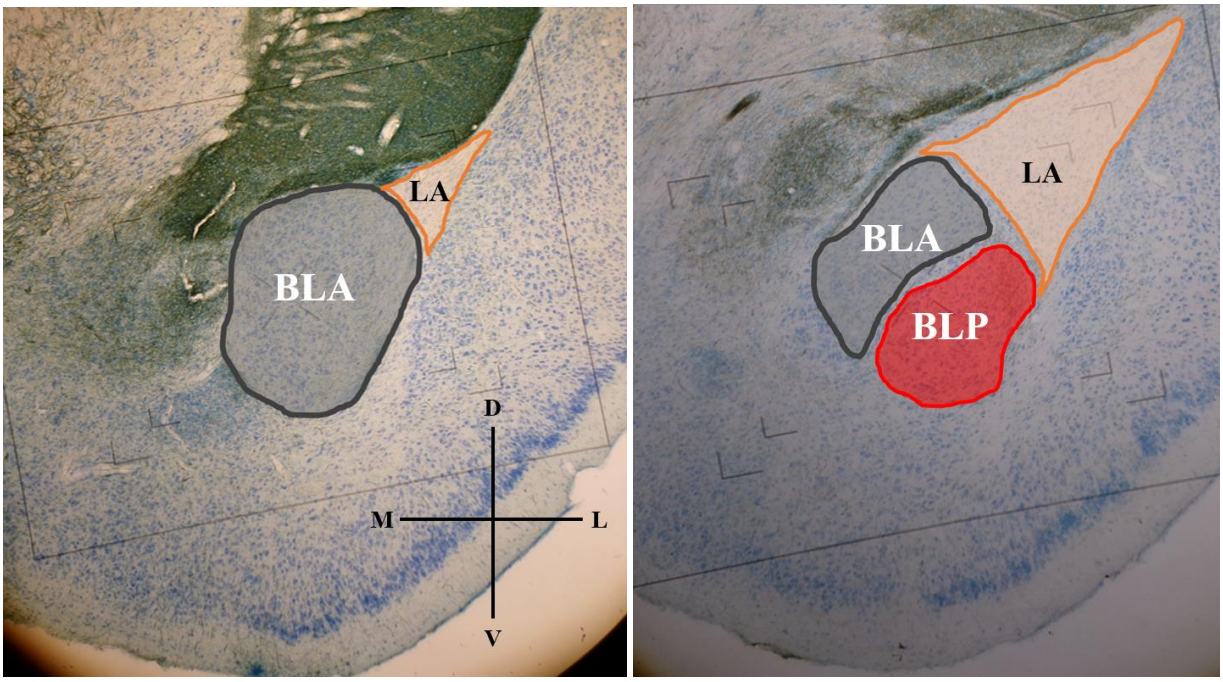

Figure 21: Images of coronal sections at 40x magnification taken of regions of interest stained with Giemsa and for TH fibers. Left, basolateral amygdala at roughly plate 26 in Radtke et al. (2016). Right, basolateral amygdala at roughly plate 29 in Radtke et al., (2016) divisions between the BLA anterior (gray) and posterior (red) regions are drawn here.

Species considerations in animal models of mood disorders and NTs

An important consideration within animal models of human conditions is the given species' timing of activity (i.e., nocturnal versus diurnal). This consideration may be especially important when investigating NT systems. Indeed, some work suggests that light can have different impacts on the release of dopamine (DA), a critical neurotransmitter in the regulation of mood and emotion, in nocturnal versus diurnal species. In a nocturnal species (mice), DA was

higher in the hypothalamus in animals exposed to long-photoperiods and was impacted only in the day-period in the striatum (greater DA release in response to longer days), while amygdalar DA was unaffected by long photoperiods. In contrast, DA in a diurnal species (chipmunks) was lower in the hypothalamus, higher in the striatum, and lower in the amygdala under long-photoperiods (Goda et al., 2015). It may be the case, thus, that an animal model of mood, emotion, or DA focused on a nocturnal species could result in misleading inferences about similar mechanisms in humans despite similar underlying circadian circuitry and visual system. For these reasons, we decided to use Mongolian gerbils (*Meriones unguiculatus*) for this work as it has shown a broad, but generally diurnal pattern of activity (Hurtado-Parrado et al., 2019), with, like humans, some significant individual variation in activity patterns (chronotype; Refinetti et al., 2016).

Current study and predictions

The current pilot study set out to determine how catecholamines, and particularly dopamine (it should be noted that our measure here cannot distinguish between dopamine and noradrenalin fibers), in the BLA are impacted by of atypically timed blue light at night relative to control or blue light in the morning in Mongolian gerbils (*Meriones unguiculatus*) across an adolescence period in a rodent species whose patterns of activity roughly match onto the human patterns of activity on the amygdala. This experiment also attempted to manipulate lighting in an ecologically valid way (i.e., lighting manipulations that attempt to parallel reasonable light exposure experienced in the modern world; it should be noted that it is not possible to simulate all possible permutations of late-night-light exposure. Here we attempt to manipulate light in a way that is not unrealistically intense or long in exposure time for what an average person may experience today). These data are important as it is not fully clear how retinofugal projections to

limbic structures might be impacted by aberrant lighting during important developmental periods. We predicted that the amygdalae of these animals will develop to look more like what is observed in human depression (i.e., reduced DA in the BLA) relative to controls and morning blue light animals in a pilot study. Specifically, we predicted that blue-enriched light at night (i.e., conditions that would strongly drive ipRGC projections at an atypical time of day) would lead to alterations in dopaminergic innervation of the BLA.

