CIRCADIAN DISRUPTION BY LIGHT AT NIGHT:
IMPLICATIONS FOR MOOD

DISSERTATION

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ABSTRACT

Life on Earth has adapted to a consistent 24-h solar cycle. Circadian rhythms in physiology and behavior remain synchronized to the environment using light as the most potent entraining cue. During the past century, however, the widespread adoption of electric light has led to ‘round-the-clock’ societies. Instead of aligning with the environment, individuals follow artificial and often erratic light cycles created by social and work schedules. In particular, exposure to artificial light at night (LAN), termed “light pollution”, has become pervasive over the past 100 years. Virtually every individual living in the U.S. and Europe experiences this aberrant light exposure, and moreover about 20% of the population performs shift work. LAN may disrupt physiological timekeeping, leading to dysregulation of internal processes and misalignment between behavior and the environment. Recent evidence suggests that individuals exposed to excessive LAN, such as night shift workers, have increased risk for depressive disorders, but the biological mechanism remains unspecified. In mammals, intrinsically photosensitive retinal ganglion cells (ipRGCs) project light information to (1) the suprachiasmatic nucleus (SCN) in the hypothalamus, regulating circadian rhythms, and (2) to limbic regions, putatively regulating mood. Thus, LAN has the potential to affect both circadian timekeeping and mood. In this dissertation, I present evidence from rodent studies supporting the novel hypothesis that night-time exposure to
light disrupts circadian organization and contributes to depressed mood. First, I consider the physiological and behavioral consequences associated with unnatural exposure to LAN. The effects of LAN on circadian output are considered in terms of locomotor activity, the diurnal cortisol rhythm, and diurnal clock protein expression in the brain in Chapter 2. The influence of LAN on behavior and brain plasticity is discussed, with particular focus on depressive-like behavior (Chapter 3) and effects of SSRI treatment (Chapter 4). Effects of LAN on structural plasticity and gene expression in the brain are described, with emphasis on potential correlates of the depressive-like behavior observed under LAN in Chapter 5. Given the prevalence of LAN exposure and its importance, strategies for reversing the effects are offered. Specifically, eliminating LAN quickly reverses behavioral and physiological effects of exposure as described in Chapter 5. In Chapter 6 I report that administration of a pharmacological cytokine inhibitor prevents depressive-like behaviors in LAN, implicating brain inflammation in the behavioral effect. Finally, I demonstrate in Chapter 7 that exposure to red wavelength LAN reduces the effects on brain and behavior, suggesting that LAN acts through specific retinal pathways involving melanopsin. Taken together, these studies demonstrate the consequences of LAN, but also outline potential avenues for prevention or intervention.
DEDICATION

This dissertation is dedicated to my family, my mentors - past and present, and my lab mates for their support and encouragement.
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As a pre-med undergraduate, I stumbled into Huda Akil’s lab at the University of Michigan looking for a little research experience and ended up staying put for four years. I thank Sarah Clinton, at that time one of Dr. Akil’s very talented post-docs, for truly inspiring me to pursue a career in research. If not for her, I would have not gone down this path. And I thank her for her continued friendship and mentorship to this day.

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TABLE OF CONTENTS

Abstract.........................................................................................................................ii

Dedication......................................................................................................................iv

Acknowledgements........................................................................................................v

Vita.................................................................................................................................vii

List of Tables..................................................................................................................xi

List of Figures................................................................................................................xii

Chapters:

1. Introduction................................................................................................................1

2. Light at Night Alters Diurnal Rhythms ................................................................. 19

3. Light at Night Alters Behavior and Brain Plasticity ............................................ 34

4. Depressive-like Behavior is Responsive to SSRI Treatment............................... 53

5. Light at Night Reversibly Alters Hippocampal Structure and Gene Expression.... 67

6. TNF Implicated in Depressive-like Behavior Provoked by Dim Light at Night..... 84

7. Effect of Wavelength of Dim Light at Night on Brain and Behavior.................... 96
8. Conclusions............................................................................................................................. 111

List of References ....................................................................................................................... 119
## LIST OF TABLES

<table>
<thead>
<tr>
<th>Table</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.1</td>
<td>52</td>
</tr>
</tbody>
</table>

3.1 Complete hippocampal morphology following LAN exposure

xi
# LIST OF FIGURES

<table>
<thead>
<tr>
<th>Figure</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.1</td>
<td>Potential pathways through which LAN may influence mood</td>
<td>18</td>
</tr>
<tr>
<td>2.1</td>
<td>Home cage locomotor activity and cortisol concentrations</td>
<td>29</td>
</tr>
<tr>
<td>2.2</td>
<td>PER1 expression in the SCN</td>
<td>30</td>
</tr>
<tr>
<td>2.3</td>
<td>PER2 expression in the SCN</td>
<td>31</td>
</tr>
<tr>
<td>2.4</td>
<td>BMAL1 expression in the SCN</td>
<td>32</td>
</tr>
<tr>
<td>2.5</td>
<td>PER1, PER2, and BMAL1 expression in CA1 hippocampus</td>
<td>33</td>
</tr>
<tr>
<td>3.1</td>
<td>Behavioral effects of LAN exposure</td>
<td>48</td>
</tr>
<tr>
<td>3.2</td>
<td>Serum cortisol concentrations following exposure to LAN</td>
<td>49</td>
</tr>
<tr>
<td>3.3</td>
<td>Hippocampal morphology following exposure to LAN</td>
<td>50</td>
</tr>
<tr>
<td>3.4</td>
<td>Correlations of depressive-like behaviors and CA1 apical dendritic spine density</td>
<td>51</td>
</tr>
<tr>
<td>4.1</td>
<td>Experimental timeline and liquid intake</td>
<td>64</td>
</tr>
<tr>
<td>4.2</td>
<td>Effect of citalopram treatment on depressive-like response with LAN exposure</td>
<td>65</td>
</tr>
<tr>
<td>4.3</td>
<td>Effect on citalopram treatment on hippocampal dendritic spine morphology with LAN</td>
<td>66</td>
</tr>
<tr>
<td>5.1</td>
<td>Schematic of experimental design</td>
<td>81</td>
</tr>
<tr>
<td>5.2</td>
<td>Home cage locomotor activity</td>
<td>82</td>
</tr>
<tr>
<td>5.3</td>
<td>Depression-like behavior and brain changes</td>
<td>83</td>
</tr>
</tbody>
</table>
6.1 Pro-inflammatory cytokine expression in the brain……………………………………95
6.2 Effect of a dominant negative TNF inhibitor on depression-like behavior and CA1 dendritic spine density……………………………………………………………………96
7.1 Nocturnal light exposure provokes depressive-like responses in the forced swim test dependent on wavelength……………………………………………………………109
7.2 Nocturnal light exposure alters neuronal morphology in the hippocampus……110
7.3 Nocturnal light exposure activates the SCN in a wavelength-dependent manner……………………………………………………………………………………………111
CHAPTER 1

INTRODUCTION

Life on Earth has adapted to a consistent 24-h solar cycle. In order to anticipate patterns in the environment, individuals use light information to synchronize internal biological rhythms to the external world. During the past ~130 years, however, the invention and widespread adoption of electric light has led to ‘round-the-clock’ societies. Instead of aligning with the environment, individuals follow artificial light cycles created by social and work schedules. In particular, exposure to artificial light at night (LAN) has become pervasive in Western society. Virtually every individual living in the U.S. and Europe experiences this aberrant light exposure, and moreover about 20% of the population performs shift work (Rajaratnam and Arendt, 2001; Navara and Nelson, 2007). Exposure to LAN obscures entrainment of biological processes to external conditions, potentially leading to misalignment between physiology, behavior, and the environment.

Chronic disruption of circadian timing may have effects beyond dysregulated biological rhythms. In mammals, intrinsically photosensitive retinal ganglion cells (ipRGCs) project light information to (1) the suprachiasmatic nucleus (SCN) in the hypothalamus, regulating circadian rhythms, and (2) to limbic regions, putatively
regulating mood. Thus, unnatural light exposure has the potential to affect both circadian timekeeping and mood. As modern life has allowed humans to manipulate lighting easily and has led to aberrant light exposure, the prevalence of major depressive disorder (MDD) has grown in parallel (Kessler et al., 1993). Accumulating evidence from the past few years suggests that nighttime light exposure may have serious consequences for circadian timing and mood. In this dissertation, I present evidence from rodent studies supporting the novel hypothesis that night-time exposure to light disrupts circadian organization and contributes to depressed mood.

**Overview of circadian function**

Circadian rhythms are generated by the SCN, an endogenous biological clock in the hypothalamus, and entrained to the external environment primarily using light information sent directly from the retina to the clock. In the absence of light input from the environment, the endogenous clock becomes out of phase with the external environment, so correctly timed light information is essential to circadian timing. In mammals, the retina is the sole mechanism of light detection, consisting of image-forming photoreceptors, called rods and cones, and non-image forming photoreceptors called ipRGCs. In contrast to rods and cones, ipRGCs are depolarized in response to light and are largely responsible for circadian photoentrainment (Hattar et al., 2002).

Light detected by ipRGCs activates a unique photopigment called melanopsin, which is maximally sensitive to light in the blue wavelength range (~480 nm) and minimally sensitive to longer, red wavelength light (>600 nm) (Lockley et al., 2003; Newman et al., 2003; Brainard et al., 2008). This means that blue wavelengths exert a
more potent influence on the circadian system. Notably, in the United States nighttime use of incandescent bulbs, which produce light of longer yellow range wavelengths, is being replaced by compact fluorescent light (CFL) bulbs, which contain the blue wavelengths that maximally activate ipRGCs (Pauley, 2004). Activated ipRGCs project to the SCN directly through the retinohypothalamic tract (RHT) and indirectly through the intergeniculate leaflet (IGL). Information sent via the RHT reaches the SCN through a single glutamatergic synapse. This process is similar in both nocturnal and diurnal species; light at night triggers FOS induction in the SCN of both (Albrecht et al., 1997; Caldelas et al., 2003).

The timing set by the SCN has wide-reaching effects for the rest of the brain and body. The SCN projects axons to the paraventricular nucleus of the hypothalamus (PVN) and to the thalamus (Figure 1.1). Thalamic relays of the SCN project to limbic regions directly involved in mood regulation, such as the prefrontal cortex, hippocampus, and amygdala. Projections through the PVN to the pineal gland regulate melatonin secretion, which is responsible for entraining peripheral clocks in cells and tissues throughout the body. And projections through the PVN to the adrenal gland regulate glucocorticoid output. From these examples, it seems reasonable to suggest that temporally aberrant cues in the environment arising from artificial LAN could cause multi-level dysfunction throughout the brain and body as systems become desynchronized.

**Melatonin**

Pineal melatonin warrants brief discussion on its own because its production is under direct control by light. Typically melatonin is rhythmically secreted during periods
of darkness from the pineal gland, where it is released directly into systemic circulation, having many different physiological roles throughout the body. Its secretion is potently inhibited by light in an intensity-dependent manner (McIntyre et al., 1989). Evidence suggests that only one hour of exposure to ~45 photopic lux can decrease plasma melatonin concentrations by ~60% in healthy human volunteers (Brainard et al., 1988). In hamsters, levels as low as 1.08 lux are sufficient to significantly suppress pineal melatonin content (Brainard et al., 1982). During the night, suppressed melatonin levels can disrupt physiological timekeeping. For example, melatonin regulates peripheral clock gene oscillations, setting and maintaining circadian time in tissues throughout the body (von Gall et al., 2002; von Gall et al., 2005). Furthermore, accumulating evidence suggests that melatonin is related to mood. Agomelatine, a melatonin-receptor agonist and serotonin (5-HT_{2c}) receptor antagonist, belongs to a new class of melatonergic antidepressants (Goodwin et al., 2009; Kennedy and Rizvi, 2010). In rodents, melatonin administration prevents stress-induced depressive-like behaviors and reductions in hippocampal dendritic complexity (Crupi et al., 2010). Melatonin has positive effects on hippocampal cell proliferation (Ramirez-Rodriguez et al., 2009) and can stimulate neurotrophin production in the brain—both of which are responses associated with antidepressants (Kong et al., 2008).

**Circadian disruption and mood**

A role for the circadian system in mood is already well-established. A host of rhythm-related disturbances have been noted in association with major depression and other mood disorders; and on the other hand, environmental perturbations of the circadian
system provoke mood disturbances in some individuals (Bunney and Bunney, 2000; McClung, 2007, 2011). Any unnatural timing of light exposure, or lack of appropriate light/dark cues in the environment, can cause misalignment between internal biological processes and the external environment, leading to impaired mood. To illustrate this point, there are several instances in which obscured environmental lighting cues lead to depressed mood.

Seasonal affective disorder (SAD) is one prominent example. At temperate latitudes, SAD affects nearly 10% of the population (Rosen et al., 1990). It is characterized by recurrent winter depression, with symptoms manifesting during the short day lengths of winter when daylight exposure is low, and symptoms remitting during the spring and summer. Lack of sufficient daytime light is thought to cause a phase shift in the rhythm of pineal melatonin secretion. Without bright morning light to inhibit melatonin production, secretion persists into the daytime, leading to desynchronization between internal timekeeping processes and the external environment. Morning bright light therapy may be used to synchronize the circadian system to the appropriate time of day.

Several groups have attempted to model SAD and other circadian mood disorders using animal models (Workman and Nelson, 2011). Nocturnal Siberian hamsters exposed to short, winter-like, day lengths develop depressive-like responses in the forced swim test and anxiety-like responses in the elevated plus maze (Workman et al., 2011). Two diurnal rodent species, fat sand rats (Psammomys obesus) and Nile grass rats (Arvicanthis niloticus), also exhibit depression-like behavior in the forced swim test after exposure to
a very short photoperiod (5 h light/19 h dark) (Ashkenazy et al., 2009a; Ashkenazy-Frolinger et al., 2010). Bright light therapy, one current treatment for SAD in human patients, reverses some of the depressive responses in fat sand rats (Ashkenazy et al., 2009b). Also, short days administered to Wistar rats provoke depression-like responses in the forced swim test (Prendergast and Kay, 2008).

Total lack of light/dark cues can have similar effects on mood and behavior. Several groups have investigated the effects of constant bright lighting on mood and cognition, given recent increases in exposure to LAN surrounding shift work, social schedules, and other aspects of modern life. Constant light administered to rodents tends to induce arrhythmia in locomotor activity, a major circadian disruption. Three weeks of constant light exposure impairs spatial learning and memory in rats (Ma et al., 2007). Constant lighting also impairs hippocampal neurogenesis and memory (Fujioka et al., 2011).

In contrast, light deprivation in the form of constant darkness may provoke depression-like changes in rodents too. Mice and rats exposed to constant darkness for several weeks develop immobility in the forced swim test, as well as other depression-like behaviors (Monje et al., 2011). There is some evidence that constant darkness causes neuronal damage to monoamine systems (Gonzalez and Aston-Jones, 2008), including increases in apoptosis in several brain regions. Other evidence suggests that the depression-like responses are IL-6 dependent and mediated through the NF-κB signaling pathway (Monje et al., 2011).
These examples serve to highlight the importance of light in regulating the circadian system and mood. Proper alignment among biological rhythms, behavior, and the environment requires a balance in the amount of light and dark to which an organism is exposed. Tipping that balance in either direction can have profound consequences for physiology and mood.

**Populations exposed to light at night**

I have presented several instances in which the lack of discrete light and dark phases in the environment leads to mood impairment. But how might artificial LAN elicit a similar effect? And which populations are susceptible to LAN exposure? Over 99% of individuals living in the U.S. and Europe experience nightly light pollution (Cinzano et al., 2001). For the average person, streetlights illuminate the bedroom, television and computer screens glow in the home at all hours, and work and social demands keep the lights on. LAN can be invasive for these individuals, but for certain populations, exposure to LAN is even more pronounced.

For example, in industrial economies, approximately 20% of the population are shift workers (Rajaratnam and Arendt, 2001). These are often factory workers, medical staff, flight attendants, and others working in environments that require bright lights during night shifts. Such bright and chronic LAN exposure provokes several physiological changes. Exposure to LAN of ≤ 200 lx, levels easily encountered during night shift work, can suppress melatonin secretion and other circadian responses in humans (Brainard et al., 1997). Night shift work also increases negative affect and feelings of helplessness (Healy et al., 1993). Furthermore, the World Health Organization
(WHO) recently cited shift work as a probable carcinogen (Stevens et al., 2011). Following this announcement, Denmark became the first nation to compensate women who developed breast cancer after working night shifts (Noone, 2010).

By the same token that night shift nurses are affected by LAN, their patients may be similarly affected. Light readings obtained at a Midwest hospital SICU demonstrate that lights were illuminated at least 30 min out of every hour throughout the night, often when no nursing or care activity was being performed (Dunn et al., 2010). LAN in this context may negatively affect patient outcome by disrupting sleep and biological rhythms (Parthasarathy and Tobin, 2004).

Though artificial LAN is most common, natural LAN may be experienced by individuals living at certain latitudes. During the summer at northern latitudes near the Arctic Circle, a phenomenon referred to as “midnight sun” occurs, in which the sun remains visible throughout the night. Scandinavian countries, for example, experience sunlight almost 24 h each day during certain times of the year. In parts of Finland’s territory lying north of the Arctic Circle, the sun does not set for 60 days during the summer. The circadian system is not adapted to such extremes in the light environment. At least one study has reported that the number of violent suicides in these regions increases dramatically during times of “midnight sun”, suggesting that even naturally occurring LAN can negatively influence mood and behavior (Bjorksten et al., 2005).

In contrast to the examples outlined here, one population in the U.S. is excluded from many types of LAN exposure. The Old Order Amish population abstains from using public power, though generators and batteries are sometimes used for powering minimal
necessary products. The Amish do not use televisions or computers, which are a major source of LAN exposure among the general population. Due to the Amish lifestyle, LAN exposure is minimal. Interestingly, the Amish display greatly reduced cancer rates compared to the general population (Westman et al., 2010). Incidence of depression and other psychiatric disorders is also reduced (Egeland and Hostetter, 1983). Of course, other lifestyle factors may contribute to better health in this population, but the Amish represent an interesting opportunity to understand the effects of modern lifestyle choices on mood.

