DIFFERENTIAL EFFECTS OF SHORT LIGHT PULSES IN THE DARK PHASE OF AN L/D CYCLE ON BEHAVIORAL DESPAIR IN MALE WISTAR RATS

by

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ABSTRACT

DIFFERENTIAL EFFECTS OF SHORT LIGHT PULSES IN THE DARK PHASE OF AN L/D CYCLE ON BEHAVIORAL DESPAIR IN MALE WISTAR RATS

The present study investigated the effect of a 10-min light pulse either early or late in the dark phase of the L/D cycle on behavioral despair in male Wistar rats. Independent groups of rats (n=8 each) maintained on a 12L/12D cycle (lights on at 06:00 h) received a 10-min light pulse (either 900 or 1350 lux provided by an incandescent lamp) 3 or 9 h after dark onset. A control group (n=8) was treated similarly except for light exposure. All animals then underwent a 15-min Forced Swim Test (FST) starting at 14:00 in the next light phase of the L/D cycle, followed by a 5-min FST 24 h later. Analysis of variance indicated that exposure to either intensity of light delivered in the late but not the early part of the dark phase of the L/D cycle has protective effect in behavioral despair as indicated by shortened durations of immobility in the second swim test compared to controls. The fact that light pulses in the early part of the dark phase had no ameliorative effect on durations of immobility suggests that the antidepressant property of the late pulses may be due to their differential effect in phase shifting the circadian rhythm. Analysis of c-Fos expression reported a significant difference between the control and light exposure groups. Light exposure at ZT21 and ZT15 induces dense c-Fos immunoreactivity in the core region of the SCN which indicates phase shifting. Slight spontaneous c-Fos immunoreactivity was detected in the shell region of the SCN of the control rats. These results suggest that light may exert its antidepressant effect via circadian phase shifting.

Keywords: depression, circadian rhythms, suprachiasmatic nucleus, light pulse, c-Fos

ÖZET

IŞIK KARANLIK DÖNGÜSÜNÜN GECE FAZINDAKİ IŞIK DARBELERİNİN ERKEK WİSTAR SIÇANLARDAKİ DAVRANIŞSAL ÇARESİZLİĞE OLAN ETKİLERİ

Bu çalışmada ışık karanlık döngüsünün gece fazında, erken ya da geç saatte verilen 10 dakikalık ışık darbesinin erkek Wistar sıçanlarda davranışsal çaresizlik olgusu üzerine etkileri incelenmiştir. 12 saat ışık ve 12 saat karanlık döngüsünde yaşayan (ışıkların açılma saati 06:00) birbirinden bağımsız gruplardaki sıçanlar (n=8) karanlık başlangıcından 3 ya da 9 saat sonra 10 dakikalık (900 ya da 1350 lükslük lamba) ışık darbesine maruz bırakılmıştır. Kontrol grubu (n=8) ışık uygulanması dışında aynı muameleyi görmüştür. Daha sonra bütün deneklere ışık karanlık döngüsünün ertesi ışık fazında saat 14:00'de 15 dakikalık zorunlu yüzme testi, bundan 24 saat sonraysa 5 dakikalık zorunlu yüzme testi uygulanmıştır. Varyans analizi ışık karanlık döngüsünün gece fazında geç saatte uygulanan farklı yoğunluklardaki ışığın, ikinci yüzme testinde azalan hareketsizlikle belirlenen davranışsal çaresizliğe karşı koruduğunu, erken saatte uygulanan ışığın korumadığını göstermiştir. Gece fazının erken saatlerinde uygulanan ışık pülslerinin hareketsiz kalma süresini azaltma etkisinin olmaması, geç saatte verilen darbelerin antidepresan etkisinin günlük ritimlerdeki faz kaymalarını etkilemesinden kaynaklandığı gösterebilir. c-Fos ekspresyonunda kontrol ve ışık grupları arasından anlamlı bir fark bulmuştur. ZT21 ve ZT15'de verilen ışık darbelerinin Suprachiasmatik çekirdeğin öz bölgesinde yoğun c-Fos aktivasyonuna neden olması faz kaymasını göstermektedir. Kontrol deneklerinde, SCN'in kabuk bölgesinde hafif spontane c-Fos aktivayonu gözlenmiştir. Bu sonuçlar ışığın antidepresan etkisini günlük faz kaymaları üzerinden gerçekleştirdiğini göstermektedir.