Methods

All experimental procedures were reviewed and approved by Emory University's Institutional Care and Use Committee (IACUC) before any animals took part in this study.

Animals

All rodents were housed in cages with water and food available *ad libitum* except for a brief food and water deprivation period during sucrose preference (described above). DAR provided cages are made of clear plastic to allow light to penetrate the walls of the cage. There is a semi-opaque filter paper placed over the top of the cage, allowing for more even distribution of light within the cage. Materials for burrowing were provided.

A total of 18 female gerbils, ordered in batches of six, were purchased from Charles River in the data presented here. Gerbils were placed in their home cages upon arrival within a holding room which exposes gerbils only to colony lighting. Following acclimation to the animal facility, animals were exposed to lighting manipulations. Experimental gerbils were housed in experimental lighting conditions until they reached roughly young adulthood at two months of age. Animals were pair-housed upon arrival until three and ½ weeks in experimental lighting. Pairs were randomly assigned to one of three experimental lighting conditions just before arrival. All experimental gerbils were roughly 28 to 32 days old upon arrival, were acclimated to the

colony for 10 days, and were placed in experimental lighting between PND 38-42. Euthanasia then occurred at roughly PND 84 after the animals were tested in a number of behavioral tasks to be reported elsewhere (Moon, Li, & Rodman, *in prep.*). These animals represented a subset (first of two cohorts) in that study.

Light manipulations

Briefly, animals were assigned to one of three light exposure paradigms: morning blue light (a two-hour light pulse at roughly 309 lx before light "onset" at 7:00 am), control lighting (colony lighting which consisted of broad spectrum light at roughly 146 lx between 7:00 am and 7:00 pm), or evening blue light (a two-hour light pulse at roughly 309 lx after light "offset" at 7:00 pm). Gerbils were randomly assigned to one of these three lighting manipulations before arriving at our lab. Gerbils were then housed in these lighting conditions from roughly 42 PND to roughly 77 PND. Additional details are provided in Moon, Li, and Rodman (*in prep.*, previous chapter).

Histology

Gerbils were deeply anesthetized with an overdose of sodium pentobarbital and perfused through the heart with heparinized saline followed by 4% paraformaldehyde solution in 0.1M phosphate buffer (PFA). Brains were then removed, postfixed overnight in PFA, transferred to brain storage buffer and kept in a -20^{o}C freezer until sectioning. Coronal sections of the brain were cut at 30 microns on a freezing microtome. Experimenters remained blind to animal group throughout the from the cutting process on to data analysis. Reference series of sections through each brain were stained with cresyl violet and the Gallyas myelin stain. Additional series of sections were set aside for staining with other markers for later studies. Sections for the current project were stained to show tyrosine hydroxylase (TH), an early step in the processing of

catecholamines that allows for the visualization of both dopamine (DA) and noradrenergic (NE). Prior to cutting, all the brains in the set were coded so that experimenters were blind to the specific group from which individual brains were taken.

To visualize TH fibers in the amygdala, 1:3 a series sections was immunostained as follows. After rinses in 0.1M phosphate buffer (PB), sections were incubated in primary antibody solution (mouse anti-TH, Sigma, 1:1000 dilution) for 14 to 18 hours in a refrigerator at roughly five degrees C. Sections were then incubated in secondary antibody solution (horse anti-mouse; biotinylated [BIgG]) for an hour at room temperature before incubation in ABC solution (avidin-biotin complex) for another hour. Lastly, sections were processed using diaminobenzidine (DAB). Additional PB rinses took place following each step. After the sections were mounted on glass slides, counterstaining to assist in visualizing structures and TH fibers was completed using Giemsa staining. To help control for any variability in reagents, timing, water quality, etc. across the project, immunohistochemistry was always performed on two brains from different experimental groups at a time.

To quantify TH fibers, three sections through the amygdala were used, spanning the extent of BLA and BLP in the structure, corresponding approximately to plates 26, 27/28, and 29 of the Radtke et al. (2016) gerbil brain atlas. Photomicrographs of the amygdala were taken at an overall magnification of 40X using a Nikon Optiphot microscope. A 6x6 grid of was then superimposed over each image in Powerpoint as a guide for systematic random sampling of TH fibers in each amygdala. To prevent possible order effects during counting, the order of sampling sites from each box of the grid for each amygdala was also randomized. Counting was performed by trained members of the Rodman lab. To ensure that there were no rater effects, fiber counts

across the three amygdala sections and across cohorts were even distributed across the research team as much as possible.