*Physiological effects of light at night exposure*

Clearly, exposure to LAN is quite pervasive in Western societies and several mechanisms exist by which this unnaturally timed exposure may influence mood. For one, at least in diurnal species, LAN is likely a sleep disruptor. There is a large literature implicating sleep disruption or deprivation in negative mood regulation (reviewed in: Tsuno et al., 2005). As many as 50% to 90% of depressed patients complain of poor sleep quality, and about 20% of insomniacs are clinically depressed (Tsuno et al., 2005). Clearly a relationship exists between disrupted sleep and depression, for which chronic exposure to LAN may be one contributor.

Furthermore, suppressed pineal melatonin secretion may contribute to mood disturbances in individuals exposed to LAN, according to the examples previously outlined. Briefly, appropriately timed melatonin secretion is essential for synchronizing circadian clocks throughout the body (von Gall et al., 2002; von Gall et al., 2005). And
some anti-depressant effects of melatonin have been demonstrated in both humans and rodents (Goodwin et al., 2009; Crupi et al., 2010).

Direct dysregulation of circadian clock genes could also underlie depression associated with LAN exposure. Some clock genes are directly regulated by light. For example, *Per1* expression in the SCN is directly stimulated by LAN (Kuhlman et al., 2003). Disruption of clock gene oscillations, particularly within limbic regions that receive projections from the SCN, may contribute to altered mood. Upsetting the daily oscillation of clock genes and their protein products may alter the function of important mood regulating systems—for example by altering expression and activity of neurotransmitter receptors implicated in mood (Reviewed in: McClung, 2007).

Similarly, diurnal variations in hormone secretion could be disrupted by LAN, particularly within the hypothalamic-pituitary-adrenal (HPA) axis. Recall that the PVN receives direct input from the SCN, which projects to the pituitary and then the adrenal. Cortisol, a major stress hormone released by the adrenal gland, has been implicated in depression and atrophy of the hippocampus when levels are chronically elevated (McEwen, 2005). In depressed patients, the diurnal rhythm of cortisol concentrations tends to be lost, and levels are consistently high throughout the day (Yehuda et al., 1996).

It is possible that LAN may act at any or all of these levels to provoke altered mood regulation. Indeed, it is likely that many layers are implicated at once, causing a complex interplay of misaligned systems. Disruption at one level could also provoke further dysregulation at another; for example, suppressed melatonin secretion could in turn further disrupt diurnal clock gene oscillations throughout the body.
Studying LAN

Before discussing evidence linking LAN to mood, it must be noted that there are several considerations to be made when choosing an animal model for studying the effects of LAN exposure. For one, a major physiological effect of nighttime lighting is likely suppression of pineal melatonin secretion. Many standard laboratory mouse strains are melatonin deficient, potentially limiting their usefulness in studies of this kind. The extent to which melatonin is implicated in LAN effects on mood is still unclear, however. LAN effects have been reported in at least one melatonin deficient mouse strain (Fonken et al., 2009). In my studies, I have chosen to use a hamster species (*Phodopus sungorus*) that is both melatonin proficient and expresses melatonin receptors in the brain, potentially increasing the translational relevancy to human physiology. A second consideration is the effect of sleep disruption in LAN exposure studies. In my studies, I use a nocturnal species that allows for separation of LAN effects from the more complicated effects of sleep disruption. LAN does indeed influence mood and physiology in at least one diurnal species, Nile grass rats, however sleep quality was not investigated in these studies, making it difficult to solely attribute the findings to LAN (Fonken et al., 2012b).

Evidence linking light at night to mood

Humans

Several studies in shift working populations have linked night work to negative affect. Among U.S. workers, regular shift work has detrimental effects for sleep and social factors, and is associated with high prevalence of MDD, with a higher rate among
women than men (Scott et al., 1997). Long term shift work is not necessary to see these effects, however. Among young student nurses performing night shift work for the first time, feelings of helplessness, loss of control, apathy, and low social support were perceived after only 3 months of night work (Healy et al., 1993). With longer term exposure (up to 20 years of shift work), there is an increased lifetime risk of MDD (Scott et al., 1997). One of the proposed treatments for problems associated with night shift work is bright light exposure during the night to phase shift the biological clock to align with the work schedule, thus improving alertness at work and facilitating sleep during the day (Czeisler et al., 1990). A limitation of this approach is the difficulty maintaining such a schedule. On weekends and “off” days, workers quickly revert back to synchronize with environmental and social cues.

Though increased depressive symptoms have been well-documented among shift workers (Healy et al., 1993), these studies are limited in establishing an exclusive link to LAN due to the other variables involved, such as sleep disruption and episodic (e.g., weekend) phase shifts. Jet lag is another modern change that is thought to be related to changes in affect (Katz et al., 2001). Patients admitted for psychiatric emergencies are more likely to exhibit depression or mania after travel across time zones compared to those admitted with no recent travel history, and those traveling westbound show the most symptoms of depression (Young, 1995). Even from a biological perspective, flight attendants working on transmeridian flights with short recovery time between trips have temporal lobe atrophy that is related to cognitive deficits (Cho, 2001).
Animal models

Human studies are limited by the number of variables, as both shift work and jet lag tend to disrupt sleep and social schedules in addition to lighting exposure. Modeling chronic LAN exposure in nonhuman animals may be the best target for initial investigations into the role of LAN and mood. Our laboratory has developed a model of chronic dim LAN exposure using Siberian hamsters. Other laboratories have investigated the effects of housing rodents in constant light (LL) without varying light intensity; however, rodents become arrhythmic under these conditions, making it difficult to obtain interpretable results (Ohta et al., 2005). Hamsters exposed to 5 lx dim LAN, however, remain entrained to the light-dim light cycle. Some changes in activity do occur, however, as homecage locomotor activity is slightly reduced during the dim light phase compared to typical dark phase activity levels and fast Fourier transformation (FFT) analysis reveals slight decrements in the strength of the 24-h rhythm, Eliminating the light at night rapidly reverses this effect (Chapter 5; Bedrosian et al., 2012b). Other changes in diurnal rhythms are also evident; LAN disrupts expression patterns in PER1 and PER2 proteins in the SCN and CA1 hippocampus (Chapter 2).

I have found that dim LAN provokes depressive-like responses in hamsters (Chapter 3; Bedrosian et al., 2011b; Bedrosian et al., 2012b). Because of the difference in activity levels during the dark or dim phase, behavioral testing for activity-dependent measures must be performed during the light phase, when activity levels are equivalent between groups. After 4 weeks of exposure to nightly 5 lx LAN, hamsters tested during the light phase display more immobility in the forced swim test, typically interpreted as
behavioral despair, and reduced preference for sucrose solution, an anhedonic-like symptom (Bedrosian et al., 2011b; Bedrosian et al., 2012b). Within 2 weeks of eliminating LAN, however, behaviors in both of these tests resemble those of hamsters exposed only to dark nights (Chapter 5; Bedrosian et al., 2012b). One method of validation for these behavioral assays of depressive-like response is to measure behavior after treatment with an antidepressant drug that is known to be effective in humans. Though Siberian hamsters housed in dim LAN for 4 weeks develop depressive-like symptoms in the forced swim test, two weeks of chronic treatment with the selective serotonin reuptake inhibitor (SSRI), citalopram, ameliorates the symptoms, supporting the validity of this model (Chapter 4; Bedrosian et al., 2012a).

The complete mechanism underlying these behavioral responses remains unknown, but I have chosen to focus my studies on the hippocampus, one brain structure implicated in the pathophysiology of MDD. Depressed patients often have hippocampal atrophy (Sheline et al., 1999; Frodl et al., 2002) and dysregulation of many hippocampal-related systems, such as stress coping and memory (Halbreich et al., 1985; McEwen, 2003; Gallassi et al., 2006). Similarly, loss of hippocampal dendritic spines and reduced dendritic complexity are observed in animal models of chronic stress and depression (Hajszan et al., 2005; Hajszan et al., 2009; Hajszan et al., 2010). In these models, expression of brain-derived neurotrophic factor (BDNF) is typically reduced in the hippocampus, but antidepressant drugs enhance its expression (Duman and Monteggia, 2006). I have observed that hamsters exposed to 4 weeks of dim LAN have reduced
dendritic spine density on hippocampal CA1 pyramidal neurons and reduced mRNA expression of BDNF in the hippocampus (Chapter 5; Bedrosian et al., 2012b).

Moreover, the hippocampus is disproportionately vulnerable to inflammation compared to other brain structures because of its high expression of receptors for pro-inflammatory cytokines such as interleukin (IL) 1β and tumor necrosis factor α (TNF) (Maier and Watkins, 1998). Neuroinflammation may play a role in depressive-like behavior provoked by a variety of circadian disruption. Exposure to constant darkness induces depression-like behavior and increased IL-6 levels, which are ameliorated by blocking NF-κB signaling. Furthermore, mice with a deletion on IL-6 are resistant to the behavioral effects of exposure to constant darkness (Monje et al., 2011). In my studies, I have observed that exposure to dim LAN increases TNF expression in the hippocampus. And further, treatment with a pharmacological inhibitor of TNF prevents depressive-like behaviors in the forced swim test after exposure to LAN (Chapter 6; Bedrosian et al., 2012b). This link between depression-like behavior provoked by circadian disruption and neuroinflammation is not surprising. A role for proinflammatory cytokines in the pathogenesis of depression has been proposed and recent evidence demonstrates a direct molecular pathway whereby disruption of circadian clock proteins enhances expression of cytokines within the brain (Narasimamurthy et al., 2012).

An important question arising from this work is how to prevent the deleterious effects of LAN when exposure cannot be avoided. One possibility may be to manipulate the wavelength of light exposure. As mentioned previously, the melanopsin-expressing cells in the retina responsible for projecting light information to the circadian system are
minimally responsive to red wavelength light. Replacing standard bulbs with red ones where possible, or using glasses that only transmit red wavelengths, may be an effective preventative against the disruptive effects of LAN. I have observed reduced effects of dim red LAN on hamster depressive responses, compared to white or blue LAN, along with reduced Fos activation in the SCN following red LAN exposure (Chapter 7).

Clearly, LAN has a variety of effects on the brain and circadian rhythms, each potentially contributing individually or in concert with others to regulate mood.

**Implications and future directions**

The incidence of depressive disorders has increased significantly in recent decades (Simon and VonKorff, 1992; Kessler et al., 1993; Compton et al., 2006), in parallel with the expansion in the use of electric LAN. Particularly, urban-dwelling individuals and night shift workers are exposed to artificial LAN on a chronic basis. For these people, LAN may be considered as a modern circadian disruptor. Given the evidence for disrupted circadian processes in depression, excessive LAN exposure could be one factor contributing to depressed mood among vulnerable individuals. Artificial and unnaturally timed light from the environment could disrupt physiological timekeeping, leading to misalignment of various biological rhythms. Studies using animal models have been useful thus far to identify a link between LAN and mood, but such a link in humans remains to be demonstrated. A first step would be to perform thorough correlational analyses through epidemiological analysis. Within this past decade, epidemiological work paved the way toward identifying the relationship between LAN and breast cancer, which is now officially recognized by the WHO and AMA. And these
studies should not only focus on shift workers, but also include individuals experiencing low levels of LAN at home. Such studies are already beginning to identify relationships between LAN and obesity (Obayashi et al., 2013). From an historical perspective, the widespread adoption of electric light occurred prior to an understanding of circadian biology. The effects of what we now know to be a major change for circadian biology were simply not considered. It is essential that we learn about the effects of modern technology on the brain and health, so that we might appropriately manage them.
Figure 1.1. Potential pathways through which LAN may influence mood.

Abbreviations- SCN: suprachiasmatic nuclei; PVN: paraventricular nucleus of the hypothalamus; PFC: prefrontal cortex; HPC: hippocampus; SCG: superior cervical ganglion
CHAPTER 2

LIGHT AT NIGHT ALTERS DIURNAL RHYTHMS

As described in Chapter 1, life on Earth has adapted to a consistent 24-h solar cycle. Biological rhythms remain entrained to the environment primarily using light. During the past century, however, the invention and widespread adoption of electric light has led to ‘round-the-clock’ societies. Instead of aligning with the environment, individuals can easily manipulate their own light exposure and follow artificial light cycles depending on work or social demands. Exposure to artificial LAN has become pervasive over the past ~130 years. Virtually every individual living in the U.S. and Europe experiences this aberrant light exposure, and moreover about 20% of the population performs shift work (Rajaratnam and Arendt, 2001; Navara and Nelson, 2007). LAN may disrupt physiological timekeeping, leading to dysregulation of internal processes and misalignment between behavior and the environment. Recent evidence suggests that individuals exposed to excessive LAN, such as night shift workers, have increased risk for depressive disorders (Fonken et al., 2009; Driesen et al., 2010; Bedrosian et al., 2011b; Bedrosian et al., 2012b), breast cancer (Hansen, 2001; Schernhammer et al., 2001; Blask et al., 2005), and metabolic ailments (Haus and
Smolensky, 2006). Given the growing list of health risks associated with excessive exposure to LAN, it is important to understand its underlying effects on physiology.

The circadian system may be influenced by LAN at several different levels. For one, nightly pineal melatonin secretion is suppressed by exposure to LAN (Brainard et al., 1982; Brainard et al., 1988), which may cause temporal misalignment between internal processes and the external environment. On another level, LAN may influence the diurnal rhythm of cortisol secretion from the adrenal gland. The SCN regulates hypothalamic-pituitary-adrenal (HPA) axis function by sending direct neural input to the PVN. Finally, the expression of circadian clock genes and proteins in the SCN is directly modulated by light information. For example, brief pulses of LAN can rapidly induce Per1 gene expression (Miyake et al., 2000), but the effects of chronic exposure remain unspecified. Disruption of the diurnal expression of clock genes and proteins may cause desynchronization of central and peripheral clocks, contributing to serious health effects (Reviewed in Hastings et al., 2003).

I investigated the effects of chronic exposure to dim (5 lx) LAN in female hamsters. This light intensity is approximately five times brighter than maximal moonlight, comparable to the levels of light pollution surrounding urban centers, and is sufficient to suppress pineal melatonin secretion in hamsters (Brainard et al., 1982). My experiments focus specifically on female hamsters because: (1) women are more likely to work evening or rotating shifts, thus encountering unnatural light exposure, and women have been implicated in many adverse health effects associated with LAN (Hansen, 2001; Williams, 2008), and (2) the effects of sleep disruption can be separated from the effects
of LAN because these are nocturnal rodents that normally sleep during the light. With this in mind, I examined diurnal patterns in (1) homecage locomotor activity, (2) serum cortisol concentrations, and (3) clock protein expression in the brain, with the hypothesis that chronic exposure to LAN would cause disruption at each of these levels. I specifically investigated clock proteins in the SCN, the brain’s master circadian clock, and the hippocampus.

Methods

Animals

Adult female Siberian hamsters (Phodopus sungorus) were obtained from our breeding colony at The Ohio State University. Hamsters were individually housed in polypropylene cages (30 cm x 15 cm x 14 cm) at a constant ambient temperature of 22±2°C and relative humidity of 50±5%. Food (Harlan Teklad 8640, Indianapolis, IN, USA) and filtered tap water were available ad libitum. Prior to starting the experiments, all hamsters (>8 weeks of age) were ovariectomized under isoflurane anesthesia in order to prevent effects of fluctuating estrogens on clock protein expression, and then allowed to recover for 1 week. This species exhibits a mixed profile that makes vaginal cytology for determining estrous stage unreliable (Dodge et al., 2002). Following the recovery period, hamsters were maintained in either control or experimental light cycles as described below. The control condition was a 16:8 h light/dark cycle (150 lux/0 lux), and the experimental condition was a 16:8 h light/dim light cycle (150 lux/5 lux). Both the bright and dim lights were typical “cool white” fluorescent bulbs. In both conditions, the bright lights were off at 15:00h (ZT12). All experimental procedures were approved by
The Ohio State University Institutional Animal Care and Use Committee and conducted in accordance with the National Institute of Health (NIH) guidelines on laboratory animal use and care.

*Homecage locomotor activity*

Homecage locomotor activity was recorded over many days using an infrared beam break system (Columbus Instruments, Columbus, OH, USA). Actigraphs were constructed using ClockLab software (Actimetrics, Wilmette, IL, USA).

*Cortisol concentrations*

Terminal blood samples were collected through heparinized capillary tubes from the retro-orbital sinus for radioimmunoassay (RIA) of cortisol concentrations. Samples were collected from separate groups of hamsters at 6 different time points every 4 hours in order to examine the diurnal pattern. Upon collection, blood samples were immediately centrifuged for 30 min at 3300 x g and 4 °C, then plasma aliquots were stored at -80 °C until assayed. Total plasma cortisol concentrations were determined in duplicate using an MP Biomedicals $^{125}$I antibody kit (Solon, OH, USA). The high and low limits of detectability of the assay were 0.1 and 100 ng/ml, respectively. The intra-assay coefficient of variation was <10%. All procedures were performed in accordance with manufacturer guidelines.

*PER1, PER2, and BMAL1 expression*

Brains were collected from separate groups of hamsters at 6 different time points every 4 hours to examine the diurnal pattern of circadian clock protein expression.
Briefly, hamsters were deeply anesthetized with an overdose of sodium pentobarbital and transcardially perfused with ice cold 0.1 M PBS (pH 7.4), followed by 4% paraformaldehyde in 0.1 M PBS (pH 7.4). Following perfusion, brains were collected, post-fixed overnight at 4 °C, transferred to 30% sucrose solution in PBS at 4 °C until sunk, and then frozen in cold isopentane and stored at -80 °C. Brains were serially sliced into 30 µm sections using a cryostat and sections were thaw-mounted onto gelatin-coated glass slides and stored at -80 °C.