Anahtar Sözcükler: depresyon, günlük ritimler, SCN, ışık darbesi, c-Fos

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LIST OF ABBREVIATIONS

AMPA	Alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid
BDNF	Brain-Derived Neurotrophic Factor
CT	Circadian Time
DM	Dorsomedial
DRN	Dorsal Raphe Nucleus
DSM-IV	Diagnostic and Statistical Manual
FST	Forced Swim Test
GABA	Gamma-aminobutyric acid
GHT	Geniculohypothalamic Tract
L/D	Light Dark
NMDA	N- methyl D- aspartate receptors
NPY	Neuropeptide Y
PACAP	Pituitary adenylate cyclase activating peptide
PVN	Paraventricular Nucleus
RHT	Retinohypothalamic Tract
SAD	Seasonal Affective Disorder
SCN	Suprachiasmatic Nucleus
SEM	Standard Error of the Mean
SD	Standard Deviation
VL	Ventrolateral
III.V	Third Ventricle
W	Watt
ZT	Zeitgeber Time

1. INTRODUCTION

1.1 Motivation and Objective

Depression is the most prevalent mental illness that affects millions of people in the world. It causes a high economic burden as it disrupts normal social and occupational functioning [1]. Thus, diagnosis and treatment of depression is very crucial, which is only possible by understanding mechanisms underlying its pathophysiology.

The pharmaceutical industry produces novel antidepressant drugs to alleviate the effects of depression. However, the effectiveness of available drugs is limited and requires long periods of time to be effective as their action mechanisms are not adequate to alleviate the pathophysiology underlying depression [2]. Searching for better antidepressant treatments takes attention on light therapy which is a non-pharmacological and biological antidepressant treatment.

Light therapy exerts its effect via modulation of circadian rhythms that enable the organism to adapt to changing conditions in the environment. Circadian rhythms can be synchronized, or entrained by external time cues [3]. Zeitgeber time (ZT) is a standardized 24-hour notation of the phase in an entrained circadian cycle in which ZT0 indicates the beginning of the light phase, and ZT12 is the beginning of the dark phase in a Light/Dark (L/D) cycle with 12hr light and 12hr dark phase [3].

The suprachiasmatic nucleus (SCN) is the master pacemaker regulating circadian rhythms [3, 4]. L/D cycle is the primary mechanism that entrains SCN [3, 5-8]. Several rhythm disturbances suggest that SCN may be malfunctioning in depressed patients [9, 10]. Controlled animal studies have reported that antidepressant treatments can alter the phase, amplitude, and patterning of circadian rhythms in a variety of measures [11].The phase delaying and period lengthening effects of light therapy are consistent with the hypothesis that disorders of circadian rhythms may be etiologically related to depression [10].

Light therapy, treatment of depression with bright light exposure, is shown to be highly effective in alleviating symptoms of both seasonal [12-19] and nonseasonal [20-23] depression.

Although light therapy is used in clinical studies, the mechanisms underlying the antidepressant action of light is unclear. Because of ethical restrictions in human studies, several animal models of depression have been developed which seem effective in mimicking the symptoms of depression in humans. Forced swim test (FST) is one of the known animal models of depression to evaluate antidepressant activities in rodents. The FST model is composed of two consecutive swim tests separated by 24 hours. The increased immobility in the second swim test is accepted as a sign of depression and is known as "behavioral despair" [24-26]. In the present study, the FST was used as an animal model of depression to evaluate the effects of light on depression. It was assumed that the antidepressant effect of light might depend on both the intensity and the application time of the light [14, 20, and 23]. A previous study explored the effect of varying length and timing of photic stimulation in the dark phase of the L/D cycle on FST and found that a 30-min light pulse delivered 8 ¼ h after dark onset (ZT21¼) on a 12 h L/D cycle reduced immobility on the second day of the testing [27]. Light pulses delivered early in the dark phase (ZT15¼) did not have an antidepressant effect on behavioral despair. This study suggests the possibility that an antidepressant effect may be attained by shorter light pulses in the dark phase of the light/dark (L/D) cycle in order to investigate the mechanisms underlying depression. Based on these findings, in this study, different intensities of brief photic stimulation at different time points at the dark phase of the light/dark cycle were evaluated. The exposure time points were selected as ZT15 and ZT21. It was demonstrated that light exposure early at night (ZT15-2100h) induces phase delays while light application late at night (ZT21-0300h) induces phase advances. The main hypothesis of this study was that the light presented at different zeitgeber times has different effects on behavioral despair compared to control animals. Based on the results of Schulz et al (2008), it was expected to find ameliorative effect of light presented late at night (ZT21) and no effect of light applied early at night (ZT15) on behavioral despair. Moreover, it was expected that to light with higher intensity would have more antidepressant effect compared to lower- intensity light.