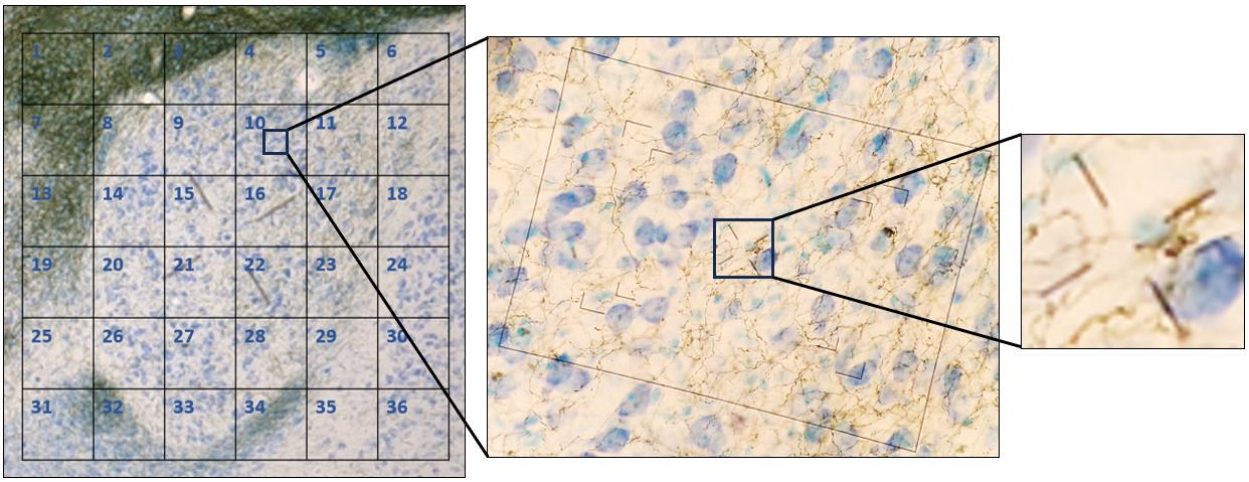

Figure 22: Counting process for TH fibers. The count order was randomized to prevent order effects. Counts were quantified as the number of times a TH fiber crossed the interior reticle on the right hand image. TH fibers can be seen as the brown colored lines around the blue cell bodies (40x magnification on left, 400x magnification on middle and right).

To perform TH fiber counts, the sections were first viewed using the 4X eyepiece while referring to the guide images with the superimposed grids. Once a sampling location had been from the grid was located in the view of the section, the center of that location was then viewed using the 40X objective (total magnification 400X; Figure 22). Fiber counts were operationalized as the number of visible TH fibers that crossed within a set of lines of a reticle within the microscope's left eyepiece that defined a virtual counting frame. For each measurement, fibers were counted only within a standard focal plane corresponding to the plane in which TH fibers within the counting frame first came in to focus as the fine focus knob on the microscope was slowly adjusted from a plane just below the tissue.

Results

Analyses were run using the statistics program SPSS. Data are presented with the standard error of the mean.

BLA

A Two-way, 2x3 (brain region by lighting condition) ANOVA revealed no overall differences in TH fibers in the BLA across our three lighting conditions (Figure 23). T-tests revealed no mean differences between evening and morning light conditions. T-tests also revealed no mean differences between the control lighting and both experimental lighting conditions combined.

BLP

A Two-way, 2x3 (brain region by lighting condition) ANOVA revealed no overall differences in TH fibers in the BLP across our three lighting conditions (Figure 23). T-tests revealed no mean differences between evening and morning light conditions. T-tests also revealed no mean differences between the control lighting and both experimental lighting conditions combined.