Sets of tissue collected at intervals of 120 µm were used for immunohistochemical detection of PER1, PER2, and BMAL1 throughout the SCN and hippocampus. Briefly, sections were incubated 10 min in warm citrate buffer (pH 6.0) for antigen retrieval, then rinsed with 0.1 M PBS, and incubated 30 min in 0.3% H₂O₂ in methanol. Sections were rinsed again in PBS, and blocked for 1 h in 1% normal goat serum + 1% bovine serum albumin in 0.1 M PBS + 0.1% Triton. Sections were then incubated overnight at room temperature with the primary antibody (PER1, 1:8,000; PER2, 1:10,000; BMAL1, 1:500; provided by David Weaver, University of Massachusetts). Next sections were rinsed, incubated for 1 hour with anti-guinea pig IgG (1:500; Southern Biotech, Birmingham, AL, USA) in 0.1 M PBS + 0.1% Triton. The signal was amplified with avidin-biotin complex (Vectastain Elite ABC Kit; Vector Laboratories, Burlingame, CA, USA) and developed using DAB. Control sections were treated the same except for omitting the primary antibody. Slides were dehydrated through a series of graded ethanol washes and cleared with xylene, then coverslipped using Permount.
Images of sections containing mid-level SCN (Bregma -0.46 to -0.58) or rostral CA1 (Bregma -1.70) were obtained at 10x magnification using a Nikon E800 bright-field microscope. Numbers of immunoreactive cells were counted in ImageJ by an observer uninformed of assignment to experimental groups, and then averaged across sections and sides of the brain to produce one value for each animal that was used for statistical analysis. All cells were counted in SCN sections and a representative sampling of cells within a 300 x 100 pixel box was counted in the CA1.

Statistical analysis

Data were analyzed by two-way analysis of variance with nighttime light condition (Dark vs. Dim) and time of day as the independent variables. Main effects were followed up with Fisher’s post-hoc comparisons. CA1 PER1 data were analyzed by unpaired Student’s t-tests to compare groups at individual time points. Statistics were performed using Statview 5.0.1 (SAS Institute, Cary, NC, USA) for Windows. Mean differences were considered statistically significant when p was ≤0.05.

Results

Locomotor activity

Locomotor activity log is presented for a representative hamster before and after exposure to LAN. Days 1-7 depict activity during exposure to dark nights and days 8-14 show activity under dim LAN. Activity was mostly contained during the dark or dim light phase in both conditions (Figure 2.1a).
Cortisol

The diurnal pattern of cortisol concentrations differed by nighttime light condition ($F_{1,46}=4.309, p<0.05$); there was a distinct peak in cortisol at ZT15, with greater concentrations in the hamsters experiencing dark nights compared to hamsters exposed to dim LAN (post-hoc, $p<0.05$; Figure 2.1b).

Clock proteins

In the SCN, I detected an interaction between light and time for PER1 expression ($F_{5,55}=2.767, p<0.05$), with greater peak expression in dark nights compared to dim LAN at ZT15 (post-hoc, $p<0.01$; Figure 2.2a-b). There was a main effect for time in PER2 expression ($F_{5,52}=2.373, p=0.05$), with greater peak expression again in dark compared to dim LAN at ZT15 (post-hoc, $p<0.05$; Figure 2.3a-b). BMAL1 expression was fairly constitutive, with no effect of light or time (Figure 2.4a-b). In the CA1 region of the hippocampus, PER1 expression was greater in dark nights compared to dim LAN at ZT3 ($t_{4}=3.056, p<0.05$; Figure 2.5a-b). There was an interaction effect in PER2 expression ($F_{5,39}=2.968, p<0.05$), revealing greater expression in dark nights at ZT11 (post-hoc, $p<0.01$; Figure 2.5c-d). There was no effect of light or time in BMAL1 expression in CA1 (Figure 2.5e-f).

Discussion

The circadian system is adapted to a rhythm of bright days and dark nights. Only in very recent history has the adoption of electric lights allowed humans to escape the natural day-night rhythm. Humans are chronically exposed to artificial LAN and negative
health consequences are only now becoming apparent. I explored the hypothesis that chronic exposure to dim LAN alters the circadian system at the level of locomotor activity patterns, the diurnal cortisol rhythm, and diurnal expression of core circadian clock proteins. My results demonstrate that, although dim LAN does not overtly influence entrainment of activity patterns, it does flatten the diurnal pattern of cortisol concentrations, and alter expression of PER1, PER2 and BMAL1 in the SCN and hippocampus. Taken together, these findings suggest that modern nighttime lighting may significantly affect physiology.

The data indicate that the daily rhythm in cortisol concentrations is abolished after chronic exposure to LAN. Typically, cortisol concentrations peak near the beginning of the active phase in both diurnal and nocturnal species. The cortisol rhythm secreted from the adrenal glands is under control of the circadian system. Light information received by the SCN is projected to the paraventricular nucleus (PVN) of the hypothalamus, which in turn releases corticotropin-releasing hormone (CRH), signaling the anterior pituitary to release adrenocorticotropic hormone (ACTH). The adrenal cortex is stimulated by ACTH to release cortisol. The lack of distinct light and dark signals to the SCN could disturb the diurnal rhythm in cortisol release, but chronic exposure to LAN may be necessary to see this effect. A 3 h light pulse of 600 lx administered to human volunteers during the night did not influence cortisol concentrations (McIntyre et al., 1992). But 8 wk exposure to constant light decreased diurnal corticosterone levels and ablated the peak in female rats (Cheifetz et al., 1968). To my knowledge, this is the first study to show that low level
nighttime illumination, as might be commonly experienced in modern society, may be sufficient to alter the diurnal cortisol rhythm.

My results also show that PER1 and PER2 expression in the SCN is altered with exposure to LAN, but BMAL1 expression remains unchanged. BMAL1 protein tends to be constitutively expressed in the mouse SCN at high levels, and its expression is unchanged by a light pulse occurring during the night (von Gall et al., 2003). This supports my observations and explains the lack of LAN influence on BMAL1 expression. PER1 and PER2, however, exhibited diurnal rhythms in hamsters exposed to a standard light-dark cycle, but the peak was reduced with dim LAN. Light pulses administered during the night are known to induce Per1 gene expression, with a more limited induction of Per2 (Miyake et al., 2000; Kuhlman et al., 2003). At the level of protein expression, a light pulse during the early night does not induce PER2 even 6 h later, and further PER2 levels are low and arrhythmic in rats exposed to constant-light (Beaule et al., 2003). The level of illumination used in my experiment may have been enough to induce arrhythmicity in PER1 and PER2 expression. It will be important to determine the amount of light required to produce this change.

In other experiments, I have found that chronic exposure to dim LAN profoundly influences the hippocampus. After four weeks, dendritic structure on CA1 neurons is altered and bdnf gene expression is reduced (Chapter 5; Bedrosian et al., 2011b; Bedrosian et al., 2012b). For that reason, I specifically investigated expression of PER1, PER2, and BMAL1 within the CA1 region of the hippocampus. The overall rhythm in PER1 and PER2 expression differed in LAN-exposed hamsters, but the pattern of
BMAL1 expression was unchanged. Specifically, in hamsters exposed to dark nights, PER1 and PER2 exhibited a small peak at the beginning of the night, which was reduced by LAN. Putatively, disrupted signals from the SCN could lead to altered clock protein expression in the other brain regions, including the hippocampus. PER2 protein is highly expressed in hippocampal pyramidal layers and Per2 mutant mice have impaired long-term potentiation and fear conditioning behavior (Wang et al., 2009).

Taken together, this study is the first to use low level nighttime illumination, similar to the conditions experienced by many individuals in modern society, to examine the effects on diurnal rhythms. 5 lx LAN is a seemingly innocuous manipulation that produces profound effects on the pattern of cortisol and clock protein expression. Since the adoption of electric light over the past ~130 years, individuals are frequently exposed to dim LAN. Televisions, computers, e-readers, and streetlight leak from outside are all potential sources of this unnatural light exposure. The circadian system is not adapted to such conditions, thus it is important that we consider the potential influence of our technology and lifestyle choices on physiology and health.
Figure 2.1. Homecage locomotor activity and cortisol concentrations.

Actigraph showing activity remains consolidated to the night after transition from dark nights to LAN (A). The rhythm in diurnal serum cortisol concentrations is abolished after exposure to LAN (B). Data are double-plotted. n=3-6 per group. *p<0.05 at ZT15.
Figure 2.2. PER1 expression in the SCN.

Representative images taken at 10x magnification show trough and peak expression at ZT3 and ZT15 (A). Peak expression at ZT15 was abolished after exposure to LAN (B). Data are double-plotted. n=3-8 per group. *p<0.05 at ZT15.
Figure 2.3. PER2 expression in the SCN.

Representative images taken at 10x magnification show trough and peak expression at ZT3 and ZT15 (A). Peak expression at ZT15 was abolished after exposure to LAN (B). Data are double-plotted. n=3-7 per group. *p<0.05 at ZT15.
Figure 2.4. BMAL1 expression in the SCN.

Representative images taken at 10x magnification show expression levels at ZT3 and ZT15 (A). Expression was constitutive and not influenced by exposure to LAN (B). Data are double-plotted. n=3-8 per group.
Figure 2.5. PER1, PER2, and BMAL1 expression in CA1 hippocampus.

LAN altered expression of PER1 (A-B) and PER2 (B-C), but did not influence BMAL1 expression levels (D-E). n=3-9 per group. Images taken at 10x magnification. *p<0.05 at time point indicated.
CHAPTER 3

LIGHT AT NIGHT ALTERS BEHAVIOR AND BRAIN PLASTICITY

The prevalence of major depression and other mood disorders has dramatically increased in recent decades; however, the factors contributing to this phenomenon remain unspecified (Simon and VonKorff, 1992; Compton et al., 2006). Better diagnoses or changing diagnostic criteria have traditionally been suggested as possible contributory factors; however, additional variables are likely involved. For instance, environmental influences play a role in the onset of depressive disorders and it is possible that recent environmental changes may partially account for the change in incidence.

One such environmental change which emerged during the 20th century is the growing prevalence of exposure to artificial LAN. The advent of electrical lighting permitted humans to stray from natural day-night cycles and this change has been widespread. In 2001, greater than 62% of the world’s population, and more than 99% of those individuals living in the U.S. and Europe, experienced brighter than normal night skies (Navara and Nelson, 2007). Furthermore, about 20% of the population in any urban economy works night shifts and is chronically exposed to night-time illumination (Rajaratnam and Arendt, 2001). Because exposure to light at night suppresses secretion of the pineal hormone melatonin, which has anti-depressant effects on mood and
hippocampal plasticity (Crupi et al., 2010), there may be a link between the rising rates of depression and nighttime light exposure.

The role of LAN in mood has been almost entirely unexplored due to a lack of animal models, and particularly in females, despite a two-fold increase in the incidence of depression among women compared to men (Kessler et al., 1993). Our laboratory recently demonstrated a depression-like phenotype in male mice exposed to constant bright light conditions (Fonken et al., 2009), but the effects of low level illumination at night, similar to what humans experience, have not yet been described. In particular, little is known of the extent to which light at night affects neural circuitry involved in mood.

The hippocampus is a critical structure in the pathophysiology of depressive disorders. Depressed patients show characteristic hippocampal atrophy (Sheline et al., 1999; Frodl et al., 2002), as well as dysregulation of many hippocampus-related systems, such as stress coping and memory (Halbreich et al., 1985; McEwen, 2003, Gallassi et al., 2006). Moreover, loss of hippocampal dendritic spines and synaptic inputs to spines are observed in animal models of depression (Hajszan et al., 2005; Hajszan et al., 2009; Hajszan et al., 2010). Interestingly, melatonin has actions directly in the hippocampus (Musshoff et al., 2002) and anti-depressant effects on mood (Crupi et al., 2010; de Bodinat et al., 2010). Furthermore, melatonin was recently shown to ameliorate the reduced spine density reported in a mouse model of depression (Crupi et al., 2010).

In order to begin investigating how light at night influences mood and hippocampal plasticity I exposed female hamsters, ovariectomized to prevent confounding estrogen effects on hippocampal morphology (Woolley and McEwen,
1992), to dim light at night, at a level sufficient to suppress melatonin secretion (Brainard et al., 1982). I hypothesized that chronic exposure to dim light at night would provoke depression-like responses in ovariectomized hamsters and alter dendritic morphology in the hippocampus.

**Materials and Methods**

**Animals**

Seventeen adult female Siberian hamsters (*Phodopus sungorus*) were obtained from our breeding colony at The Ohio State University. Hamsters were individually housed in polypropylene cages (30 cm x 15 cm x 14 cm) at a constant ambient temperature of 22±2°C and relative humidity of 50±5%. Food (Harlan Teklad 8640, Indianapolis, IN, USA) and filtered tap water were available *ad libitum*. Prior to starting the experiments, all hamsters (>8 weeks of age) were ovariectomized under isoflurane anesthesia and allowed to recover for ≥3 weeks. Following the recovery period, hamsters were maintained for 8 weeks in either control or experimental lighting conditions.

Control animals (N=8) remained in the standard colony room under a 16:8 light/dark cycle (150 lux/0 lux), whereas the experimental group (N=9) was housed in a separate room on a 16:8 light/dim light schedule (150 lux/5 lux). Both the bright and dim lights were typical fluorescent bulbs of the same wavelength. In both conditions, the bright lights were illuminated at 23:00 h. All experimental procedures were approved by The Ohio State University Institutional Animal Care and Use Committee.
Experimental design

After 8 weeks in lighting conditions, hamsters were tested for depression- and anxiety-like behaviors. Behavioral testing was performed between 08:00 and 12:00 and hamsters were allowed to habituate to the test room 30 min prior to testing. Tests were performed in the following order: 1) elevated-plus maze, 2) forced swim test, 3) sucrose anhedonia. Our experience with these tests at The Ohio State University Behavioral Phenotyping Center indicates no test order effects on these behavioral assessments. A baseline blood sample was taken after 4 weeks in lighting conditions and a terminal blood sample and brains were collected after 11 weeks (1 week after the conclusion of behavioral testing).

Behavioral tests

To assess depression-like behavioral responses in the forced swim test (Porsolt et al., 1977), hamsters were placed in a 4 L glass beaker filled with room-temperature water (22 ± 1°C) inside an opaque cylindrical tank for 7 min. Behavior was recorded on video and subsequently scored with Observer software (Noldus, Wageningen, The Netherlands) by an observer unaware of assignment to experimental groups. The behaviors scored were: 1) climbing (i.e., vigorous swimming or scratching directed at the wall of the tank), 2) swimming (i.e., horizontal movement in the tank), and 3) floating/immobility (i.e., minimal movement necessary to keep head elevated above water surface).

Sucrose intake was determined by measuring consumption of a 1% sucrose solution over 24 hours. To acclimate the animals to the novel solution, hamsters were presented with a bottle containing normal drinking water and the bottle containing
sucrose solution over the weekend and left undisturbed for three days. On the fourth day each bottle was weighed, replaced in the cage, and then subsequently weighed again 24 hours later. To control for possible side preferences, placement of the bottles in the cage was counterbalanced.

Anxiety-like behavior was tested in the elevated plus maze, which is a plus-shaped apparatus, elevated above the floor with two dark enclosed arms and two open (i.e., anxiogenic) arms. Each hamster was placed in the center of the test apparatus to begin and behavior was recorded on video for 5 minutes. An uninformed observer later scored videotapes for percent time spent in the open arms of the maze using Observer software.

Cortisol assays

After 4 weeks in experimental lighting conditions and at the conclusion of the study (11 weeks), blood samples were collected through heparinized capillary tubes from the retro-orbital sinus for radioimmunoassay (RIA) of cortisol concentrations. Samples were immediately centrifuged at 4°C for 30 min at 3300g and plasma aliquots were stored at -80°C until assayed. Total plasma cortisol concentrations were determined in duplicate using an ICN Diagnostics 125I double antibody kit (Costa Mesa, CA, USA). The high and low limits of detectability of the assay were 1200 and 3 ng/ml, respectively. The intra-assay coefficient of variation was <10%. All procedures were performed in accordance with the manufacturer guidelines.
Analysis of hippocampal morphology

All hamsters were deeply anesthetized with isoflurane vapor and rapidly decapitated on the same day between 10:00 and 12:00 h. Brains were quickly dissected and processed for Golgi-Cox staining using a Rapid GolgiStain Kit (FD NeuroTechnologies). Briefly, whole brains were submerged in Golgi-Cox solution and stored for 14 days in the dark, followed by a 30% sucrose solution for 4 days. Brains were then rapidly frozen and 100 µm coronal sections were sliced on a cryostat and collected onto gelatin-coated glass slides. The stain was developed in NH₄OH for 10 minutes and sections were counterstained with cresyl violet. Finally, slides were dehydrated through a series of graded ethanol washes, cleared with xylene, coverslipped with Permount, and dried in the dark for at least 1 week.

Neurons impregnated with the Golgi-Cox solution were chosen within the CA1, CA3, and dentate gyrus (DG) regions of the hippocampus. Only neurons that were fully impregnated, not obscured by neighboring neurons, and had no obviously truncated dendrites were chosen for analysis. For each animal, six randomly chosen, representative neurons from different sections were completely traced at 20x (N.A. 0.75) using Neurolucida 8 software (MicroBrightField, Williston, VT, USA) for PC and a Nikon Eclipse E800 brightfield microscope. Dendritic spines were traced in each neuron at 100x (N.A. 1.30) in 4 apical and 4 basilar randomly chosen, representative dendrite segments of at least 20 µm in length, and at least 50 µm distal to the cell body. Morphological characteristics were analyzed using Neurolucida Explorer software (MicroBrightField,
Williston, VT, USA) and consisted of: 1) dendritic length, 2) cell body area, 3) cell body perimeter, and 4) dendritic spine density.

**Statistical Analyses**

Behavioral data from the forced swim test and elevated plus maze, as well as data from cortisol RIA and neuronal traces, were compared for group differences (LD vs. dim LAN) using unpaired Student’s *t*-tests. One animal died during the course of the study. In the case of immobility in the forced swim test, data were log transformed prior to analyses due to inequality of variances and one outlier was removed. Sucrose intake data were analyzed using two-way ANOVA with lighting condition (Dark vs. Dim) and liquid (water vs. sucrose solution) as the independent variables. Main effects were followed up with Fisher’s Exact Test for post-hoc comparisons. Correlations between depression-like behaviors and dendritic spine density were also calculated. Statistics were performed using Statview 5.0.1 for Windows. Mean differences were considered statistically significant when *p* was <0.05.