The mechanism underlying the ameliorative effect of light is unclear but there are hypotheses about its function. It has been hypothesized that the antidepressant effect of light therapy may be due to suppression of melatonin production [14, 28], photon over exposure [16, 28], activation of the brain serotonergic system [11, 29, 30], and circadian phase shifting [13, 18, 23, 28, 31, 32]. Analysis of the photic induction of c-Fos immunoreactivity within the SCN has been used to study the neurochemical mechanisms involved in the endogenous circadian clock [33]. Following light stimulus, c-Fos is expressed in the SCN and induced several signal transduction pathways [31]. Light induces c-Fos in the SCN only in mid-subjective night when it also phase shifts circadian rhythmicity [34]. To elucidate the mechanism underlying the antidepressant effect of short light pulses in the present study, histological analysis of c-Fos staining was performed.

1.2 Contribution of the Thesis

Light therapy is often used in clinical studies as an antidepressant treatment [20]. However, there is an ongoing debate on the exact nature by which light therapy exerts its ameliorative effect in depression [14, 28, and 30].The histological findings of this study indicated that the phase shifting mechanism might be involved in the antidepressant effect of light. Light therapy is used with different intensities and durations and most of the studies have found controversial results about the optimal treatment to produce antidepressant action. For example, the original regimen of light therapy produces antidepressant effects at 2500 lux for 3 hours in morning and evening sessions [16] while later studies used 10,000 lux for 30 minutes, or 2500-5000 lux for longer durations [17, 20] Moreover, as the intensity and duration of light exposure increase, side effects of light are also augmented [35].The present findings indicate that only 10 minutes of light exposure at ZT21 is sufficient to alleviate symptoms of behavioral despair. Moreover, our findings support previous findings that ZT21 is the critical circadian time point which enabled minimum light exposure to produce positive effects [27]. These findings will lead to a better understanding of the mechanism underlying the pathophysiology of depression and its treatment.

2. DEPRESSION

Depression is the most prevalent, recurrent and disabling mental illness that affects 17% of the population in the USA [36] causing a high economic burden as it disrupts normal social and occupational functioning [1]. Depression is a complex disease in which psychological, behavioral and physiological systems become abnormal. The term depression is used to describe a plurality of illnesses that have some core symptoms in common [37]. For this thesis, the term depression will be used to indicate Major Depressive Disorder [1].

2.1 Classification and Diagnosis

Depression has been diagnosed as 'major depressive disorder' based on symptomatic criteria set forth in the Diagnostic and Statistical Manual (DSM). Diagnostic criteria for major depression are summarized below. A diagnosis of major depression is made when a certain number of the below symptoms are reported for longer than a 2 week period of time, and when the symptoms disrupt normal social and occupational functioning [1].