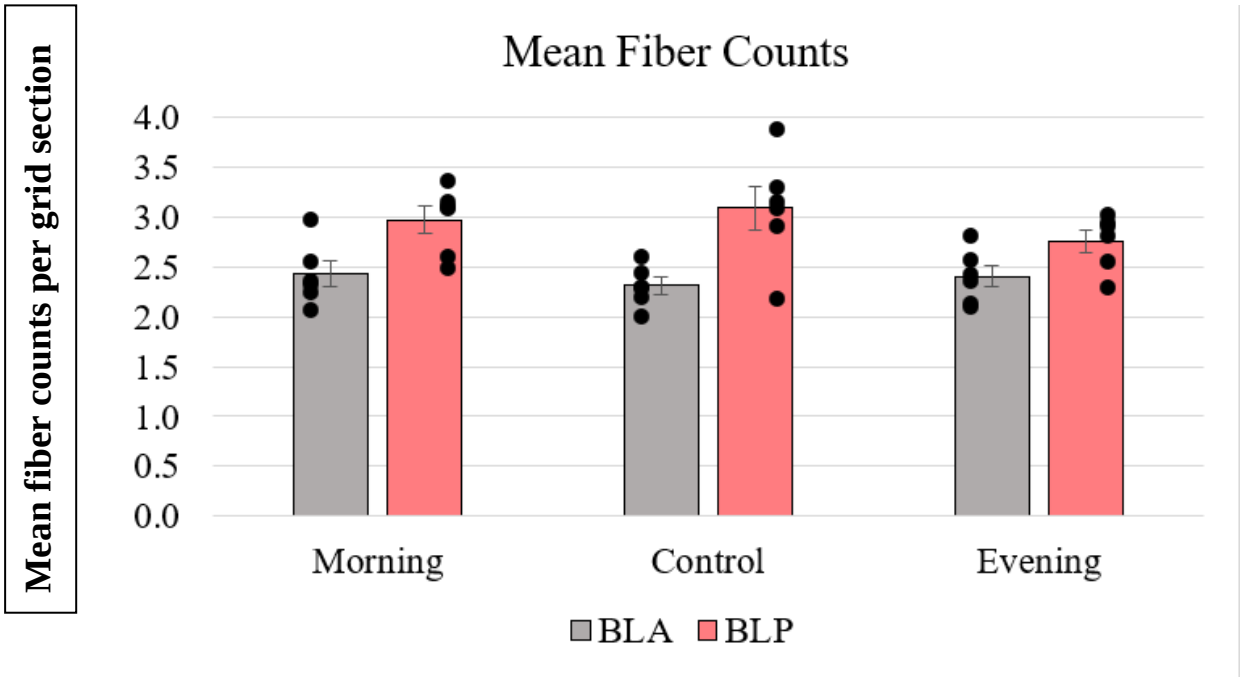

Figure 23: Mean differences in average number of fibers per grid box in the BLA (gray) and BLP (red) across our lighting conditions. Dots are individual means for each animal.

Ratio of average of BLA/BLP

In order to control for any possible differences between groups in overall shrinkage or other aspects of tissue quality that might have impacted TH fiber density, we also derived a ratio of TH-positive fibers in the nuclei of interest in each animal. This ratio would reveal a potential redistribution of TH fibers (and thus potentially DA modulation) between the BLA and BLP as a result of the experimental manipulations. In a prior study, we found such a redistribution of parvalbumin-immunoreactive fibers in monkey amygdala after early damage to the hippocampus (Barokas et al., *in prep.*). The ratio between the average number of BLA and BLP fibers counted per grid section was thus also calculated for each animal. A one-way ANOVA revealed no overall differences in the ratio of average TH fibers in the BLA and BLP across our three lighting conditions (Figure 24). T-tests revealed no mean differences between evening and morning light conditions, or between each of these conditions and the control. T-tests also revealed no mean differences between the control lighting and both experimental lighting conditions combined.

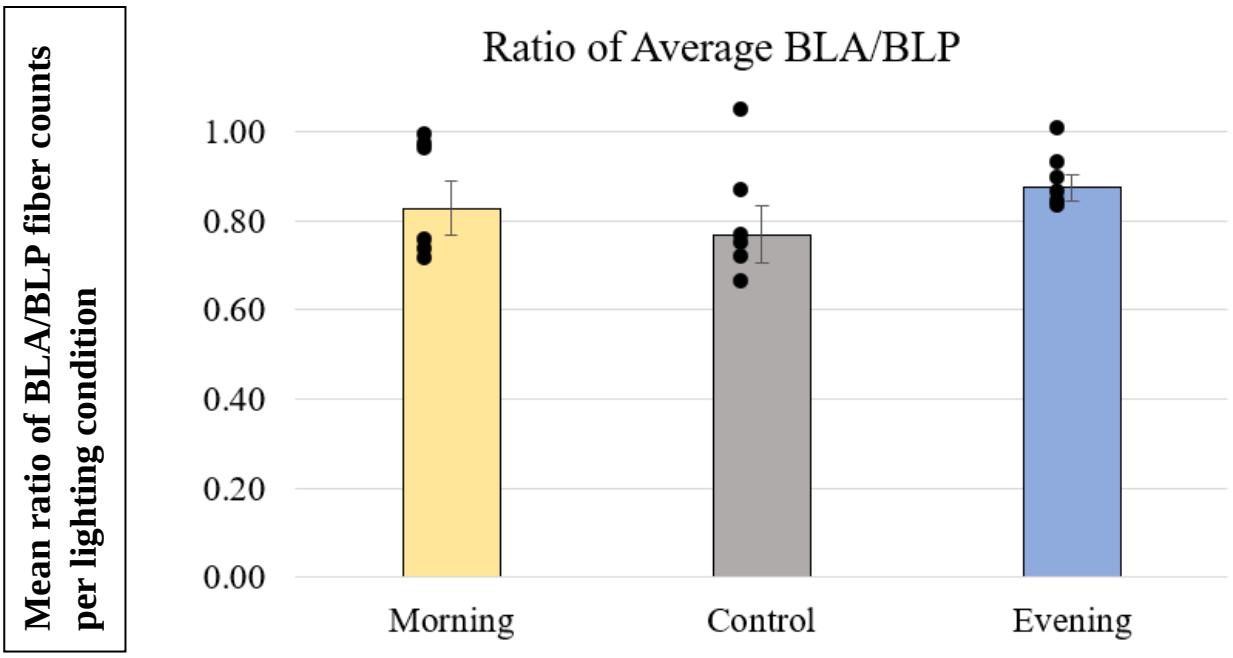

Figure 24: Ratios of mean differences in average number of fibers per grid box in the BLA and BLP across our lighting conditions. Dots are individual means for each animal.