**Results**

**Depression- and anxiety-like behaviors**

Depression- and anxiety-like responses were observed in dim LAN using three different behavioral assays (Figure 3.1). In the forced swim test, hamsters exposed to dim LAN spent more time immobile (*t*<sub>14</sub> = 1.969, *p*<0.05; Figure 3.1a) and less time climbing to escape (*t*<sub>14</sub> = -2.329, *p*<0.05; Figure 3.1b) compared to hamsters exposed to dark nights, indicating a depression-like phenotype. Furthermore, in a test of sucrose intake,
two-way ANOVA revealed a main effect of lighting treatment \((F_{1,28} = 8.319, p<0.01)\), and post-hoc tests revealed that hamsters in dim LAN consumed less sucrose solution than hamsters in dark \((p<0.01; \text{Figure 3.1c})\), supporting the notion of an anhedonic-like state in LAN. Water intake was equivalent between groups \((p>0.05)\). In contrast, hamsters exposed to LAN demonstrated less anxiety-like behavior in the elevated plus maze, as they spent greater percent time in the anxiogenic open arms of the maze compared with hamsters in dark nights \((t_{12} = 2.232, p<0.05; \text{Figure 3.1d})\).

**Cortisol concentrations**

Plasma cortisol concentrations were determined during the light phase after 4 and 11 weeks in dark or dim conditions \((\text{Figure 3.2})\). There were no group differences in cortisol concentrations after 4 weeks in lighting conditions, nor at the conclusion of behavioral testing \((p>0.05; \text{Figure 3.2})\). Moreover, the duration of exposure to light at night also did not change cortisol concentrations, as there were no differences between the 4\(^{th}\) week and 11\(^{th}\) week samples \((p>0.05)\).

**Hippocampal cell morphology**

Hippocampal neurons from CA1, CA3 and DG were traced and analyzed for morphological characteristics \((\text{Figure 3.3})\). Two brains were lost due to crumbling during the sectioning process and one animal died during the course of the study, so \(N=6\) dark and \(N=8\) dim brains in total were used for morphological analyses. In the CA1 region, hamsters exposed to dim LAN had significantly reduced dendritic spine density in both apical \((t_{12} = -3.465, p<0.01; \text{Figure 3.3c})\) and basilar \((t_{12} = -2.708, p<0.05; \text{Figure 3.3f})\)
dendrites. The reduction in spine density on CA1 apical dendrites significantly correlated with immobility in the forced swim test ($r = -0.578$, $p<0.05$; Figure 3.4a) and sucrose intake ($r = 0.581$, $p<0.05$; Figure 3.4b). The groups did not differ in any other measures of cell morphology in CA1 and no differences were observed in CA3 or the DG ($p>0.05$ for all comparisons; Table 3.1).

**Discussion**

Exposure to dim LAN is sufficient to provoke a depression-like phenotype in ovariectomized female hamsters, as well as low anxiety-like responses. In addition, dim LAN reduces the density of dendritic spines, the primary sites of synaptic contacts, in hippocampal CA1 pyramidal cells. These changes were observed independently of changes in baseline plasma cortisol concentrations. Other measures of neuronal plasticity, such as changes in dendritic length and cell body size, were not observed, suggesting that light at night specifically affects the connectivity of hippocampal neurons rather than cell size or other aspects of morphology. Moreover, the density of CA1 dendritic spines significantly correlated with depression-like behaviors in both behavioral assays, suggesting a functional relationship.

**Behavior Differences**

Hamsters chronically exposed to dim light at night exhibited more immobility, generally interpreted as behavioral despair (Porsolt et al., 1977), in the forced swim test and consumed less sucrose solution in a test of sucrose intake, which is interpreted as an anhedonic-like response (Willner et al., 1992). Taken together, these behavioral
responses suggest a depression-like state. LAN exposed hamsters also spent more time in the open arms of the elevated plus maze, indicating a reduced anxiety-like response. These results are consistent with our previous observation that constant bright light provokes a depression-like phenotype and reduced anxiety in male mice (Fonken et al., 2009). One possible explanation for the findings is that dim light at night is stressful and alters baseline circulating glucocorticoid concentrations, which may in turn influence behavior. Though previously I observed differences in the diurnal rhythms of cortisol concentrations (Chapter 2), I found that cortisol concentrations were equivalent between groups at the time of testing both after 4 weeks in lighting conditions and at the conclusion of the study.

The observation of increased depression-like behavior in coincidence with attenuated anxiety is unexpected, given that depression and anxiety are often comorbid in humans (Fawcett and Kravitz, 1983). It is possible that, in my model, the elevated plus maze is more reflective of a novelty-seeking or impulsive aspect of the phenotype rather than anxiety per se. In this case, hamsters exposed to dim LAN may be displaying a phenotype more reminiscent of depressive subtypes which coincide with impulsive risk-taking behavior, such as substance abuse disorders or suicide attempts. Indeed, some studies regarding rodent chronic stress models have shown anhedonia to coexist with heightened novelty-seeking behavior (Shumake et al., 2005; Li et al., 2010); however, further behavioral testing is necessary to either support or refute these relations in my model.
It is important to note that I have previously shown no differences in light phase activity levels of animals chronically exposed to dim light at night compared to those housed in a standard light-dark cycle (Chapter 2). Therefore, despite the activity-dependent nature of two of my behavioral measures, the present findings are unlikely to be confounded by differences in amount of activity.

Although the precise mechanism underlying the link between light at night and mood remains to be specified, one putative mechanism could be via suppression of pineal melatonin. Temporal organization of physiological processes relies largely on light information being transduced into a hormonal signal and circulated throughout the body. During the day, light is received by the photoreceptive retinal ganglion cells of the eye and then transmitted via the retinohypothalamic tract to the suprachiasmatic nuclei (SCN), the master circadian pacemaker. The SCN controls production and secretion of the pineal hormone, melatonin, which is secreted into the bloodstream only during the night, making it a useful physiological cue for nighttime (Reiter, 1993). However, exposure to light at night robustly suppresses melatonin secretion, thus distorting the body’s time of day information (Navara and Nelson, 2007). In my study, 5 lux dim light at night was likely sufficient to suppress melatonin; illumination levels as low as 1.08 lux inhibit pineal melatonin production in Syrian hamsters (Mesocricitus auratus) (Brainard et al., 1982).

As mentioned in Chapter 1, melatonin may play a role in mood. Agomelatine, a melatonin-receptor agonist which also antagonizes serotonin (5-HT$_{2c}$) receptors, is an effective anti-depressant (Goodwin et al., 2009; Kennedy and Rizvi, 2010). Furthermore,
melatonin itself has immunomodulatory effects on neuroinflammation (Maldonado et al., 2009), as well as the ability to re-entrain disrupted circadian rhythms (Pandi-Perumal et al., 2006), both potential anti-depressive mechanisms. Given this link between melatonin and depression, as well as the melatonin-suppressing effect of light at night, it is plausible that the observed behavioral changes may be related to melatonin suppression. Future studies will determine whether the reported effects are reversible by returning hamsters to standard light-dark exposure, thus restoring melatonin secretion among other effects, after chronic exposure to LAN.

**Hippocampal Plasticity**

A large literature demonstrates the morphological changes that hippocampal pyramidal cells undergo when exposed to stress and glucocorticoids (For review: McEwen, 2008). However, to the best of my knowledge, this is the first study to document LAN as a sufficient stimulus to induce changes in hippocampal plasticity. I measured dendritic spine density on segments at least 50µm from the cell body, thus targeting my analysis to the primary sites of excitatory neuronal input (Megias et al., 2001; von Bohlen Und Halbach, 2009). Therefore, my observation that dim LAN reduces dendritic spine density is likely reflective of reduced excitatory input to hippocampal CA1 pyramidal cells. It is noteworthy that dendritic spine morphology relays information about the strength and maturity of the spine and associated synapse (Yoshihara et al., 2009); however, in the present study I measured density of total spines and did not distinguish between morphological classifications of spines. This is an area that will be addressed in Chapter 7.
The observation that these changes correlate with the depressive behavioral responses suggests a functional role of decreased CA1 spine density in depressive affect induced by light at night. In support of this, studies show that in female rodents, reduced spine density in the hippocampus correlates with learned helplessness (Hajszan et al., 2010). Hippocampal spine density has also been linked to several neuropsychiatric disorders, and particularly depression via the neurotrophin hypothesis, which suggests that depressive pathology involves altered plasticity of neuronal pathways (Reviewed in: Altar, 1999). Reduced spine density has been associated with MDD in humans (Law et al., 2004) and antidepressant treatment increases CA1 spine density in rats (Hajszan et al., 2005; Norrholm and Ouimet, 2001). Antidepressant treatment also increases brain-derived neurotrophic factor (bdnf) mRNA in rat brain (Altar, 1999). Putatively, insufficient neurotrophic support in depressive disorders could cause structural disorganization in the brain (Angelucci et al., 2005). Rapid changes in spine density in other brain regions, such as prefrontal cortex, have also been implicated in depression (Li et al., 2009). I restricted my analysis to the hippocampus after chronic light treatment in my study, but future studies could investigate structural changes to the prefrontal cortex, as well as the rapidity of these changes.

The mechanism of reduced CA1 dendritic spine density in dim LAN could involve suppression of pineal melatonin production. Melatonin ameliorates corticosterone-induced reduction of spines in the hippocampus of male mice (Crupi et al., 2010) and increases hippocampal neurogenesis (Ramirez-Rodriguez et al., 2009), thus an insufficiency of melatonin in hamsters exposed to LAN may underlie my observations.
Estradiol increases hippocampus dendritic spine density (Woolley and McEwen, 1992), but I avoided this potential confound in the current study by ovariectomy. On the basis of results obtained in this study, future studies could be aimed at elucidating the exact role of melatonin in the current findings, as well as determining whether female steroid hormones and light at night may interact to influence hippocampal plasticity. In contrast, however, CA3 and DG neurons failed to show any morphological changes in response to dim light at night. CA3 neurons are generally resistant to remodeling in response to stress in female rodents compared to males (Galea et al., 1997). A similar effect of sex could account for my finding that CA3 and DG neurons were more resistant to change in response to dim light at night than neurons in CA1.

In conclusion, over the past century exposure to nighttime illumination and the incidence of depressive disorders have increased in tandem, yet there has been little research into a possible connection, despite increasing evidence of the anti-depressant and neuroprotective actions of melatonin. Here I demonstrate that dim light at night is sufficient to provoke depression-like behaviors, which correlate with reduced density of CA1 dendritic spines in the hippocampus of female hamsters. These findings warrant further research into light at night as a putative environmental contributor to depressive disorders.
Figure 3.1. Behavioral effects of LAN exposure.

Depression- and anxiety-like behaviors in the forced swim test (A-B), sucrose intake test (C), and elevated plus maze (D). Hamsters exposed to dim light at night spent more time engaged in immobility, or behavioral despair, in the forced swim test (A) and less time climbing to escape (B) compared with hamsters exposed to dark nights. Hamsters in dim nights consumed less sucrose solution in a test of sucrose intake, a behavioral measure of anhedonia, whereas water consumption was equivalent between groups (C). Dim light reduced anxiety in the elevated plus maze and hamsters spent a greater percent time in the open arms than hamsters in dark (D). Graphs depict mean ± SEM. * indicates p<0.05.
Baseline serum cortisol concentrations at 4 weeks and 11 weeks (terminal) of experimental light cycles. At this morning timepoint, there was no effect of light condition on cortisol concentrations, nor an effect of the duration of time spent in the condition (i.e., 4 week vs. 11 week; p>0.05 in each comparison).

**Figure 3.2.** Serum cortisol concentrations following exposure to LAN.
Figure 3.3. Hippocampal morphology following exposure to LAN.

Dendritic spine density on apical and basilar dendrites in the CA1 region of the hippocampus. Hamsters exposed to dim light at night significantly reduced dendritic spine density on both apical (A-C) and basilar (D-F) dendrites. Graphs depict mean ± SEM. * indicates p<0.05.
Figure 3.4. Correlations of depressive-like behaviors and CA1 apical dendritic spine density.

Sucrose intake positively correlated with spine density on CA1 apical dendrites such that less consumption (i.e., high anhedonic symptoms) correlated with reduced spine density (A). Immobility in the forced swim test negatively correlated with apical dendritic spine density in CA1 such that more float time (i.e., high behavioral despair) correlated with reduced spine density (B).
Table 3.1. Complete hippocampal morphology following LAN exposure.

<table>
<thead>
<tr>
<th></th>
<th>Apical Dendrite Length</th>
<th>Basal Dendrite Length</th>
<th>Cell Body Perimeter</th>
<th>Cell Body Area</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dim</td>
<td>1344.6 ± 124.1</td>
<td>993.8 ± 100.7</td>
<td>69.4 ± 2.3</td>
<td>275.2 ± 14.7</td>
</tr>
<tr>
<td>Dark</td>
<td>1472.7 ± 113.7</td>
<td>1145.5 ± 40.2</td>
<td>67.6 ± 2.5</td>
<td>271.0 ± 11.4</td>
</tr>
</tbody>
</table>

Morphological characteristics of hippocampal CA1, CA3, and dentate gyrus (DG) neurons represented as mean micrometers ± SEM.
CHAPTER 4

DEPRESSIVE-LIKE BEHAVIOR IS RESPONSIVE TO SSRI TREATMENT

Exposure to light at night (LAN) is a relatively new phenomenon in human history, having arisen only since the widespread adoption of the electric light bulb about one hundred and thirty years ago. LAN has allowed humans to cultivate 24-h societies; 99% of individuals living in the U.S. or Europe experience nightly light pollution (Navara and Nelson, 2007) and about 20% of workers in any urban economy work night shifts (Rajaratnam and Arendt, 2001). LAN appears to disrupt circadian organization either by suppression of pineal melatonin secretion, or inappropriate phase relationships among various molecular, cellular, and physiological responses. Pineal melatonin production occurs during the night in both diurnal and nocturnal species; exposure to light robustly suppresses its secretion. Dysregulation of the daily melatonin rhythm distorts the body’s time of day information, which could disrupt circadian clock gene expression and hormone secretion (Stehle et al., 2003), which have been linked to mood (Bunney and Bunney, 2000). Shift workers and individuals exposed to disruptive LAN are at increased risk of mood disorders (Meyrer et al., 2009; Driesen et al., 2010; Rajaratnam et al., 2011; Rosenberg and Doghramji, 2011), but the precise mechanism linking LAN to mood remains unspecified.
The hippocampus is one brain structure involved in the pathophysiology of major depression. Depressed individuals show characteristic hippocampal atrophy (Sheline et al., 1999; Frodl et al., 2002), and depression symptoms have been linked to reductions in the complexity of hippocampal neurons, including reduced numbers of dendritic spines (Hajszan et al., 2005; Hajszan et al., 2009; Hajszan et al., 2010). Antidepressant treatment with a selective serotonin reuptake inhibitor (SSRI) ameliorates dendritic spine deficits in rats (Hajszan et al., 2005). Melatonin receptors are present in the rodent hippocampus and melatonin itself modulates neuronal excitability in the hippocampus (Musshoff et al., 2002). Furthermore, melatonin administration prevents the depressive-like behavior and reduction in hippocampal neuron complexity observed under a chronic stress paradigm (Crupi et al., 2010). These observations make the hippocampus an interesting target for studies in my model.

Citalopram is a selective serotonin reuptake inhibitor with high selectivity for the serotonin transporter over other transporter proteins. It is an effective and commonly prescribed treatment for depression, but distinct from other pharmacotherapeutics in its class because it also has linear pharmacokinetics (Keller, 2000). Evidence suggests citalopram does not affect spontaneous locomotor activity in rodents (Cervo et al., 2005), making it an attractive choice for behavioral studies. Whether SSRI treatment is effective for treating depression specifically related to circadian disruption by exposure to LAN, however, remains unknown.

In Chapter 3, I demonstrated that chronic exposure to dim LAN provokes depressive-like behaviors in ovariectomized female hamsters and reduces the number of
dendritic spines on hippocampus CA1 pyramidal neurons (Bedrosian et al., 2011b). Hamsters were ovariectomized in order to prevent any confounding effects of fluctuating estrogen levels on hippocampal morphology (Woolley and McEwen, 1992). The effect of chronic SSRI treatment in this model has remained unspecified. In order to investigate this question, I hypothesized that 2 weeks of treatment with citalopram would improve depressive-like behavior and the reduction in dendritic spine density observed under LAN.

Methods

Animals

Forty adult female Siberian hamsters (Phodopus sungorus) were obtained from our breeding colony at The Ohio State University. This species is nocturnal and exhibits a robust nightly melatonin rhythm. Hamsters were individually housed in polypropylene cages (30 cm x 15 cm x 14 cm) at a constant ambient temperature of 22 ± 2°C and relative humidity of 50 ± 5%. Food (Harlan Teklad 8640, Indianapolis, IN, USA) and filtered tap water were available ad libitum. Prior to starting the experiments, all hamsters (8 weeks of age) were ovariectomized under isoflurane anesthesia and allowed to recover for 1 week. Following the recovery period, hamsters were maintained in either control (N=20) or experimental (N=20) lighting conditions as described below. The control condition was the same as the standard colony room, which was a 16:8 light/dark cycle (150 lux/0 lux), and the experimental condition was a 16:8 light/dim light cycle (150 lux/5 lux). Both the bright and dim lights were typical “cool white” fluorescent bulbs. In both conditions, the bright lights were illuminated at 22:00 h. All experimental
procedures were approved by The Ohio State University Institutional Animal Care and Use Committee.

**Drug Administration**

After 4 weeks in experimental lighting conditions, citalopram (0.16g/L) was administered in the drinking water with 1% saccharin to mask any taste of the drug, according to previous studies (Warner-Schmidt et al., 2011b; Warner-Schmidt et al., 2011a). Control groups (N=10 from each lighting condition) drank 1% saccharin solution. Chronic treatment lasted 18 d and behavioral testing and tissue collection occurred during the last 4 d of treatment. Water consumption was measured twice weekly. Hamsters remained in experimental lighting conditions throughout the duration of the study.