- Depressed mood
- Irritability
- Low self esteem
- Feelings of hopelessness, worthlessness, and guilt
- Decreased ability to concentrate and think
- Decreased or increased appetite
- Weight loss or weight gain
- Insomnia or hypersomnia
- Low energy, fatigue, or increased agitation
- Decreased interest in pleasurable stimuli (e.g. sex, food, social interactions)
- Recurrent thoughts of death and suicide

The term “Seasonal Affective Disorder” (SAD) was first used by Norman Rosenthal in 1984 [16]. SAD is a type of major depression in which seasonal changes occur in mood, activity, weight, sleep, appetite and social life [20]. A diagnosis of SAD is made when there is a regular onset and remission of depressive symptoms during particular times of the year, when there are two major depressive episodes in the last 2 years with no intervening non-seasonal episodes, and when seasonal episodes outnumber non-seasonal episodes over the individual’s lifetime [1].

2.2 Pharmacological and Non-pharmacological Treatment of Depression

Several treatment methods have been developed to cure depression. Major treatment methods are based on pharmacotherapy, electroconvulsive, sleep-wake and light therapies [2, 21, 38, and 39]. Major antidepressant drugs exert their effects via increasing synaptic levels of monoamines [40]. However, their limited effects indicate the presence of other mechanisms rather than monoamine abnormalities in the pathophysiology of depression. Furthermore, it was demonstrated that several noncompetitive NMDAR antagonist such as ketamine and MK-801 and positive modulators of AMPA receptors have rapid antidepressant effect on several animal models of depression [2, 41, and 42]. Additionally, as a non-pharmacological method, electroconvulsive therapy is preferred when patients do not respond to conventional drug treatments [43].

There are several hypotheses about the mechanisms underlying pharmacological and non-pharmacological antidepressant treatments. One of them is the neurotrophin hypothesis. Neurotrophins are small endogenous proteins which function as growth factors [44]. Among neurotrophins, brain-derived neurotrophic factor (BDNF) may be involved in the antidepressant action of several antidepressant treatments. BDNF is a neurotrophic factor that has a variety of effects such as survival of neurons during development of nervous system and enabling neurogenesis in adult life [45]. BDNF is upregulated by different classes of antidepressants such as selective serotonin reuptake inhibitors, norepinephrine selective reuptake inhibitors, monoamine oxidase inhibitors; atypical antidepressants and electroconvulsive shock [46]. BDNF upregulation in hippocampus is measured in the antidepressant action of NMDA antagonists such as memantine [47] and

ketamine [48]. BNDF infusion into several brain regions decreases depressive symptoms in several animal models of depression such as FST and tail suspension test [49, 50]

Recent studies indicate that the sleep-wake therapy and light therapy are effective antidepressant treatments [20, 21, and 38]. Even though the mechanism underlying the antidepressant action of light therapy is unclear, it was assumed that the mechanism is based on modulation of circadian rhythms.

3. CIRCADIAN RHYTHMS

Circadian rhythms enable the organism to adapt to changing conditions in the environment. Circadian rhythms that are expressed in the absence of any 24 hour external signal are called free running, with a period about 24 hours (Latin: circa=about; dies=day). The persistence of free running rhythms indicates the presence of an internal biological clock. Circadian rhythms can be synchronized, or entrained by external time cues. If a shift in external cues occurs, the rhythms will be entrained to the new cues [3].

Circadian time (CT) is a standardized 24-hour notation of the phase in a circadian cycle that represents an estimation of the organism's subjective time. CT0 indicates the beginning of a subjective day, and CT12 is the beginning of a subjective night when the rhythms are allowed to free run. Zeitgeber time (ZT-Zeitgeber, "time giver" in German) is a standardized 24-hour notation of the phase in an entrained circadian cycle in which ZT0 indicates the beginning of the light phase, and ZT12 is the beginning of the dark phase in a Light/Dark (L/D) cycle with 12hr light and 12hr dark phase [3].

In its simplest configuration, the circadian system is composed of a central circadian clock, or pacemaker, a set of input pathways mediating the effects of various environmental synchronizers (such as light and darkness) on the pacemaker, and a set of output pathways conveying pacemaker signals to other regulatory systems [11]. Any stimulus that affects any of these components may alter the expression of circadian rhythms.