Discussions, limitations, and future directions

Our data presented here do not suggest that atypically timed blue light has an impact on TH-positive fiber densities in the BLA and BLP in overall average fiber counts per animal, nor in the ratio of average fiber counts in the BLA to BLP. These data do not support our hypothesis that atypically timed blue light over adolescence, a critical developmental period, leads the BLA to develop in such a way that it resembles the BLA of depressed individuals (e.g., possibly reduced TH fibers in the BLA, which could, but does not necessarily equate to reduced DA). When considered in light of behavioral data from Moon et al. (*in prep.*) that suggest atypically timed blue light impacts aggressive and social behaviors more so than depressive behaviors, it may be that blue light impacts behavior outside of conventional RGC projections to the lateral amygdala and then the BLA. Indeed, the behavioral profile observed by Moon et al. (*in prep*) suggests that other sub-nuclei of the amygdala that are more involved in social and aggressive

behaviors, such as the medial amygdala (MeA; Petrulis 2020), or structures outside of the amygdala, may play a more central role in shifts in behavior associated with atypically timed blue light.

Our pilot study here was limited in a number of ways. First, given the use of TH staining as our marker for catecholamines, we were unable to differentiate between dopamine and noradrenaline fibers in the BLA. The locus coeruleus (LoC) produces NE and NE producing cells also appear to be sensitive to developmental perturbations (Saboory, Ghasemi, & Mehranfard, 2020). The decision to use TH staining rather than other, more targeted stains for DA (e.g., dopamine-beta hydroxylase or DA itself) was based on a number of considerations. The use of TH as a stain for catecholamines is well established in the literature and the vast majority of immunohistochemical studies of DA in the BLA have used TH staining (McDonald, 2023). Thus, interpretations of TH staining in the BLA are more intuitive as they have been more vetted. Additionally, shifts in TH are typically associated with shifts in DA and are often used as a proxy for DA itself (Pinard, Muller, Mascagni, & McDonald, 2008), thus making it likely that fiber density counts presented here are associated with true DA fibers.

Second, we focused our investigation on DA in the BLA. It is possible that many structures, including the MeA, may be involved in any behaviors affected by atypically timed blue light. Additionally, other neurocircuitry may be involved in the impacts of light on affective behavior. For example, parvalbumin (PV) circuits, which are primarily inhibitory, have been shown to be sensitive to early life perturbations in the BLA (Barokas et al., *in prep.*). PV interneurons in the BLA appear to be targets of DA neurons (Pinard et al., 2008); thus, targeting PV circuitry may elucidate more indirect, but important, DA sensitivity of BLA structures. Third, we were limited in overall power for the study based on previous work and expected

effect sizes. This may have prevented us from identifying any real effects within our data. Fourth, while DA is a critical neurotransmitter system known to be atypical in individuals with mood disorders and may be sensitive to aberrant lighting, other neurotransmitter systems may be as involved or even more involved. Serotonin (5-HT) is often linked to several aspects of depression, and the dorsal raphe nucleus (DRN) receives retinofugal inhibitory innervations likely via Y-like retinal ganglion cells (Pickard, So, & Pu, 2015). Another neurotransmitter system worth investigation that is involved in fear responses and projects to the BLA would be the noradrenaline system (NE; McCall et al., 2017). Other endogenous chemicals that are more consistently tied to social behaviors, such as vasopressin-oxytocin nonapeptides, which is released by the MeA in certain species (Kelly & Goodson, 2014), may be another important group to consider, as previous work by Moon et al. (*in prep.*) and the current work suggest that socially relevant structures and behaviors may be impacted by blue light at atypical times of the day. Thus, there are many structures and endogenous chemicals outside of dopamine and the BLA worth investigating that may help to explain our work.

Future work may look to address several of the limitations mentioned here. Primarily, a larger sample size may help to reveal real effects within our data. More targeted DA immunohistochemistry techniques (e.g., for dopamine-beta hydroxylase or DA receptors or transporters) may also allow for more definitive conclusions about the types of fiber densities observed in experiments Other NT systems mentioned above may also prove helpful in understanding how aberrant light impacts the brain and subsequent behavior.

Conclusion – pilot study

Based on limited sampling, the data here do not support our a priori hypothesis that exposure to atypically timed blue light across an adolescence period will lead the BLA to

develop in such a way that it resembles the amygdala in depressed individuals (i.e., reduced TH fibers). As work has demonstrated that similar lighting paradigms can lead to changes in behavior (Moon et al., *in prep.*), it is likely that there are changes in the brain that were not identified here. There are many structures that may be responsible for previously identified behavioral effects of aberrant blue light, and future work should attempt to determine which retinofugal pathways and brain structures are involved.