**Forced swim test**

To assess depression-like behavioral responses in the forced swim test (Porsolt et al., 1977), hamsters were placed in an opaque cylindrical tank filled with room-temperature water (22 ± 1°C) for 10 min. Behavior was recorded on video and the full 10 min of test time was subsequently scored with Observer software (Noldus, Wageningen, The Netherlands) by an observer unaware of assignment to experimental groups. The behaviors scored were: 1) climbing (i.e., vigorous swimming or scratching directed at the wall of the tank), 2) swimming (i.e., horizontal movement in the tank), and 3) floating/immobility (i.e., minimal movement necessary to keep head elevated above water surface).
**Analysis of hippocampal morphology**

Hamsters were deeply anesthetized with isoflurane vapors and rapidly decapitated between 10:00 and 12:00 h a day after the conclusion of behavior testing. Brains were quickly removed and 20 brains were randomly chosen to be processed for Golgi-Cox staining using a Rapid GolgiStain Kit (FD NeuroTechnologies) as previously described (Bedrosian et al., 2011b). Briefly, brains were submerged in Golgi-Cox solution and stored for 14 days in the dark, followed by a 30% sucrose solution for 4 days. Brains were then rapidly frozen and 100 µm coronal sections were sliced on a cryostat and collected onto gelatin-coated glass slides. The stain was developed in NH₄OH for 10 min and sections were counterstained with cresyl violet. Finally, slides were dehydrated through a series of graded ethanol washes, cleared with xylene, coverslipped with Permount, and dried in the dark for at least 1 wk.

Neurons impregnated with the Golgi-Cox solution were chosen within the CA1 region of the hippocampus based on our previously observed differences in this region. Only neurons that were fully impregnated, not obscured by neighboring neurons, and had no obviously truncated dendrites were chosen for analysis. For each animal, 4-6 randomly chosen, representative neurons from different sections were chosen. Dendritic spines were traced in each neuron at 100x (N.A. 1.30) in 4 apical and 4 basilar randomly chosen, representative dendrite segments of at least 20 µm in length, and at least 50 µm distal to the cell body, using Neurolucida 8 software (MicroBrightField, Williston, VT, USA) for PC and a Nikon Eclipse E800 brightfield microscope. Dendritic spine density
was analyzed using Neurolucida Explorer software (MicroBrightField, Williston, VT, USA).

Statistical analyses

Water consumption was compared for each day using one-way ANOVA with treatment (vehicle vs. citalopram) as the independent variable. Behavior and brain morphology data were analyzed using two-way ANOVA with lighting condition (dark vs. dim LAN) and drug treatment (vehicle vs. citalopram) as the independent variables. Main effects were followed up with Fisher’s post-hoc comparisons. Statistics were performed using Statview 5.0.1 for Windows PC. Mean differences were considered statistically significant when p \leq 0.05.

Results

Water consumption

Water intake was sampled twice weekly throughout the 2 wk of citalopram treatment by weighing bottles at baseline and then again 24 h later. Over the first 24 h of treatment, hamsters receiving citalopram in the water consumed less than hamsters receiving vehicle only \((F_{1,37}=14.46, p<0.05)\). At all subsequent sampling dates, however, hamsters consumed equivalent amounts of water regardless of treatment \((p>0.05\) in all cases), averaging approximately 4 ml per day (Figure 4.1).

Depressive-like behavior

Hamsters were tested for depressive-like behaviors in the forced swim test by assessing time spent climbing versus immobile and latency to float. Hamsters exposed to
dark nights spent more time in escape-directed behavior (i.e., climbing) compared to hamsters housed in dim LAN ($F_{1,33}=5.052, p<0.05$). There was also a main effect of treatment ($F_{1,33}=4.177, p<0.05$). As a planned comparison, I found that citalopram-treated hamsters in dim LAN spent a greater duration climbing compared to vehicle-treated hamsters in dim LAN (post-hoc, $p<0.05$; Figure 4.2a). Immobility, generally interpreted as behavioral despair in this test, was increased in hamsters housed in dim LAN ($F_{1,33}=4.826, p<0.05$). There was also an effect of treatment ($F_{1,33}=4.300, p<0.05$) and a light x treatment interaction effect ($F_{1,33}=4.856, p<0.05$). Citalopram-treated hamsters in dim LAN spent less time immobile than vehicle-treated hamsters in dim LAN (post-hoc, $p<0.05$; Figure 4.2b). Furthermore, latency to first become immobile was reduced in hamsters in dim LAN ($F_{1,33}=17.720, p<0.05$) with an effect of treatment ($F_{1,33}=9.277, p<0.05$) and a light x treatment interaction ($F_{1,33}=8.261, p<0.05$). Citalopram-treated hamsters in dim LAN had a greater latency to float compared to vehicle-treated hamsters in dim LAN (post-hoc, $p<0.05$; Figure 4.2c).

**Hippocampal morphology**

Dendritic spine density on hippocampal CA1 pyramidal neurons was reduced in dim LAN compared to dark nights on both apical ($F_{1,15}=6.781, p<0.05$) and basilar ($F_{1,15}=8.899, p<0.05$) dendrites, without an effect of citalopram treatment (Figure 4.3a-b). Following up this effect with planned comparison post-hoc analyses, however, revealed a subtle distinction. Vehicle-treated hamsters in dark vs. dim LAN had significantly different levels of spine density (post-hoc, $p<0.05$), whereas citalopram-treated hamsters
in dim LAN were not significantly different from either dark or dim LAN vehicle-treated groups, suggesting a slight improvement with treatment.

**Discussion**

Exposure to artificial LAN has grown in prevalence during the past 100 years, which has allowed humans to stray from natural day-night cycles, potentially provoking circadian dysregulation and changes in physiology and behavior. Our previous studies have shown that LAN provokes depressive-like behavior in rodents (Fonken et al., 2009; Bedrosian et al., 2011b). The response of this phenomenon to an SSRI, the standard in depression treatment, has remained unknown. In this study, I demonstrate for the first time that the selective serotonin reuptake inhibitor, citalopram, prevents the expression of depressive-like behaviors in the forced swim test after chronic LAN exposure, without affecting non-depressed hamsters housed without LAN. Citalopram did not, however, fully restore hippocampal dendritic spine density after LAN exposure. This study suggests that our LAN paradigm can serve as a depression model in which antidepressant treatment is selectively effective in hamsters showing depressive-like behavior.

Citalopram is a selective serotonin reuptake inhibitor with high selectivity for 5-HT over other transmitters. Citalopram inhibits uptake of 5-HT 3,400 times more potently than of norepinephrine and 22,000 times more potently than of dopamine, making it more selective than many other SSRIs, including fluoxetine, paroxetine, and sertraline (Keller, 2000). I chose citalopram because it is an effective and commonly prescribed treatment for depression, but distinct from other pharmacotherapeutics in its class because it also has linear pharmacokinetics (Keller, 2000). Evidence suggests
citalopram does not affect spontaneous locomotor activity in rodents (Cervo et al., 2005), thus would not bias my results from the forced swim test. This notion is further supported by the finding that citalopram did not affect forced swim test behavior in hamsters exposed to dark nights.

SSRI treatment promotes dendritic complexity of hippocampal neurons and formation of pyramidal dendritic spine synapses (Hajszan et al., 2005; Bessa et al., 2009; Banasr et al., 2011). Dendritic spines are highly plastic structures, capable of changing within minutes in response to certain environmental stimuli (Fischer et al., 1998). In this study, dim LAN reduced the number of dendritic spines on both apical and basilar dendrites of CA1 pyramidal neurons. Because I targeted my analysis to dendritic segments at least 50 µm from the cell body, I restricted the analysis to the primary sites of excitatory neuronal input (Megias et al., 2001; von Bohlen Und Halbach, 2009). Thus, my results may reflect diminished excitatory input to CA1 pyramidal neurons in LAN. Under the dim LAN conditions tested in this experiment, only slight increases in CA1 dendritic spine density were observed with citalopram treatment, but not full recovery of dark levels. This may reflect other constraints on dendritic spine density or other factors, such as stress hormone concentrations or lack of melatonin, which cannot be overcome by SSRI treatment alone. Importantly, I measured overall spine numbers and did not distinguish between morphological classifications of spines in this study. Because morphology provides information about the strength and maturity of the spine and its associated synapse (Yoshihara et al., 2009), distinguishing by spine type is an interesting area for investigation and may reveal more nuanced effects of citalopram treatment.
On a broader level, the upstream mechanism linking LAN to behavioral and neuronal changes remains unspecified. One possibility is that reduced melatonin secretion under LAN conditions provokes a cascade of downstream effects. Temporal organization of physiological processes relies largely on the transduction of light information into a hormonal signal that is circulated throughout the body. During the day, light received by the intrinsically photoreceptive retinal ganglion cells of the eye is transmitted via the retinohypothalamic tract to the suprachiasmatic nuclei (SCN). The SCN in turn regulates production and secretion of the pineal hormone, melatonin, which is secreted into the bloodstream during the dark, making it a useful physiological cue for nighttime (Reiter, 1993). Exposure to LAN, however, suppresses production of melatonin, thus distorting natural time of day information (Navara and Nelson, 2007). In this study, I used 5 lux LAN, that is likely sufficient to suppress melatonin levels. Light levels as low as 1.08 lux inhibit pineal melatonin production in Syrian hamsters (Brainard et al., 1982). Importantly, melatonin has been implicated in mood. In rodents, melatonin administration prevents stress-induced depression-like behaviors and reductions in hippocampal dendritic complexity (Crupi et al., 2010).

Overall, the results of this study validate our LAN paradigm as a depression model in which depression-like behavior in dim LAN is selectively improved by citalopram treatment, without any effect of the drug on hamsters in dark nights. This is the first study, to my knowledge, to validate the forced swim test using an SSRI in this particular species. The results also suggest that SSRI treatment may be equally effective for treating depression related to LAN, in populations such as shift workers, as for
treating general major depression. Nonetheless, more attention should be given to LAN as a potential circadian disruptor with downstream effects on physiology and mood.
**Figure 4.1.** Experimental timeline and liquid intake.

Experimental timeline for treatment and testing (A). Water intake sampled four times throughout the two weeks of treatment differed only during the first 24 h in citalopram vs. vehicle groups (B). OVX: ovariectomy; *p<0.05 vehicle vs. citalopram, day 1.
Figure 4.2. Effect of citalopram treatment on depressive-like response with LAN exposure.

Hamsters housed in dim LAN spent less time climbing in the forced swim test, but citalopram treatment ameliorated this deficit (A). Dim LAN also provoked more time spent immobile, but citalopram treatment reduced this response (B). Latency to immobility was reduced in dim LAN hamsters, but was enhanced with citalopram (C). *p<0.05 post-hoc, dim LAN vehicle vs. each other group.
Figure 4.3. Effect on citalopram treatment on hippocampal dendritic spine morphology with LAN.

Representative photomicrographs of apical dendritic spines in dark (top) and dim LAN (bottom) in CA1 (A). CA1 dendritic spine density was reduced in hamsters housed in dim LAN on both apical (B) and basilar (C) dendrites. Spine density on citalopram-treated hamsters in dim LAN was not significantly different from either dark or dim LAN vehicle-treated groups, suggesting a slight improvement with treatment. #p<0.05 planned comparisons between vehicle groups.
CHAPTER 5

LIGHT AT NIGHT REVERSIBLY ALTERS HIPPOCAMPAL STRUCTURE AND GENE EXPRESSION

Major depressive disorder (MDD) poses an enormous burden worldwide and is subject of intensive research, yet its etiology remains poorly defined. Rates of major depression have increased in recent decades and women are twice as likely as men to develop the disorder (Simon and VonKorff, 1992; Kessler et al., 1993; Compton et al., 2006). Better diagnoses or changing diagnostic criteria have traditionally been suggested as possible contributors to this phenomenon; however, additional variables are likely involved. For instance, environmental influences play a role in the onset of depressive pathology and it is possible that recent environmental changes may partially account for the increasing MDD incidence.

Chronic exposure to artificial light at night (LAN) was recently identified to pose several health risks for humans. Accumulating evidence suggests that LAN may contribute to the risk of breast cancer, heart disease, obesity, and mood disorders, though its relationship with mood is understudied (Ha and Park, 2005; Dumont and Beaulieu, 2007; Kloog et al., 2008; Wyse et al., 2011). Electric light has permitted humans to cultivate 24-hour societies; such unnatural conditions almost certainly have multiple
repercussions for physiology and mood, likely acting foremost as a circadian disruptor. As artificial LAN is a relatively new phenomenon in human history, having arisen only since the widespread adoption of the electric light bulb about one hundred thirty years ago, the mechanisms underlying its physiological implications remain unspecified.

The hippocampus is a critical structure in the pathophysiology of MDD. Depressed patients show characteristic hippocampal atrophy (Sheline et al., 1999; Frodl et al., 2002) and dysregulation of many hippocampal-related systems, such as stress coping and memory (Halbreich et al., 1985; McEwen, 2003; Gallassi et al., 2006). Similarly, loss of hippocampal dendritic spines is observed in animal models of chronic stress and depression (Hajszan et al., 2005; Hajszan et al., 2009; Hajszan et al., 2010). In these stress models, expression of brain-derived neurotrophic factor (BDNF) is reduced in the hippocampus, but antidepressant drugs enhance its expression (Duman and Monteggia, 2006).

Suppression of pineal melatonin secretion by LAN is one putative mechanism linking it to depressive affect and the hippocampus. Melatonin is produced during the night in both diurnal and nocturnal species; exposure to light suppresses its secretion. Melatonergic antidepressants improve mood in depressed patients (Hickie and Rogers, 2011). In rodents, melatonin administered under chronic stress conditions prevents the development of depression-like behaviors and reduced hippocampal plasticity (Crupi et al., 2010).

In this study, I investigated the effects of chronic exposure to 5 lux LAN on depression-like behaviors and dendritic spine density of CA1 pyramidal neurons in
female hamsters. This level of illumination is approximately five times brighter than maximal moonlight, comparable to the levels of light pollution surrounding urban centers, and is sufficient to suppress melatonin production in hamsters (Brainard et al., 1982). I also determined whether these changes are reversible when the LAN stimulus is removed. Furthermore, I characterized gene expression of BDNF in the hippocampus. I hypothesized that dim LAN provokes reversible changes in locomotor activity patterns, depression-like behaviors, hippocampal BDNF gene expression levels, and spine density on hippocampal neurons.

**Materials and Methods**

**Animals**

Adult female Siberian hamsters (*Phodopus sungorus*) were obtained from our breeding colony at The Ohio State University. Hamsters were individually housed in polypropylene cages (30 cm x 15 cm x 14 cm) at a constant ambient temperature of 22±2°C and relative humidity of 50±5%. Food (Harlan Teklad 8640, Indianapolis, IN, USA) and filtered tap water were available *ad libitum*. Prior to starting the experiments, all hamsters (8 weeks of age) were ovariectomized under isoflurane anesthesia to control for fluctuating steroid concentrations, which could confound measures of dendritic spine density (Woolley and McEwen, 1992), and then allowed to recover for 1 week. Following the recovery period, hamsters were maintained in either control or experimental lighting conditions as described below. The control condition was the same as the standard colony room, which was a 16:8 h light/dark cycle (150 lux/0 lux), and the experimental condition was a 16:8 h light/dim light schedule (150 lux/5 lux). Both the
bright and dim lights were typical fluorescent bulbs of the same wavelength. In both conditions, the bright lights were illuminated at 22:00 h. All experimental procedures were approved by The Ohio State University Institutional Animal Care and Use Committee.

Experimental Design

The aim of this experiment was to investigate and reverse the depressive effects of LAN by returning hamsters to a standard light/dark cycle after 4 weeks of nightly exposure to dim light. One control group was maintained for the 8 week duration of the study in the standard light/dark cycle (Dark; N=6) and a second group experienced the dim LAN for the last 4 weeks of the experiment (Dim; N=7). In order to determine a time course for the reversal, three experimental groups were used (see Figure 5.1). Each of these groups experienced dim LAN for 4 weeks, and then was returned to the standard light/dark cycle for either 1 week (Dim-1 wk Dark; N=7), 2 weeks (Dim-2 wk Dark; N=7), or 4 weeks (Dim-4 wk Dark; N=7) before behavioral testing began. Homecage activity, immobility in the forced swim test, and sucrose preference were determined as described below. Following testing, brains were collected for Golgi-Cox staining and qPCR. Figure 5.1 shows a schematic of the experimental design.

Behavioral assays

Homecage locomotor activity data were recorded using an infrared beam break system (Columbus Instruments, Columbus, OH). Actigraphs were constructed using ClockLab software (Actimetrics) and data were analyzed for fast Fourier transform (FFT)
power where applicable. To assess depression-like behavioral responses in Porsolt’s forced swim test (Porsolt et al., 1977), hamsters were placed in an opaque cylindrical tank filled with room-temperature water (22 ± 1°C) for 10 min. I have validated the forced swim test for hamster depressive responses in our laboratory. Testing occurred during the light phase between 08:00 and 12:00 for two reasons: 1) locomotor activity levels were different between groups during the dark phase, but not the light phase, and the forced swim test is an activity-dependent measure of depression; and 2) experimental light manipulations occurred during the dark phase, but conditions were equivalent during the light phase. As above, behavior was recorded on video and subsequently scored with Observer software (Noldus, Wageningen, Netherlands) by an observer unaware of assignment to experimental groups. The behaviors scored were: 1) climbing (i.e., vigorous swimming or scratching directed at the wall of the tank), 2) swimming (i.e., horizontal movement in the tank), and 3) floating/immobility (i.e., minimal movement necessary to keep head elevated above water surface). Sucrose preference was determined by measuring intake of a 1% sucrose solution over 24 h. Reduced sucrose preference is interpreted as an anhedonic response and is interpreted as a depressive-like state (Willner et al., 1992). To acclimatize the animals to the novel solution, hamsters were presented with a bottle containing normal drinking water and the bottle containing sucrose solution over the weekend and left undisturbed for three days. On the fourth day each bottle was weighed, replaced in the cage, and then subsequently weighed again 24 hours later. To control for possible side preferences, placement of the bottles in each cage was counterbalanced.
Analysis of hippocampal morphology

Hamsters were deeply anesthetized with isoflurane vapors and rapidly decapitated between 10:00 and 12:00 h at the conclusion of behavior testing. Brains were quickly removed and dissected into two hemispheres. One hemisphere from each brain was randomly chosen to be processed for Golgi-Cox staining using a Rapid GolgiStain Kit (FD NeuroTechnologies). Briefly, brains were submerged in Golgi-Cox solution and stored for 14 days in the dark, followed by a 30% sucrose solution for 4 days. Brains were then rapidly frozen and 100 µm coronal sections were sliced on a cryostat and collected onto gelatin-coated glass slides. The stain was developed in NH₄OH for 10 min and sections were counterstained with cresyl violet. Finally, slides were dehydrated through a series of graded ethanol washes, cleared with xylene, coverslipped with Permount, and dried in the dark for at least 1 week.