3.1 Circadian Rhythms and SCN

Lesioning and transplanting studies in the early 1970s demonstrated that the SCN is the master pacemaker regulating circadian rhythms [3, 4, 51] It regulates nearly all 24 hour rhythms such as body temperature, sleep-wake cycle and endocrine and physiological rhythms and strikes a balance between internal and external environment [3, 8, 52, 53]. Light/dark cycle is the primary mechanism that entrains SCN that can be modulated by

feeding and activity cycles, temperature and socials interactions as well as light exposure [3, 5-8]

SCN, located in a distinct region of the hypothalamus, has two major functions. The primary function is to generate endogenous rhythms with a period of 24 hours. The secondary function is to entrain the physiology and behavior of the individual to certain types of stimuli. Neurons in the SCN function in synchrony in order to produce coherent output [54, 55].There are three major neural pathways that relay information to the SCN (see figure 3.1). The major one is the retinohypothalamic tract (RHT) Light reaches the SCN directly from retinal ganglion cells through the RHT. Pituitary adenylate cyclase activating polypeptide (PACAP) and glutamate are main neuromessengers in this pathway [55]. The second pathway is the geniculohypothalamic tract (GHT) and emerges from the intergeniculate leaflet (IGL) located in the thalamus. Main neurochemicals are neuropeptide Y (NPY) and GABA in the GHT. The third pathway emanates from the raphe nuclei of the midbrain which functions in the regulation of mood, sleep, arousal and other behavioral functions [55]. RHT and GHT pathways mediate the effects of light on the SCN, while highly dense serotonergic pathways from the raphe nuclei mediate behavioral regulation in response to non-photic stimuli such as locomotor activity in the SCN [11, 29].

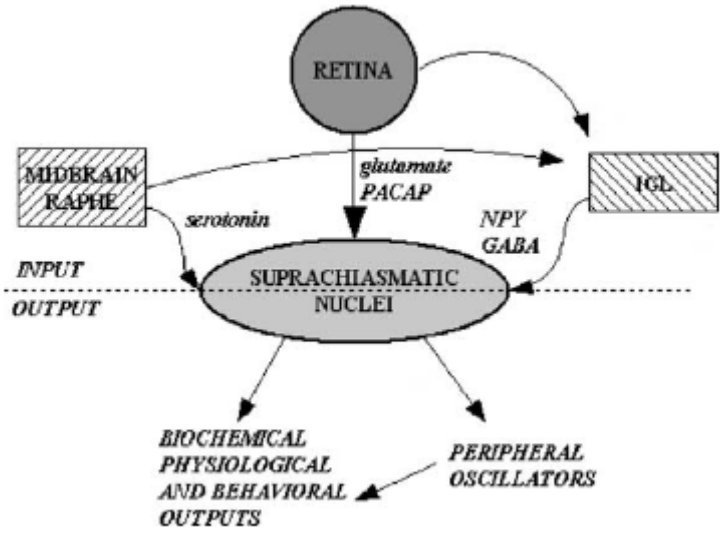

Figure 3.1 Schematic representation of the input pathways to the SCN [30].

The SCN has three main output pathways [8, 56] (see figure 3.2). The first output pathway is the largest one that extends dorsally from the SCN to the subparaventricular zone (SPZ) which is just ventral to the paraventricular nucleus (PVN) of the hypothalamus [8]. The second output pathway is to the dorsomedial and ventromedial nuclei of the hypothalamus which functions in the regulation of sleep-wake cycle, feeding, and locomotor activity and in some cases corticosteroid secretion [8, 52]. The third output pathway courses dorsally into the paraventricular nucleus of the thalamus, however only a small portion of fibers extend from the SCN into this region [8, 52]