General Dissertation Discussion

Across two studies and a pilot project, my dissertation investigated two facets of my main research question, namely, how does light impact mood and emotion in the short-term (1), as well as across the lifespan (2)? First, in experiment 1, we provided evidence that a 20-minute, blue-saturated light exposure paradigm can subtly impact a selected measure of emotional states (i.e., facial expression processing) compared to exposure to a light that is not blue-saturated for 20-minutes. Given how social humans are and how critical it is that we correctly identify social cues, this subtle shifting may impact the way individuals interpret their social world. Indeed, social deficits have often been associated with mood disorders such as depression (Segrin 2000). Correctly interpreting the intentions of another human via their facial expression is immensely important. We suggest that these effects are related to activation of visual pathways to structures involved in emotion processing such as the amygdala via either ipRGCs or via conventional RGCs.

In experiment 2, we investigated whether atypically timed blue light during adolescence phase, a period critical for development, can impact long-lasting affective behaviors in a diurnal rodent species, the Mongolian gerbil (*Meriones unguiculatus*). Through repeated activation of the ipRGC system (and/or conventional RGCs) with blue light during atypical times of the day-night cycle, networks relevant to depression and/or anxiety (i.e., amygdala connectivity with ACC, insula, and an antagonistic interconnection with the mPFC, activation of the LHb, activation of the LoC, etc.) may develop to become slightly overactive as is seen in individuals with depression. We thus predicted that gerbils so exposed might manifest depressive-like behaviors. While not in line with our hypotheses, gerbils reared in evening blue light demonstrated increased aggressive and altered social behaviors and fewer instances of escape

behavior when escape was not possible. Increased aggressive behaviors or irregular social behaviors may put one at greater risk for mood disorders.

In a pilot study on the neuroanatomical impacts of atypically timed blue light, we found that tyrosine hydroxylase (TH) fibers in the BLA were not differentially dense across our lighting conditions. This may indicate that DA and/or NE fibers in the BLA are *not* the primary drivers responsible for our behavioral data in experiment 2. As behavior is driven by the activity in the brain, it is likely that some other region(s) (potentially the MeA or the hypothalamus) is(are) responsible for behaviors observed here. Further work should attempt to identify these structures.

Taken together, the data across the experiments highlight subtle but meaningful changes in social processing following exposure to blue light. These changes may, in turn, increase the risk of the development of some type of mood disorder. When considering the ever-increasing presence of artificial light across the entirety of the 24-hour, day-night cycle in urbanized (and even rural) settings, it is apparent that we must consider the impacts that these stimuli have on the psychological condition outside of providing enough light to see. In fact, previous work from our lab has demonstrated that individuals who were tested in the evening interpret the intensity of facial expressions slightly differently relative to participants tested in the morning (Recht, Moon, & Rodman, *in prep*), suggesting that there is something about the evening hours or evening light that is critical for social processing.

An alternative explanation for our behavioral data in experiment 2 that implicates the hypothalamus is intriguing. The behavioral evidence from experiment 2 suggests increased aggressive and social drive but *reduced* effort in circumstances where effort cannot alter the animal's outcome (e.g., unable to escape) following exposure to atypically timed blue light. It

seems unlikely that the BLA or the MeA alone are primary drivers of these observed behaviors as this suite of behaviors is broader than behaviors associated with either structure. The lateral and ventromedial hypothalamus receive direct ipRGC innervations (Hattar et al., 2006) and both are implicated in feeding behaviors while the ventromedial hypothalamus has also been associated with aggression and circadian energy regulation (Petrovich 2018; Nisbett, 1972; Orozco-Solis et al., 2016; Olivier, 1977). Activation of these hypothalamic regions at atypical times of the day-night cycle throughout adolescence may have altered their functionality, leading to broader changes in behavior than would be expected from amygdalar dysfunction.

It is unlikely the case that atypically timed blue light, in isolation, leads to the development of a mood disorder, but it may be one of many risk factors that contribute to the observed rise in the presence of mood disorders in younger, urban living people. Indeed, the diathesis-stress model posits that there are many factors that can increase one's risk for the development of a psychological disorder if the individual is genetically vulnerable said disorder (Colodro-Conde et al., 2018); mistimed blue light may be one such contributing factor.

Possible alternative pathways involved in behavior impacts of lighting

Based on data from our pilot study, it is unclear if shifts in DA innervations within the BLA are responsible for the behavioral effects observed in experiments 1 and 2. As described earlier, we now know that the retina projects widely outside of image-forming regions of the brain. ipRGCs project heavily to the master circadian clock of the suprachiasmatic nucleus of the hypothalamus (SCN; Vandewalle et al., 2009) and regulate circadian rhythms. Desynchronization of circadian rhythms can have profound impacts on mood, emotion, and physical health in individuals who work in environments such as shift workers, where light exposure is essentially the opposite of natural, solar light (Juda, Vetter, & Roenneberg, 2013).