Neurons impregnated with the Golgi-Cox solution were chosen within the CA1 region of the hippocampus based on our previously observed differences in this region. Only neurons that were fully impregnated, not obscured by neighboring neurons, and had no obviously truncated dendrites were chosen for analysis. All analyses and selection of neurons were performed by an experimenter unaware of assignment to experimental groups. For each animal, 4-6 randomly selected representative neurons from different sections were chosen. Dendritic spines were traced in each neuron at 100x (N.A. 1.30) in 4 apical and 4 basilar randomly chosen representative dendrite segments of at least 20 µm in length, and at least 50 µm distal to the cell body, using Neurolucida 8 software (MicroBrightField, Williston, VT, USA) for PC and a Nikon Eclipse E800 brightfield.
microscope. Dendritic spine density was analyzed using Neurolucida Explorer software (MicroBrightField, Williston, VT, USA).

**Bdnf gene expression**

Hippocampal *bdnf* gene expression was assayed using quantitative real-time PCR. The randomly-chosen brain hemisphere not used for hippocampal morphology was frozen in RNAlater (Applied Biosystems, Foster City, CA) at -80°C until use. Total RNA was extracted from ≤30 mg of individual hippocampi using a homogenizer (Ultra-Turrax TB; IKA Works, Inc., Wilmington, NC, USA) with an RNeasy Mini Kit (Qiagen) according to the manufacturer’s guidelines. Extracted RNA was suspended in 30 µL RNase-free water and RNA concentration was determined by spectrophotometer (NanoDrop-1000, Nanodrop Technologies, Wilmington, DE, USA). RNA samples were stored at -80°C until further analysis. cDNA was created via reverse transcription of 2 µg RNA with MMLV Reverse Transcriptase enzyme (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's guidelines. The *Bdnf* probe sequence (labeled with 6-FAM) was CCGAACTACCCAGTC, the forward primer was AAGGCACTGGAACTCGCAAT and the reverse primer was CCATAGTAAGCGCCCGAACA. A TaqMan 18S ribosomal primer and probe set (labeled with VIC, Applied Biosystems) was used as the control gene for relative quantification. Amplification was performed on an Applied Biosystems 7500 Fast Real-Time PCR System by using TaqMan Universal PCR Master Mix. cDNA samples were run at 1:10 dilution. The universal two-step RT-PCR cycling conditions used were: 50°C for 2 min, 95°C for 10 min, followed by 40 cycles of 95°C for 15 s and 60°C for 1 min.
Relative gene expression of individual samples run in duplicate was calculated by comparison to a standard curve consisting of serial dilutions of *P. sungorus* hippocampal cDNA (1:10, 1:100, 1:1000, 1:10000) followed by normalization to 18S rRNA gene expression.

**Statistical analysis**

Independent groups were compared against the control group by planned comparisons using unpaired Student’s *t*-tests. Immobility data in the forced swim test were log transformed prior to analyses due to inequality of variances and some brains were lost due to crumbling during sectioning for Golgi-Cox staining. Statistics were performed using Statview 5.0.1 for Windows PC. Mean differences were considered statistically significant when *p* was ≤0.05.

**Results**

**Behavior**

Homecage locomotor activity was monitored for each group during the last week of the experiment prior to behavioral testing. Chronic exposure to dim LAN reduced dark phase activity relative to hamsters housed in a standard light-dark cycle (*t*<sub>12</sub>=-1.927, *p*<0.05), but had no effect on activity in the light phase (*p*>0.05; Figure 5.2a). Actigraphs show composite activity data over several days from hamsters housed in each lighting condition (Figure 5.2a inset). Dim LAN reduced the 24 h FFT power of the locomotor activity rhythm (*t*<sub>12</sub>=-4.599, *p*<0.001), but within 1 week of return to the light-dark cycle this measure was restored and 1 wk, 2 wk, and 4 wk groups did not differ from hamsters
exposed to dark nights the entire duration of the study (p>0.05 in all cases; Figure 5.2b). Depression-like behaviors were measured using Porsolt’s forced swim test and sucrose preference. Time spent immobile in the forced swim test was increased after 4 weeks exposure to dim LAN (t_{11}=2.081, p<0.05; Figure 5.3a). Within 1 week of return to the light-dark cycle, hamsters displayed an intermediate duration of immobility that was not different from either control group (p>0.05). At 2 weeks and 4 weeks, hamsters spent significantly less time immobile than hamsters exposed to dim LAN (t_{12}=2.346, p<0.05 and t_{13}=3.185, p<0.01, respectively), but equivalent to the group exposed to dark nights (p>0.05). Sucrose preference was reduced by exposure to dim LAN (t_{11}=-2.769, p<0.05; Figure 5.3b). At 2 and 4 weeks of return to the standard light-dark cycle, hamsters exhibited greater sucrose preference than the group exposed to LAN (t_{12}=-1.584, p<0.05 and t_{12}=-2.424, p<0.05, respectively), but equivalent to the group exposed to dark nights (p>0.05).

**Hippocampal morphology and bdnf expression**

Hippocampal bdnf gene expression was reduced in hamsters exposed to dim LAN (t_{9}=3.962, p<0.01), but restored to a level equivalent to the dark nights group in all three groups that returned to dark nights after dim LAN exposure (p>0.05 in all cases; Figure 5.3c).

Dim LAN reduced apical dendritic spine density on CA1 pyramidal cells relative to hamsters exposed to dark nights (t_{12}=-1.837, p<0.05). At 1 week after returning to dark nights from dim LAN, hamsters had an intermediate spine density indistinguishable from dark- and dim-exposed groups (p>0.05 for both comparisons; Figure 5.3d). After 2 weeks
of return to dark nights, hamsters had restored dendritic spine density compared to those in dim LAN ($t_{12}=-3.113$, $p<0.01$) and were statistically equivalent to hamsters exposed only to dark nights ($p>0.05$).

Discussion

The prevalence of major depression has increased in recent decades and women are more susceptible than men; however, a complete set of contributing factors remains unspecified. On the one hand, genetic predisposition plays a role in the onset of major depression, but the increase in incidence over the past several decades has occurred too rapidly for genetic shifts in human populations to entirely account for the phenomenon. Environmental influences also play a role in the onset of depressive disorders, so it is possible that environmental changes may partially account for the change in incidence. Many of these factors have existed for centuries, whereas others are relatively new. One relatively new environmental change which emerged during the 20th century is the growing prevalence of exposure to artificial LAN. The advent of electrical lighting permitted humans to stray from natural day-night cycles, potentially provoking circadian dysregulation and consequent changes in physiology and behavior.

I tested the hypothesis that LAN provokes depression-like changes using female hamsters as a model system. My results demonstrate that hamsters exposed to chronic dim LAN alter locomotor activity patterns, depression-like behaviors, reduce hippocampal $bdnf$ expression, and reduce CA1 dendritic spine density. These changes were reversed within 2 weeks of removing the LAN.
Light is a potent entraining cue for the circadian system, and circadian abnormalities are prominent features of depressive disorders (Benca et al., 2009). Homecage locomotor activity of hamsters exposed to a standard light-dark cycle peaked during the dark phase and dropped to very low levels during the light phase. In contrast, my results demonstrate that hamsters exposed to dim nocturnal illumination reduced activity levels during the dark phase, but light phase activity levels were equivalent to hamsters without light at night. It is important to note that sleep occurs during the light phase in this nocturnal hamster species, and thus the LAN manipulation likely does not disrupt sleep. The observation that LAN did not disrupt light phase activity lends support to this notion. Additional power spectrum analysis of the 24 h activity rhythm using FFT revealed decreased strength of the 24 h rhythm in hamsters exposed to LAN, but this was reversed within one week of removing the LAN.

Depression-like behaviors were evaluated using Porsolt’s forced swim test and sucrose preference. Hamsters exposed to chronic LAN exhibited more immobility, generally interpreted as behavioral despair (Porsolt et al., 1977), and consumed less sucrose solution in a test of sucrose intake, which is interpreted as an anhedonic-like response (Willner et al., 1992). These represent both activity-dependent and activity-independent depression-like responses, and are consistent with previous studies (Fonken et al., 2009; Bedrosian et al., 2011b). My prior work demonstrated that baseline circulating cortisol is equivalent at the time of testing, but the diurnal patterns are different in dark- vs. LAN-exposed hamsters (Chapter 2; Bedrosian et al., 2011b).
Cortisol could be a contributor to these behavioral changes, though glucocorticoid stress response under LAN remains to be studied.

LAN also reduced hippocampus CA1 dendritic spine density and \textit{bdnf} mRNA expression. I targeted my analysis to dendritic segments at least 50 µm from the cell body, thus capturing the primary sites of excitatory neuronal input (Megias et al., 2001; von Bohlen Und Halbach, 2009). My observation of reduced dendritic spine density is likely reflective of diminished excitatory input to CA1 pyramidal neurons. Dendritic spines are highly plastic structures, changing on the order of minutes in response to environmental stimuli (Fischer et al., 1998). Indeed, spine density was quickly restored upon removing the LAN stimulus in my study. It is important to note that spine morphology also changes rapidly and provides information about the strength and maturity of the spine and its associated synapse (Yoshihara et al., 2009). In the present study I measured total spines and did not distinguish between morphological classifications, but this is an interesting area for future study and will be addressed in Chapter 7.

Hippocampal pyramidal neurons exposed to stress and glucocorticoids undergo morphological changes (for review: McEwen, 2008). Furthermore, reduced spine density in the hippocampus of female rodents correlates with learned helplessness behavior (Hajszan et al., 2010). Reduced spine density has been associated with major depression in humans (Law et al., 2004), and antidepressant treatment increases CA1 spine density (Norrholm and Ouimet, 2001; Hajszan et al., 2005) and \textit{bdnf} mRNA in rats (Altar, 1999). Insufficient neurotrophic support in depressive disorders may cause structural
disorganization in the brain (Angelucci et al., 2005). My finding of reduced bdnf expression in the hippocampus under LAN is consistent with this hypothesis.

Furthermore, the time course of the reversal of depression-like behavior and reduced spine density follows the time course for restoration of bdnf expression. In this study I restricted my analysis to the hippocampus; however, it is noteworthy that recent reports show that depression-like symptoms and reduction in spine density and bdnf in prefrontal cortex neurons can be rapidly reversed by ketamine treatment (Garcia et al., 2008; Li et al., 2010). Therefore, the prefrontal cortex represents an interesting target for future studies using the LAN model.

From a broader perspective, however, the upstream mechanism linking LAN to neuronal changes remains unspecified. One likely candidate linking depressed affect with the hippocampus is the suppression of pineal melatonin secretion caused by LAN. Temporal organization of physiological processes relies largely on the transduction of light information into a hormonal signal that is circulated throughout the body. During the day, light received by the intrinsically photoreceptive retinal ganglion cells of the eye is transmitted via the retinohypothalamic tract to the suprachiasmatic nuclei (SCN). The SCN in turn regulates production and secretion of the pineal hormone, melatonin, which is secreted into the bloodstream only during the dark, making it a useful physiological cue for nighttime (Reiter, 1993). Exposure to LAN, however, robustly suppresses melatonin secretion and thus distorts the body’s time of day information (Navara and Nelson, 2007). The level of illumination used for this study (5 lux LAN) was likely sufficient to suppress melatonin; levels as low as 1.08 lux inhibit pineal melatonin
production in Syrian hamsters (Brainard et al., 1982). Accumulating evidence suggests a role for melatonin in mood. For example, agomelatine, a melatonin-receptor agonist and serotonin (5-HT$_{2C}$) receptor antagonist, is an effective antidepressant (Goodwin et al., 2009; Kennedy and Rizvi, 2010). In rodents, melatonin administration prevents stress-induced depression-like behaviors and reductions in hippocampal dendritic complexity (Crupi et al., 2010).

Overall, my findings suggest that chronic exposure to low levels of LAN may be one contributor to rising rates of MDD in recent decades. Given the growing prevalence of LAN, attention must be given to the physiological effects of this circadian disruptor. This study should direct future research into the effects of LAN on humans.
Figure 5.1. Schematic of experimental design.
Figure 5.2. Homecage locomotor activity.

Hamsters housed in dim LAN reduced dark-phase activity compared to controls (A).
Inset shows composite actigraphs from hamsters exposed to dark nights (top) versus dim LAN (bottom). FFT power was reduced in dim-exposed hamsters compared to dark, but restored within 1 week of removing the LAN (B). *P<0.05.
Figure 5.3. Depression-like behavior and brain changes.

Hamsters exposed to dim LAN spend more time immobile in the forced swim test compared to hamsters exposed to dark nights, a phenomenon that is reversed 2 weeks and 4 weeks following removal of the LAN (A). Dim LAN reduces sucrose preference, but it is restored 2 and 4 weeks following return to dark nights (B). Hippocampal bdnf mRNA expression is reduced by LAN, but is reversed within a week of return to dark nights (C). CA1 spine density on apical dendrites of hippocampal pyramidal neurons was reduced in dim LAN compared to dark nights, but 2 weeks after removal of LAN the levels were equivalent to hamsters exposed to only to dark nights (D). *P<0.05.
Recent evidence has supported a role for inflammation in depressive disorders. In humans, one third of patients treated with recombinant human cytokines develop major depressive disorders (Raison et al., 2006). Similarly, MDD is more prevalent in patients with inflammatory disorders as compared to the general population (Steptoe, 2007). Endotoxin (lipopolysaccharide) administration to rats provokes proinflammatory cytokine expression and depression-like behaviors (Yirmiya, 1996), and it has been suggested that inhibitors of these cytokines might alleviate depressive symptoms (Dantzer et al., 1999). In humans, COX-2 inhibitors reduce production of proinflammatory cytokines and have positive effects on depression symptoms (Muller et al., 2006).

Inflammatory cytokine response is linked to the pathogenesis of affective disorders and changes in dendritic morphology of CA1 pyramidal neurons in the hippocampus (Richwine et al., 2008). Moreover, the hippocampus is a structure disproportionately vulnerable to inflammation because of its high expression of receptors
for pro-inflammatory cytokines such as interleukin (IL) 1β and tumor necrosis factor α (TNF) (Maier and Watkins, 1998).

Recently, a molecular mechanism directly linking circadian clock disruption to brain inflammation (i.e., proinflammatory cytokine expression) was identified (Narasimamurthy et al., 2012). In this experiment, I investigated whether LAN exposure would provoke expression of TNF or IL-1β gene expression within the hippocampus. Further, I asked whether administering an intracerebroventricular (icv) TNF inhibitor under LAN might influence depressive-like responses under LAN exposure. I hypothesized that enhanced TNF expression may have a role in the depression-like phenotype seen under LAN and that a specific icv inhibitor might prevent depression-like behavior from developing.

**Materials and Methods**

**Animals**

Adult female Siberian hamsters (*Phodopus sungorus*) were obtained from our breeding colony at The Ohio State University. Hamsters were individually housed in polypropylene cages (30 cm x 15 cm x 14 cm) at a constant ambient temperature of 22±2°C and relative humidity of 50±5%. Food (Harlan Teklad 8640, Indianapolis, IN, USA) and filtered tap water were available *ad libitum*. Prior to starting the experiments, all hamsters (8 weeks of age) were ovariectomized under isoflurane anesthesia to control for fluctuating steroid concentrations, which could confound measures of dendritic spine density (Woolley and McEwen, 1992), and then allowed to recover for 1 week. Following the recovery period, hamsters were maintained in either control or
experimental lighting conditions as described below. The control condition was the same as the standard colony room, which was a 16:8 h light/dark cycle (150 lux/0 lux), and the experimental condition was a 16:8 h light/dim light schedule (150 lux/5 lux). Both the bright and dim lights were typical fluorescent bulbs of the same wavelength. In both conditions, the bright lights were illuminated at 22:00 h. All experimental procedures were approved by The Ohio State University Institutional Animal Care and Use Committee.

Experimental Design

The aim of this experiment was to first measure gene expression of TNF and IL-1β in the hippocampus, and then to determine whether inhibiting TNF signaling prevents the depression-like behavior and changes in dendritic spine density previously observed. In the first experiment, hamsters were exposed to four weeks of dim LAN and then brains were collected for qPCR analyses. In the second experiment, each hamster was implanted with an osmotic mini-pump connected to a cannula directed into the lateral ventricle to continuously administer the TNF inhibitor, XPro1595 (Xencor, Monrovia, CA, USA). XPro1595 is a highly selective dominant-negative inhibitor of soluble TNF (Zalevsky et al., 2007). Hamsters were anesthetized with isoflurane vapor and a stereotaxic apparatus was used to implant a 28 gauge cannula into the lateral ventricle (cannula position: -0.1 posterior and -0.9 lateral to bregma, extending 2.35 mm below the skull; Plastics One, Roanoke, VA, USA). The cannula was connected by tubing to an Alzet minipump (Model 2006, Durect, Cupertino, CA, USA) implanted subcutaneously in the scapular region that delivered either saline or 5 mg/ml XPro1595 at a rate of 0.15 µl/h for the
duration of the experiment (operational time of pumps ≥6 wk). From the day following surgery, one control group was maintained in a standard light-dark cycle (N=26) and another in dim LAN (N=32) as described above. Four weeks later hamsters were tested for depression-like behavior in the forced swim test and brains were collected for analysis of dendritic spine density in the hippocampal subfield CA1.