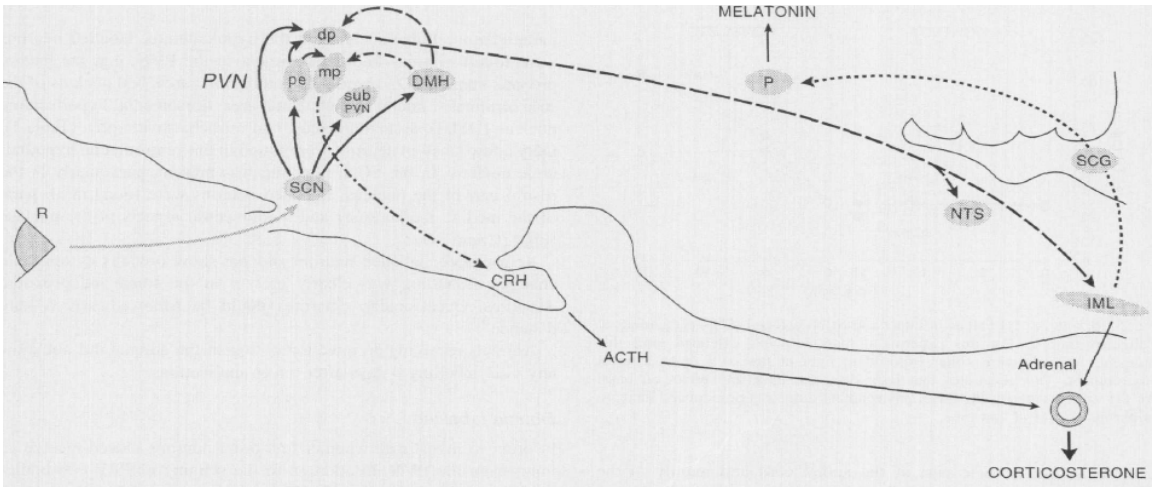

Figure 3.2 Schematic representation of the output pathways of the SCN [57]. From the retina (R), light information is transmitted to the SCN which transmits the signal to the particular nuclei around PVN. The pineal (P) is connected to the retina via a multi-synaptic pathway originating in the retinal ganglion cells and involving the SCN, the PVN and superior cervical ganglion (SCG). The pineal is responsible for production of melatonin. Output pathways of the SCN also regulate secretion of corticosterone from the adrenal gland.

3.2 Circadian Rhythms, Depression, and Light

Recent studies demonstrate that circadian rhythms are altered in depression [14, 18, 19, 23, 32]. Severe disturbances of the sleep-wake rhythm as well as changes in hormonal and body temperature rhythms are reported in depression [3, 8, and 52]. These rhythm disturbances suggest that the biological clock may be malfunctioning in depressed patients. The mechanism underlying the relationship between depression and circadian rhythms is unclear. The disrupted rhythms may cause depression or alternatively depression may

cause rhythm disturbances [58]. Phase delaying and period lengthening effects of antidepressant drugs and lithium are consistent with the hypothesis that disorders of circadian rhythms may be etiologically related to depression [10].Controlled animal studies have reported that antidepressant drug treatments can alter the phase, amplitude, and patterning of circadian rhythms in a variety of measures [11].

In addition to antidepressant drugs, it has been shown that light therapy that is implemented at the appropriate intensity, duration and time of the day may have therapeutic effects on the symptoms of seasonal affective disorder [12-18] and nonseasonal depression [20-23]. Nonseasonal depressed patients may display signs of both phase advances and phase delays [21]. Light exposure early in the night results in phase delays, whereas light late at night causes phase advances of the SCN [3,6]. It was hypothesized that evening light corrects phase advances, whereas morning light corrects phase delays [13]. Thus, the timing of light relative to a photosensitive interval is critical for its antidepressant effect.

4. LIGHT THERAPY

Light therapy is mostly used in clinical studies for the treatment of SAD, although more recent studies point its effectiveness in nonseasonal depression as well [12-23]. It has been generally administered by the outpatient himself or herself at home at the scheduled time with homemade units, such as light boxes [61, 18]. Patients sit in front of the light box to be exposed to light. The original regimen of light therapy that had significant antidepressant effect in clinical studies used 2500 lux for 3 hours in the morning and evening sessions [16]. Later studies demonstrated that more intense light has significant antidepressant effect in shorter durations such as 10000 lux for 30 minutes [20, 59]. Additionally, it was reported that bright light treatment is comparable in terms of its efficiency with most antidepressant medications [60].

There is a debate about the exposure time of light. Some of the studies did not find any significant difference of the time in terms of antidepressant effects [22, 61, 62] while others found a superiority for morning light treatment over evening treatment both in SAD and nonseasonal depression [12, 15, 17, 18]. It was found that most of the depressed patients have phase delays in their daily rhythms. When phototherapy is administered to these patients in the morning, its effect was superior compared to evening light treatment [12, 15, 17, and 18]. The specific hypothesis is that morning light is more effective as it causes phase advances which recover phase delays in depressed patients.