The retina, via ipRGCs, also projects to the lateral habenula (LHb) which has been associated with learned helplessness (Li et al., 2011), and disruptions of ipRGC input to the LHb has been associated with mood-like alterations (Fernandez et al., 2018) in experimental animals. Additionally, indirect retinal pathways to the locus coeruleus (LoC) via the SCN may increase stress-like behaviors as well as impact noradrenergic (NE) systems (Deurveilher & Semba 2005). Thus, there is ample opportunity for visual information to impact mood and emotion outside of projections to amygdalar sub-nuclei.

All of these structures and pathways do have the potential to impact mood and emotion in specific ways. However, behavioral data here do not support long-term changes in the activity of all of the aforementioned structures. If the LoC, via NE producing cell projections to the BLA (McCall et al., 2017), had been a primary contributor to behavioral effects, we would have expected to see increased stress- or anxiety-like behaviors, such as less time spent in the open arms of the EPM or less time spent in the center or middle portions of the M-OFT. It is true that TH stains for both DA and NE producing cells and their projections, and it is thus impossible to dissociate DA and NE terminal fields in our study; however, based on our behavioral effects, it seems unlikely that NE is the primary driver. Regarding projections to the SCN, circadian phase shifting may have occurred in the evening blue light condition, resulting in a more evening-oriented phenotype. Evening orientation has been associated with several psychological phenomena related to mood disorders or general psychological disorders (Jonason, Jones, & Lyons, 2013). However, if evening orientation were driving behavioral shifts examined here, we might have expected to see more depressive-like behaviors such as reduced sociability in the evening blue light conditions. Indeed, evening oriented chronotypes have been associated with

depression in humans (Bauducco, Richardson, & Gradisar, 2020), but this phenotype was not observed in our animals.

Relatively recently, the habenula in general and the perihabenular (PHb) nucleus specifically have been identified as structures critical for the impact of light on mood outside of circadian impacts of light. It is established that the lateral habenula (LHb) receives direct innervation from ipRGCs and is broadly implicated in responses to unexpected, negative feedback and learned helplessness (Li et al., 2011). In addition, the LHb exerts control on monoamine systems. As mentioned above, monoamines like DA and 5-HT are heavily involved in mood and emotion regulation. The LHb directly impacts both DA and 5-HT via projections to the VTA and DRN, respectively. It is thought that, in depression, LHb activity inhibits DA and 5-HT release (Yang, Wang, Hu, & Hu, 2018). Further, Fernandez et al. (2018) identified a previously uncharacterized retinal pathway to the PHb. The PHb has been recently identified as a separate thalamic region near the pineal gland that projects to the ventromedial prefrontal cortex (vmPFC; Figure 25). The authors discovered that activation of this region leads to anxiety-like behaviors in the forced-swim and tail-suspension tasks. Further work has determined that the PHb exhibits both short- and long-range GABAergic inhibitory projections and that short-range inhibitory connections may desynchronize local networks if lighting is irregular. This desynchronization may, in turn, impact longer-range inhibitory connections, altering mood and emotion (Weil et al., 2022). As it is a much more recently identified structure, it is less clear whether the PHb is involved in habenular projections to monoamine systems, but it is likely involved as well. Based on current evidence, it is not apparent whether the LHb or the PHb are interconnected with other mood and emotion regulating regions such as the amygdala, but both

regions may work together as a somewhat redundant network to process emotionally salient stimuli.

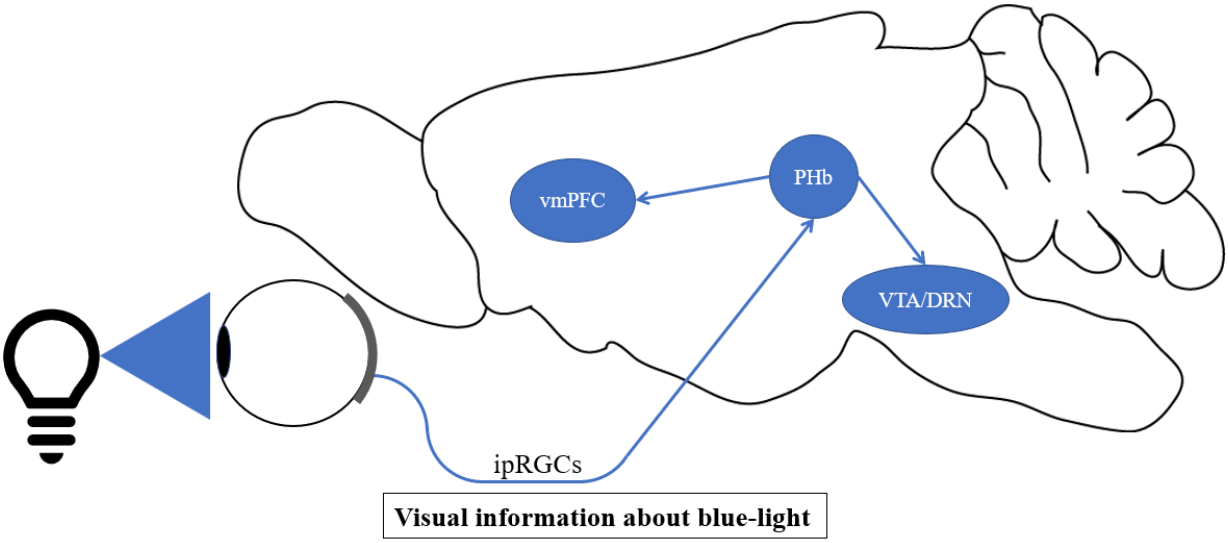

Figure 25: Possible ipRGC pathway to PHb, adapted from Fernandez et al. 2018.