Cytokine gene expression

Hippocampal TNF and IL-1β gene expression were assayed using quantitative real-time PCR. Brains were dissected after rapid decapitation and frozen in RNAlater (Applied Biosystems, Foster City, CA) at -80°C until use. Total RNA was extracted from ≤30 mg of individual hippocampi using a homogenizer (Ultra-Turrax TB; IKA Works, Inc., Wilmington, NC, USA) with an RNeasy Mini Kit (Qiagen) according to the manufacturer’s guidelines. Extracted RNA was suspended in 30 µL RNase-free water and RNA concentration was determined by spectrophotometer (NanoDrop-1000, Nanodrop Technologies, Wilmington, DE, USA). RNA samples were stored at -80°C until further analysis. cDNA was created via reverse transcription of 2 µg RNA with MMLV Reverse Transcriptase enzyme (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's guidelines. Previously synthesized primers and probes for Phodopus sungorus labeled with 6-FAM were used for tnf and Il-1β (Pyter et al., 2005). A TaqMan 18S ribosomal primer and probe set (labeled with VIC, Applied Biosystems) was used as the control gene for relative quantification. Amplification was performed on an Applied Biosystems 7500 Fast Real-Time PCR System by using TaqMan Universal PCR Master Mix. cDNA samples were run at 1:10 dilution. The universal two-step RT-PCR cycling
conditions used were: 50°C for 2 min, 95°C for 10 min, followed by 40 cycles of 95°C for 15 s and 60°C for 1 min. Relative gene expression of individual samples run in duplicate was calculated by comparison to a standard curve consisting of serial dilutions of *P. sungorus* hippocampal cDNA (1:10, 1:100, 1:1000, 1:10000) followed by normalization to 18S rRNA gene expression.

Forced swim test

To assess depression-like behavioral responses in Porsolt’s forced swim test (Porsolt et al., 1977), hamsters were placed in an opaque cylindrical tank filled with room-temperature water (22 ± 1°C) for 10 min. We have validated the forced swim test for hamster depressive responses in our laboratory. Testing occurred during the light phase between 08:00 and 12:00 for two reasons: 1) locomotor activity levels were different between groups during the dark phase, but not the light phase, and the forced swim test is an activity-dependent measure of depression; and 2) experimental light manipulations occurred during the dark phase, but conditions were equivalent during the light phase. Behavior was recorded on video and subsequently scored with Observer software (Noldus, Wageningen, Netherlands) by an observer unaware of assignment to experimental groups. The behaviors scored were: 1) climbing (i.e., vigorous swimming or scratching directed at the wall of the tank), 2) swimming (i.e., horizontal movement in the tank), and 3) floating/immobility (i.e., minimal movement necessary to keep head elevated above water surface).
Analysis of hippocampal morphology

Hamsters were deeply anesthetized with isoflurane vapors and rapidly decapitated between 10:00 and 12:00 h at the conclusion of behavior testing. Brains were quickly removed and processed for Golgi-Cox staining using a Rapid GolgiStain Kit (FD NeuroTechnologies). Briefly, brains were submerged in Golgi-Cox solution and stored for 14 days in the dark, followed by a 30% sucrose solution for 4 days. Brains were then rapidly frozen and 100 µm coronal sections were sliced on a cryostat and collected onto gelatin-coated glass slides. The stain was developed in NH$_4$OH for 10 min and sections were counterstained with cresyl violet. Finally, slides were dehydrated through a series of graded ethanol washes, cleared with xylene, coverslipped with Permount, and dried in the dark for at least 1 week.

Neurons impregnated with the Golgi-Cox solution were chosen within the CA1 region of the hippocampus based on our previously observed differences in this region. Only neurons that were fully impregnated, not obscured by neighboring neurons, and had no obviously truncated dendrites were chosen for analysis. All analyses and selection of neurons were performed by an experimenter unaware of assignment to experimental groups. For each animal, 4-6 randomly selected representative neurons from different sections were chosen. Dendritic spines were traced in each neuron at 100x (N.A. 1.30) in 4 apical and 4 basilar randomly chosen representative dendrite segments of at least 20 µm in length, and at least 50 µm distal to the cell body, using Neurolucida 8 software (MicroBrightField, Williston, VT, USA) for PC and a Nikon Eclipse E800 brightfield
microscope. Dendritic spine density was analyzed using Neurolucida Explorer software (MicroBrightField, Williston, VT, USA).

Statistical analyses

qPCR data were analyzed by Student’s $t$-test with lighting condition as the independent variable. Treatment data were analyzed using two-way ANOVA with lighting condition (dark vs. dim LAN) and drug treatment (vehicle vs. XPro1595) as the independent variables. Main effects were followed up with Fisher’s post-hoc comparisons. During behavior testing for the second experiment, a technical problem with the VCR caused loss of data from 13 animals. Final analyzed sample sizes were: N=10 dark veh, N=10 dim veh, N=13 dark DN-TNF, N=12 dim DN-TNF. Statistics were performed using Statview 5.0.1 for Windows PC. Mean differences were considered statistically significant when $p$ was $\leq 0.05$.

Results

Tnf and Il-1β expression

Exposure to dim LAN increased $tnf$ gene expression in the hippocampus compared to the standard light-dark cycle ($t_{10}=1.955$, $p<0.05$). All three groups that returned to the dark nights had expression levels equivalent to the group exposed only to dark nights ($p>0.05$; Figure 6.1a). Exposure to LAN did not affect $Il-1β$ gene expression (Figure 6.1b).
Behavior with TNF inhibitor

I assessed depression-like behaviors after chronic treatment with XPro1595, a dominant negative TNF inhibitor. In the forced swim test there was a main effect of the light condition ($F_{1,41}=4.824$, $p<0.05$) and also a light-by-treatment interaction ($F_{1,41}=9.149$, $p<0.05$) on duration immobile. LAN increased the duration of immobility in vehicle-treated hamsters compared to all three other groups (post-hoc, $p<0.05$ in each case; Figure 6.2a). Hamsters treated with XPro1595 in LAN did not differ in float duration from those exposed to dark nights ($p>0.05$). XPro1595 provoked a slight, but not statistically significant, increase in float duration for hamsters in dark nights ($p>0.05$). XPro1595 treatment also affected latency to first become immobile in the forced swim test. There was a significant effect of treatment ($F_{1,41}=9.436$, $p<0.05$) and an interaction effect of light by treatment ($F_{1,41}=5.371$, $p<0.05$). Hamsters in dim LAN treated with vehicle had reduced latency to float compared to all other groups (post-hoc, $p<0.05$ in each case; Figure 6.2b).

Hippocampal Morphology

There was a main effect of light condition on apical dendritic spine density on CA1 pyramidal neurons ($F_{1,25}=5.851$, $p<0.05$), with an overall reduction of spines on neurons of hamsters exposed to dim LAN compared to dark (Figure 6.2c), but no effect of treatment with XPro1595.

Discussion

Considerable evidence indicates a role for the immune system in psychiatric diseases, especially depression. Immune function is dysregulated in patients with major depression.
Similarly, administration of proinflammatory cytokines induces depressive symptoms in humans and animals, and several medical illnesses characterized by chronic inflammation are accompanied by depression (Yirmiya et al., 1999). Direct effects of LAN on immune function have been reported in both nocturnal and diurnal rodents (Bedrosian et al., 2011a; Fonken et al., 2012a). Furthermore, LAN suppresses melatonin, which has been shown to reduce pro-inflammatory cytokine levels in the brain and periphery (Tyagi et al., 2010; Ochoa et al., 2011). In this study, hippocampal mRNA expression of TNF, but not Il-1β, was increased in hamsters exposed to LAN.

Inflammatory cytokine response is linked to the pathogenesis of affective disorders and changes in dendritic morphology of CA1 pyramidal neurons in the hippocampus (Richwine et al., 2008). Because tnf mRNA expression was elevated in the hippocampus of hamsters exposed to LAN, I investigated whether it might be directly implicated in the depressive-like behaviors and hippocampal CA1 morphology changes observed in this model. Interestingly, chronic icv infusion of the dominant negative TNF inhibitor, XPro1595, prevented the development of depressive-like symptoms under LAN in the forced swim test, although it did not affect CA1 apical dendritic spine density. These findings suggest the pro-inflammatory soluble form of TNF is tentatively involved in the depression-like behavior provoked by chronic exposure to LAN, but further investigation will be required to understand its role in the hippocampus. Because administration was icv, versus directly into the hippocampus, it is possible that XPro1595 had an effect on other brain regions which also contributed to the diminished depression-
like symptoms. XPro1595 is a specific inhibitor of soluble TNF, unlike etanercept or infliximab which are general inhibitors of both soluble and transmembrane forms. I specifically used XPro1595 versus one of the anti-TNF antibodies, which are not selective for proinflammatory soluble TNF, in order to make this distinction. Both forms of TNF are encoded by the same gene so my PCR data do not distinguish between them. My findings using XPro1595, however, specifically implicate soluble TNF in LAN depression-like behavior. It is possible that transmembrane TNF is involved in changes in spine density. Future studies could address this by comparing the effects of XPro1595 to a non-selective TNF inhibitor.

Furthermore, because XPro1595 treatment did not block reductions in dendritic spine density observed under LAN, but did block depression-like behavior in the forced swim test, this suggests that reduced spine density is not a necessary component in eliciting depression-like behavior under LAN. Perhaps reduced hippocampal BDNF expression, in the presence of an enhanced inflammatory microenvironment, may alter behavior and spine density independently. The results of this study suggest a putative scenario in which LAN may provoke excessive TNF expression, which in turn may elicit depression-like behaviors independent of changes in dendritic spine density. The effects of LAN on depression using TNF knock-out or overexpression techniques would be interesting to investigate; unfortunately transgenic models using Siberian hamster are not readily available and inbred mice are not an ideal model for these studies because many strains are melatonin-deficient. Particularly in regard to inflammation, and specifically
TNF, further investigation is necessary into the mechanistic and potentially therapeutic role for TNF in depression.
Figure 6.1. Pro-inflammatory cytokine expression in the brain.

Hamsters exposed to dim LAN increased TNF expression in the hippocampus compared to hamsters in dark nights. All reversal groups had equivalent expression levels to dark controls (A). There were no differences in IL-1β expression in the hippocampus (B).

*P<0.05.
**Figure 6.2.** Effect of a dominant negative TNF inhibitor on depression-like behavior and CA1 dendritic spine density.

Hamsters housed in dim LAN spent more time immobile in the forced swim test. Hamsters exposed to LAN and treated with a DN-TNF inhibitor spent less time immobile than their vehicle treated counterparts (A) and have increased latency to float (B). Dim LAN reduced CA1 apical dendrite spine density but treatment with the TNF inhibitor, XPro1595, did not have an effect (C). *P<0.05, #P<0.05 Dim vehicle vs. Dim DN-TNF.
CHAPTER 7

EFFECT OF WAVELENGTH OF DIM LIGHT AT NIGHT ON BRAIN AND BEHAVIOR

Humans and other organisms have adapted to a 24-h solar cycle. Endogenous circadian rhythms in physiology and behavior are synchronized to this cycle, using light information as the most potent entraining cue. In mammals, ambient light is detected by the retina and signaled to the brain through retinal ganglion cells. One population, called intrinsically photosensitive retinal ganglion cells (ipRGCs), projects both to the suprachiasmatic nucleus (SCN) in the hypothalamus, regulating circadian rhythms, and to limbic regions, putatively regulating mood (Karatsoreos and McEwen, 2011). Thus, nocturnal light exposure has the potential to affect both circadian timekeeping and mood.

Unnatural lighting, particularly exposure to electric light at night (LAN), is a relatively new phenomenon in human history. During the past century, environmental lighting conditions have shifted from natural light-dark cycles resulting from the rotation of the Earth, to artificial and often erratic light cycles created by social schedules. Exposure to excessive LAN, and particularly to blue wavelength light, has become widespread within industrialized societies (Navara and Nelson, 2007). Sources include computer and television screens, urban light pollution, shift work, compact fluorescent
light (CFL) bulbs, and transmeridian travel. Importantly, ipRGCs are maximally sensitive to blue wavelength light (~480 nm) and minimally sensitive to red wavelength light (>600 nm) due to melanopsin expression (Lockley et al., 2003; Newman et al., 2003; Brainard et al., 2008), meaning that modern sources of LAN may be particularly disruptive to circadian function.

Night-time light exposure results in desynchrony between the biological clock and the external environment, which may lead to health consequences or altered mood regulation (Healy et al., 1993). For example, night shift workers have increased risk for mood disorders (Dumont and Beaulieu, 2007). Further, circadian abnormalities are prominent features of depressive disorders, and changes in the daily light cycle provoke depressed mood, as in the case of seasonal affective disorder (Bunney and Bunney, 2000; McClung, 2011). Moreover, evidence suggests that depressive responses associated with circadian disruption provoke structural changes to neurons in limbic regions that receive ipRGC projections, such as the hippocampus (Bedrosian et al., 2011b; Workman et al., 2011).

In this experiment, I hypothesized that nocturnal light exposure (i.e., dim LAN) would induce rodent depressive-like responses and alter neuronal structure. Because red wavelength light minimally activates melanopsin, I further hypothesized that exposure to red LAN would have less effect on brain and behavior as compared to shorter wavelength blue LAN, or white LAN which contains blue wavelengths. I hypothesized that red LAN would not activate melanopsin, and consequently the SCN, to the same degree as blue wavelength light. In a second experiment, I observed Fos activation of the SCN following
light pulses of different wavelengths in order to support this hypothesis. My experiments focus specifically on female hamsters because (1) women are both more likely to work evening or rotating shifts, thus encountering unnatural light exposure, and have greater risk for mood disorders compared to men (Kessler et al., 1993; Williams, 2008), (2) I can discount the effects of sleep disruption from LAN because these are nocturnal creatures that normally sleep during the light, and (3) they share the same ipRGCs and projections as humans.

Materials and Methods

Animals

Adult female Siberian hamsters (Phodopus sungorus) from our breeding colony at the Ohio State University were individually housed in polypropylene cages (30 cm x 15 cm x 14 cm) at a constant ambient temperature of 22±2°C and relative humidity of 50±5%. Food (Harlan Teklad 8640, Indianapolis, IN, USA) and filtered tap water were available ad libitum. Prior to starting the experiments, all hamsters (>8 weeks of age) were ovariectomized under isoflurane anesthesia to prevent known effects of cycling estrogens on neuronal morphology (Woolley and McEwen, 1992), then allowed to recover for 1 week. Following the recovery period, hamsters were maintained in either control or experimental light cycles as described below. All experimental procedures were approved by The Ohio State University Institutional Animal Care and Use Committee and were conducted in accordance with the National Institutes of Health (NIH) guidelines.
**Chronic exposure to different wavelengths**

The aim of the first experiment was to determine the effects of chronic exposure to different wavelengths of LAN on depressive-like behavior and hippocampal morphology. Nighttime conditions consisted of no light (Dark; N=10), dim red light (rLAN; >600 nm; N=10), dim white light (wLAN; broad spectrum; N=10), or dim blue light (bLAN; peak at ~480 nm; N=10). Hamsters were exposed to 4 weeks of experimental light environments prior to testing. All light cycles consisted of 16 h of bright white light (150 lx) and 8 h of either dark (0 lx) or dim light (5 lx). Both the bright and dim white lights were typical “cool white” fluorescent bulbs. The dim red and dim blue lights were produced using Rosco Supergel light filters in Medium Red and Italian Blue (Rosco Laboratories, Stamford, CT, USA). Spectral transmission for each is presented in Figure 7.1a. In each condition, the bright lights were illuminated at 22:00 h.

**Behavioral assays**

To assess depression-like behavioral responses in the Porsolt forced swim test (Porsolt et al., 1977), hamsters were placed in an opaque cylindrical tank filled with room-temperature water (22 ± 1°C) for 10 min. I have previously validated the forced swim test for hamster depressive responses in our laboratory (Bedrosian et al., 2012c). Testing was performed during the light phase between 08:00 and 12:00 because experimental light manipulations occurred during the dark phase, but conditions were equivalent during the light phase. Behavior was recorded on video-tape and subsequently scored with Observer software (Noldus, Wageningen, Netherlands) by an observer unaware of assignment to experimental groups. The behaviors scored were: 1) climbing
(i.e., vigorous swimming or scratching directed at the wall of the tank), 2) swimming (i.e., horizontal movement in the tank), and 3) floating/immobility (i.e., minimal movement necessary to keep head elevated above water surface).

**Analysis of hippocampal morphology**

Hamsters were deeply anesthetized with isoflurane vapors and rapidly decapitated between 10:00 and 12:00 h at the conclusion of behavioral testing. Brains were quickly removed and processed for Golgi-Cox staining using a Rapid GolgiStain Kit (FD NeuroTechnologies). Briefly, brains were submerged in Golgi-Cox solution and stored for 14 days in the dark, followed by a 30% sucrose solution for 4 days. Brains were then rapidly frozen and 100 µm coronal sections were sliced on a cryostat and collected onto gelatin-coated glass slides. The stain was developed in NH₄OH for 10 min and sections were counterstained with cresyl violet. Finally, slides were dehydrated through a series of graded ethanol washes, cleared with xylene, coverslipped with Permount, and dried in the dark for at least 1 week.

Neurons impregnated with the Golgi-Cox solution were chosen within the CA1 region of the hippocampus for analysis of dendritic spine density, based on our previous findings (Bedrosian et al., 2011b). All analyses and selection of neurons were performed by an experimenter unaware of assignment to experimental groups. For each animal, 4-6 randomly selected representative neurons from different sections were chosen, but only neurons that were fully impregnated, not obscured by neighboring neurons, and had no obviously truncated dendrites were chosen for analysis. Dendritic spines were traced in each neuron at 100x (N.A. 1.30) in 4 apical and 4 basilar representative dendrite
segments of at least 20 µm in length, and at least 50 µm distal to the cell body, using Neurolucida 8 software (MicroBrightField, Williston, VT, USA) for PC and a Nikon Eclipse E800 brightfield microscope. Dendritic spine density was analyzed using Neurolucida Explorer software (MicroBrightField, Williston, VT, USA).

**Acute exposure to different wavelengths**

The aim of the second experiment was to determine Fos activation of the SCN by different wavelengths of LAN. Hamsters were housed in constant darkness (DD) for three days and then administered one 30 min light pulse beginning at approximately CT13. Homecage locomotor activity data were recorded using an infrared beam break system (Columbus Instruments, Columbus, OH). Actigraphs were constructed using ClockLab software (Actimetrics).