General limitations and future directions

It should be noted that none of the studies presented in this dissertation directly measured nor manipulated the ipRGC system or any relevant retinal pathways. Thus, it is not possible to know with certainty whether any observed behavioral effects are via ipRGCs or conventional RGCs. As discussed above, there are also many structures outside of the amygdala that could theoretically result in several of the observed behaviors. Future work should address these limitations and tease apart exactly which pathway is responsible for which of the behavioral alterations observed.

Teasing apart retinal projections responsible for behavioral effects

There are many approaches that allow researchers to trace, or retrace, neural projections. Relevant here, it may be possible to trace ipRGC and conventional RGC afferents to structures of

interest and then measure subsequent changes in TH or myelination, etc. Retrograde and anterograde traces have been used to track retinofugal projections in the past, but specifically pairing these tracers with observed changes in TH and behavior may provide more concrete evidence of which RGCs are responsible for the effects observed here.

A second possible approach is to ablate ipRGCs or melanopsin altogether and compare behavioral and neuroanatomical changes observed to wildtypes. Ablation of melanopsin cells via an attenuated diphtheria toxin in mice has been used before to understand its functionality (Güler et al., 2008). Ablating melanopsin would allow us to determine if behavioral changes in the melanopsin lacking animals are specifically tied to the loss of these pathways. In essence, the same experimental design as experiment 2 could be run with all animals in evening blue light. The primary manipulation would be that in one group of gerbils melanopsin is ablated, while it is not in the other. If behavioral effects persist following the ablation of melanopsin in our paradigm, any effects can be associated with conventional RGCs and their projections. To our knowledge, this approach has never specifically been used to tease apart mood and emotion relevant changes following atypical exposure to blue light.

A third type of approach would be to stimulate ipRGC cells or amygdala substructures of interest directly via DREADDs or optogenetics, similar to Fernandez et al. (2018) and their study on the PHb. This artificial stimulation of the cells would make it possible to specifically stimulate the ipRGC pathway or ipRGC targets at different times of the day and compare differences in structures such as the amygdala, habenula, or PFC across the timing of activation of these pathways. This technique would also allow us to know with certainty that we were stimulating certain retinal cells or ipRGC targets and not others. Here, we are inferring that our blue-saturated lighting conditions activated ipRGCs either at specific times of the day-night

cycle or relative to another, warmer light. Again, these techniques have been used to understand retinofugal pathways in previous work (Fernandez at al., 2018), but specifically using them to better understand how the timing of the activation of this system will help us to better understand the more complex issue of the timing of blue light exposure (i.e., blue light in the morning has anti-depressive effect, evidence provided here suggests blue light in the evening may negatively impact mood or emotion).

A fourth relevant approach could employ human subjects and the "silent substitution" lighting method developed by Spitschan and Woedlers (2018). Silent substitution involves exposing participants to lights with highly specific and narrow spectral properties ("metamers") to either excite or not excite certain retinal cells. These light sources are particularly appealing as they are perceived as identical stimuli, but activate different retinal pathways. This may allow one to differentiate between the impacts of ipRGCs and conventional RGCs during tasks such as our facial expression processing task in experiment 1 that are relevant to emotional states. For example, an experiment may implement two different lighting metamer conditions, one that drives ipRGCs maximally, and another that drives ipRGCs minimally before having participants complete the FEP. As the lighting conditions are equated across intensity as well as overall spectral composition, this manipulation negates any potential confounds such as variation in spatial frequency based on expression type (Gao & Maurer 2011)

Conclusion

Data from two experiments and a pilot study provide evidence that blue light can have subtle, but important impacts on our mood or emotional states, and particularly as they relate to social cues. First, even short exposure to blue light can negatively shift how we interpret social cues (facial expressions) which may, in turn, alter how we interact with others. Issues with facial

expression processing have also been tied to depression. Second, extended exposure to blue light in the evening during adolescence, a critical developmental period, subtly, but importantly, impacted mood relevant behaviors in gerbils. Increased aggressive and social investigatory behaviors were observed in gerbils. Interestingly, energy conservation-like behaviors were also observed, suggesting a broader suite of behaviors that are sensitive to atypically times blue light. Data from our pilot study did not provide evidence that changes in DA innervations in the BLA are responsible for these behavioral effects. Future work could employ a diversity of approaches to identify the primary regions involved. Considering these data and how common light exposure well into the evening is in modern societies, it is clear that we must consider how these light sources impact both immediate as well as longer-term mood and emotion states, especially in younger individuals.

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