The light pulses consisted of bright white light (150 lx W; N=4), dim white light (5 lx W; N=4), dim red light (5 lx R; N=4), or dim blue light (5 lx B; N=4). A control group received no light pulse and remained in darkness (Dark; N=4). Light sources and filters were the same as described for the chronic exposure experiment. One hour after the start of the light pulse, hamsters were deeply anesthetized with an overdose of sodium pentobarbital and transcardially perfused with ice cold 0.1M PBS (pH 7.4), followed by 4% paraformaldehyde in 0.1 M PBS (pH 7.4). Following perfusion, brains were collected, post-fixed overnight at 4 °C, transferred to 30% sucrose solution in PBS at 4 °C until sunk, and then frozen in cold isopentane and stored at -80 °C. Brains were serially sliced into 30 µm sections using a cryostat and sections were thaw-mounted onto gelatin-coated glass slides and stored at -80 °C.
Fos immunohistochemistry

Sets of tissue collected at intervals of 120 µm were used for immunohistochemical detection of Fos throughout the SCN. Briefly, sections were incubated 10 min in sodium borohydride, then rinsed with 0.1 M PBS, and blocked for 1 h in 1% normal goat serum in 0.1 M PBS + 0.1% Triton-X. Sections were then incubated overnight at room temperature with the primary antibody (1:1000 Ab-5, Millipore). Next, sections were rinsed, incubated for 1 hour with anti-goat IgG (1:500; Vector Laboratories, Burlingame, CA) in 0.1 M PBS + 0.1% Triton-X. The signal was amplified with avidin-biotin complex (Vectastain Elite ABC Kit; Vector Laboratories, Burlingame, CA, USA) and developed using DAB. Slides were dehydrated through a series of graded ethanol washes and cleared with xylene, then coverslipped using Permount.

Images of sections containing the SCN (Bregma -0.46 to -0.58) were obtained at 10x magnification using a Nikon E800 bright-field microscope. Numbers of immunoreactive cells were counted in ImageJ by an observer uninformed of treatment groups, and then averaged across sections and sides of the brain to produce one value for each animal that was used for statistical analysis.

Statistical analysis

Behavior and immunohistochemistry data were analyzed using independent t-tests and planned comparisons, with light conditions as the independent variables. Dendritic spine density was analyzed using two-way ANOVA with light and spine shape as the independent variables. Main effects were followed with Fisher’s post-hoc tests where applicable. Statistics were performed using Statview 5.0.1 (SAS Institute, Cary, NC,
USA) for Windows. Mean differences were considered statistically significant when p was ≤0.05.

Results

Chronic exposure to different wavelengths

Immobility in the forced swim test was analyzed as a measure of depressive-like response among hamsters exposed to the four light conditions (Figure 7.1). Hamsters exposed to wLAN ($t_{14}=2.082$, $p=0.03$) or bLAN ($t_{14}=2.026$, $p=0.03$) had more float bouts compared to hamsters exposed to dark. Moreover, hamsters exposed to bLAN also engaged in more float bouts than those in rLAN ($t_{17}=-1.712$, $p=0.05$; Figure 7.1a). Hamsters exposed to dim LAN ($t_{14}=2.159$, $p=0.02$) or bLAN ($t_{13}=2.128$, $p=0.03$) spent more time immobile compared to those exposed to dark (Figure 7.1b).

I analyzed spine density on apical and basilar dendritic branches of CA1 pyramidal neurons (Figure 7.2a). Light influenced both apical ($F_{3,31}=9.936$, $p<0.0001$) and basilar ($F_{3,31}=8.827$, $p<0.0001$) dendritic spine density, with interactions between light and spine shape for apical ($F_{3,31}=2.548$, $p=.002$) and basilar ($F_{3,31}=2.197$, $p=0.008$) branches. On apical dendrites, wLAN reduced total spine density compared to dark ($p=0.0006$) and rLAN ($p=0.03$), and similarly bLAN reduced total spine density compared to dark ($p=0.0004$) and rLAN ($p=0.012$). wLAN specifically reduced the density of thin spines ($p=0.03$) and mushroom spines ($p=0.03$) compared to hamsters exposed to dark nights. bLAN also reduced the density of thin ($p=0.04$) and mushroom spines ($p=0.02$) versus dark. On apical dendrites, wLAN reduced total spine density compared to dark ($p=0.01$) and rLAN ($p=0.05$), and similarly bLAN reduced total spine
density compared to dark (p=0.004) and rLAN (p=0.0228). wLAN specifically reduced the density of thin spines (p=0.04) and mushroom spines (p=0.0426) compared to dark. bLAN also reduced the density of thin (p=0.01) and mushroom spines (p=0.03) versus dark (Figure 7.2b-d).

**Acute exposure to different wavelengths**

One 30 min light pulse was administered beginning at CT13 to hamsters housed in DD (Figure 7.3a). Brains were collected 1 h after the start of the light pulse and processed for c-Fos immunoreactivity, and then positively stained cells were counted in the SCN as guided by brain atlas coordinates (Franklin and Paxinos, 2007) (Figure 7.3b). Fos induction was greatest in the SCN of hamsters exposed to the blue or bright white light pulses (Figure 7.3c-d). The blue pulse elicited significantly greater Fos-ir compared to dark ($t_6=-3.844, p=0.004$), red ($t_6=2.752, p=0.02$), and dim white ($t_6=1.877, p=0.05$). The bright white pulse elicited more Fos-ir compared to the dark ($t_5=4.035, p=0.005$) and red ($t_5=2.395, p=0.03$) pulse.

**Discussion**

Night-time light exposure, particularly to blue wavelengths, has become widespread in modern societies. This opens the possibility for negative effects on circadian and mood regulation, given that melanopsin-expressing retinal ganglion cells are highly sensitive to blue wavelength light, and project to both circadian and limbic brain regions. In my first experiment, I demonstrate that depressive-like responses and altered neuronal morphology are provoked in response to dim LAN, and that the effect is
dependent on the wavelength of light exposure, as blue wavelengths produce a greater magnitude of response. In my second experiment, I demonstrate that blue wavelength light induces more Fos activity in the SCN compared to red wavelength light, suggesting that the ipRGC pathway that projects through the SCN to limbic regions such as the hippocampus may indeed have a role in this phenomenon.

Light is known to play a role in mood disorders, but typically it is thought to act indirectly by disrupting circadian entrainment or sleep. My results suggest that inappropriately timed light exposure can have direct effects on mood. Dim LAN does not influence entrainment of locomotor activity rhythms to the light-dim light cycle (Bedrosian et al., 2012b) and the LAN manipulation occurs during the active period for this nocturnal rodent species, making it unlikely that sleep during the inactive phase was disrupted. Additionally, depressive-like responses provoked by dim LAN are rapidly reversed by simply eliminating nighttime light exposure, providing further evidence for a direct effect of light (Bedrosian et al., 2012b).

Nocturnal light exposure may affect mood regulating brain regions, such as the hippocampus, through projections from light detecting ipRGCs to limbic regions via the SCN (Karatsoreos and McEwen, 2011). ipRGCs are maximally sensitive to blue wavelength light (~480 nm) and minimally sensitive to red wavelength light (>600 nm) (Newman et al., 2003). Blue LAN, and white LAN that contains blue wavelengths, induced greater Fos expression in the SCN compared to red LAN or darkness, demonstrating that the levels of blue and white light exposure used in this experiment activate ipRGCs and the SCN. This activation by LAN is inappropriately timed and may
lead to changes affecting the projection targets of the SCN, such as limbic regions like the hippocampus. Indeed, evidence suggests that blue wavelength light in particular can influence the hippocampus. Exposure to blue light increases human hippocampus activity measured by fMRI as compared to longer wavelength green light and the differences are detectable almost immediately following light onset (Vandewalle et al., 2007). This provides some evidence that light is a modulator of cognitive brain function in humans (Vandewalle et al., 2009).

I investigated structural plasticity of the hippocampus in response to chronic LAN exposure. Neurotrophic support, in particular expression of BDNF, is reduced in the hippocampi of rodents exposed to LAN (Chapter 5; Bedrosian et al., 2012b). Further, dendritic spines are highly plastic and may change rapidly in response to cues from the environment (Fischer et al., 1998). I measured dendritic spine density on segments more than 50 µm from the cell body so as to capture the primary sites of excitatory neuronal input (Megias et al., 2001; von Bohlen Und Halbach, 2009). I also analyzed spines based on shape, as this provides information about the strength and maturity of the associated synapse (Yoshihara et al., 2009). Spine density was reduced after exposure to blue or white LAN, and the effect was driven by reductions in mushroom and thin spines in particular, the most common spine shape observed. This observation raises the possibility that LAN exposure reduces the number of newly forming spines, as thin spines are thought to be young and possibly involved in learning and cognitive function (Yoshihara et al., 2009). In support of this notion, LAN impairs performance in learning and memory tasks in rodents (Fonken et al., 2012b).
Taken together, my results demonstrate an important role for nocturnal light exposure in mood regulation and point to the broader implications of modern lighting on the brain and behavior. Computers, televisions, e-readers, and CFL bulbs emit short wavelength light and nighttime use is widespread, raising questions about the implications of such technology. My results highlight the importance of wavelength in mood-related responses to light. Such observations should direct efforts to minimize the deleterious effects of aberrant light exposure through manipulation of wavelength in individuals exposed to excessive LAN.
Figure 7.1. Nocturnal light exposure provokes depressive-like responses in the forced swim test dependent on wavelength.

Spectral distribution of light transmitted through blue and red filters and experimental design are presented (A). Exposure to 5 lux white or blue LAN increased the number of float bouts (B) and increased overall time spent immobile (C) in the forced swim test. a- \( p \leq 0.05 \) compared to Dark; b- \( p \leq 0.05 \) compared to rLAN.
Figure 7.2. Nocturnal light exposure alters neuronal morphology in the hippocampus.

Dendritic spines on both basilar and apical branches of CA1 pyramidal neurons (A) were quantified based on spine shape (B). Exposure to 5 lx white or blue LAN reduced density of thin and mushroom spines on apical (C) and basilar (D) dendrites. * p<0.05. t- thin, m- mushroom, s- stubby, b- branched, d- detached.
Figure 7.3. Nocturnal light exposure activates the SCN in a wavelength-dependent manner.

A 30 min light pulse administered after several days of exposure to DD (A) provoked c-Fos expression in the SCN which was quantified using immunohistochemistry (B). Exposure to 5 lx white, 5 lx blue, or 150 lx white light induced Fos expression, whereas red light did not (C-D). a- $p \leq 0.05$ compared to Dark; b- $p \leq 0.05$ compared to 5 lx R; c- $p \leq 0.05$ compared to 5 lx W.
CHAPTER 8

CONCLUSIONS

During the past century, the invention and widespread adoption of electric light has led to ‘round-the-clock’ societies. Instead of aligning physiology and behavior with the environment, individuals follow artificial and often erratic light cycles created by social and work schedules. Exposure to artificial light at night (LAN) has become pervasive in Western society and its consequences are only now becoming understood. In this dissertation, I hypothesized that dim LAN would disrupt circadian organization and contribute to depressed mood. My initial experiments outlined the consequences of chronic LAN exposure on behavior, circadian output, and brain plasticity. Next I described a potential mechanism through which LAN increases brain inflammation and contributes to depressive-like behavioral responses. Finally, I demonstrated methods of prevention and intervention to avoid deleterious health effects associated with LAN.

Consequences

*Light at night provokes depressive-like behaviors.*

Substantial evidence supports a role for the circadian system in mood regulation, and environmental perturbations of the circadian system are known to cause mood
disturbances in some individuals. Any unnatural timing of light exposure can cause misalignment between internal biological processes and the external environment, leading to health consequences. In order to investigate the effects of chronic nighttime lighting, I developed a model in which hamsters (*Phodopus sungorus*) were exposed to dim light (5 lx) every night for four weeks. This level of illumination is approximately five times brighter than maximal moonlight, comparable to the levels of light pollution surrounding urban centers, and is sufficient to suppress pineal melatonin production in hamsters (Brainard et al., 1982). Following the period of nighttime light exposure, hamsters exhibited more immobility in Porsolt’s forced swim test and reduced preference for sucrose solution in a test of sucrose preference compared to hamsters exposed to dark nights throughout the experiment (Bedrosian et al., 2011b). Both of these results indicate that LAN provokes a depressive-like response. These behavioral tests, and particularly the forced swim test, have been validated in rats and mice to show that treatment with a selective serotonin reuptake inhibitor (SSRI) is effective in reducing the immobility response and restoring typical behavior. And indeed, hamsters treated with citalopram during exposure to LAN reduce immobility in the forced swim test, supporting the validity of this model (Bedrosian et al., 2012c).

*Light at night alters circadian output*

One advantage of using nocturnal hamsters for these studies is that I was able to separate the effects of light on the circadian system from the more complex effects of sleep disruption. This is because the light manipulation occurred during the hamsters’ active and awake phase. Substantial evidence supports a relationship between sleep
disruption and mood, but the direct effects of aberrant light exposure on mood and circadian output are not fully understood. I recorded homecage locomotor activity and then collected blood and brain samples around the clock after four weeks exposure to LAN. Outwardly, hamsters remained entrained to the light cycle, with activity consolidated during the dim light phase. Nonetheless, the diurnal fluctuation in serum cortisol concentrations was blunted, as were the expression patterns of PER1 and PER2 proteins in the SCN (Chapter 2). This finding parallels the flattened cortisol rhythm associated with major depression in human patients. And disorganization of the diurnal oscillations of circadian clock proteins in the SCN could contribute to further disruption at other levels. This experiment was important because it demonstrated that even dim LAN can profoundly influence the circadian system.

**Light at night reduces brain plasticity**

The hippocampus is a critical structure in the pathophysiology of major depression. Depressed patients have characteristic hippocampal atrophy and dysregulation of hippocampal-related systems, such as stress coping and memory. Reduced structural plasticity, in terms of hippocampal dendritic spine density and dendritic complexity, is observed in animal models of chronic stress and depression. Hamsters exposed to four weeks of LAN reduced hippocampal gene expression of brain-derived neurotrophic factor (BDNF), a neurotrophic factor implicated in neuronal growth and survival (Bedrosian et al., 2012b). Following from that, the structural complexity of CA1 neurons was reduced after exposure to LAN. The density of dendritic spines was reduced on both apical and basilar dendrites, in a manner that correlated with the severity
of depressive-like behavior (Bedrosian et al., 2011b). Overall, LAN provoked hippocampal abnormalities related to the depressive-like response (Chapters 3-5).

Potential Mechanism

Neuroinflammation is implicated in depressive-like behaviors under light at night

Inflammation has been implicated in depressive disorders, and recently a molecular pathway directly linking disrupted circadian clock genes to brain inflammation was described (Narasimamurthy et al., 2012). In hamsters exposed to LAN, expression of the proinflammatory cytokine, tumor necrosis factor (TNF), was elevated in the hippocampus (Bedrosian et al., 2012b). To determine whether greater levels of TNF in the brain may be implicated in the depressive-like behaviors and reduced hippocampal dendritic spine density observed after LAN exposure, I implanted each hamster with a cannula connected to an osmotic minipump for continuous infusion of either a TNF inhibitor or saline. Following four weeks of parallel treatment and LAN exposure, hamsters receiving the TNF inhibitor in LAN spent less time immobile in the forced swim test than their saline-treated counterparts (Bedrosian et al., 2012b). Dendritic spine density on CA1 neurons was improved, but not completely restored, suggesting that neuroinflammation may be only one of a few potential contributors acting in concert to provoke depressive-behavior associated with LAN (Chapters 6-7).
Prevention and Intervention

Strategies for preventing and reversing effects of light at night

Through my dissertation work, I am convinced that this seemingly innocuous exposure to dim nighttime lighting can have profound effects on the brain and behavior. Nonetheless, I am no Luddite and I do not propose elimination of LAN. Rather, I suggest strategies to effectively manage our technology to minimize the health consequences. For one, my studies suggest that the effects of LAN are reversible in many instances. When nighttime light was eliminated after four weeks of exposure, hamsters tested in the forced swim test recovered typical behavioral responses as soon as two weeks later (Chapter 5; Bedrosian et al., 2012b). Gene expression levels of BDNF and dendritic spine density were also quickly recovered. This suggests that for individuals falling asleep to a television or encountering excessive streetlight leaking into the bedroom, eliminating these sources of LAN could have fast-acting beneficial effects. For night shift workers, or others who cannot avoid LAN, another strategy may be to manage the particular wavelength of light exposure. Red wavelength light is minimally disruptive to the circadian system, whereas blue wavelength light has the most potent effects. This is due to the sensitivity of melanopsin-expressing ipRGCs in the retina that carry light information to the SCN. Hamsters exposed to four weeks of red LAN did not develop the same depressive-like response or structural changes to hippocampal neurons as hamsters exposed to white or blue LAN. And a brief pulse of red LAN does not activate c-Fos in the SCN to the same degree as white or blue light (Chapter 7). For night shift workers,
glasses that block blue wavelengths, or lights shifted to the red-orange end of the spectrum may help to prevent deleterious effects of chronic nighttime lighting.

**Implications**

Exposure to LAN is a wide-reaching phenomenon that has been mostly neglected until very recently as a potential biological disruptor. As accumulating evidence links LAN to risk of breast cancer, obesity, mood disorders, and other ailments, it is critical that we consider its effects on the circadian system, general physiology, and mood. In this dissertation, I speculate about melatonin as the major upstream mechanism associated with the effects that I have observed. The direct suppression of pineal melatonin by LAN could contribute to the reported changes in locomotor activity, clock protein expression, behavior, brain plasticity, and neuroinflammation. With melatonin in mind, we may consider LAN as an endocrine disruptor as well as a circadian disruptor. Though not within the scope of this dissertation, in future studies it will be important to investigate depressive-like responses provoked by LAN at this overarching level.

The goal of this dissertation was to investigate the novel hypothesis that chronic exposure to dim LAN disrupts circadian timekeeping and mood. I believe that this body of work contributes important knowledge about the effects of LAN in a hamster model. It is my hope that epidemiological work in the future might investigate whether there exists a correlation between light exposure in the home or in work settings with mood disorders, and particularly depressive symptoms, and further contrast these populations with those exposed to very little artificial LAN, such as the Amish. Establishment of such a link
within human populations, taken together with the evidence presented in this dissertation, would make a strong case for the deleterious effects of LAN on mood. Going forward, it will be important to consider the effects of our technology on biological processes that may be influenced by subtle environmental changes.
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