Nevertheless, considerable variety of side effects has been reported based on several clinical studies on light therapy. The most common side effects are nausea, headache, vision problems, hypomania, anxiety, irritability, agitation, mood swings, manic episode, and suicide attempt [35, 59, and 63]. Generally, light therapy has been usually used in parallel with medication. These side effects may not be due to light therapy alone but to combination of the therapy and drugs [35].

4.1 The Use of Light Therapy in Animal Models of Depression

Animal models of depression have been developed to elucidate the mechanisms underlying depression as well as to test drugs for their antidepressant effect before clinical applications. The validity of these models heavily relies on how they predict the antidepressant effects [64]. As it is not ethical to use humans in investigating the central mechanisms of depression by invasive methods, several animal models of depression such as forced swim test (FST) and tail suspension test are used [25, 26].

The FST is an animal model of depression which is used to evaluate antidepressant activities in rodents [25, 26]. The FST is composed of two consecutive swim tests separated by 24 hours. The first test is also known as the conditioning swim test with duration of 15 minutes. The second test is also known as the test swim and its duration is 5 minutes [65]. The FST is conducted in a cylinder tank of 45cm height and 30 cm diameter which is filled with water at 25 ^{o}C to a level of 15-18cm in the original FST and 25-30cm in the modified FST [24, 25, 26, and 66]. When animals are forced to swim in this cylinder, initially they try to escape and develop struggling behaviors such as head shaking, diving and jumping; afterwards they tend to become motionless or in other words immobile. Durations of immobility is longer in the test swim because of previous exposure to the conditioning swim. The significantly increased immobility in the test swim compared to the conditioning swim is accepted as a sign of depression and is known as "behavioral despair". Animals given antidepressant drugs exhibit less immobility and display more struggling behaviors in the test swim compared to controls. The behavioral activation by antidepressants in the forced swim test is not due to generalized locomotor stimulation as these drugs usually decrease locomotor activity under basal conditions [26, 64].

There are few laboratories which study the antidepressant effect of light in animal models of depression. A pioneering study conducted in 2000 by Molina-Hernandez and Tellez- Alcantra showed the antidepressant effect of light on behavioral despair model of depression. The standard 12-hour Ligth/12-hour Dark (12L/12D) cycle of animals was changed via manipulating illumination to evaluate the effects of photoperiod on behavioral despair. Animals were maintained under either a long (14L/10D) or short photoperiod (5L/19D) procedure for 30 days or treated with tricyclic antidepressants for 30 days under

normal photoperiod (12L/12D). The study demonstrated that animals exposed to long photoperiod displayed less immobility similar to animals that were given antidepressant treatment, while the group maintained on short photoperiod displayed longer immobility compared to both long photoperiod and drug treated groups [67].

Another experiment showed the antidepressant effect of a single day of constant light on behavioral despair. In this study, animals were kept under light in the dark phase of a 12L/12D daily lighting schedule resulting effectively in a 24h light schedule for a single day. Animals in the 12-hour light treatment group displayed shorter immobility in the test swim compared to the control group [42]. Another study conducted by the same group explored the effect of varying length and timing of photic stimulation in the dark phase of the L/D cycle on behavioral despair. Light pulses were given either 2 hr or 30 min in length in early, middle or late hours of the dark phase. Results revealed that a 30-min light pulse delivered 8 ¼ h after dark onset (ZT20¼) on a 12 h L/D cycle significantly reduced immobility on the second day of the testing compared to the controls. Light pulses delivered early in the dark phase (ZT15¼) did not have an antidepressant effect. These findings show that photic stimulation may have antidepressant effect on behavioral despair if it is applied in the appropriate time for appropriate duration [27]. Overall, the authors suggested that antidepressant effect may be attained by shorter light pulses.

4.2 The Hypothetical Mechanisms Underlying the Antidepressant Action of Light Therapy

The mechanism underlying the ameliorative effect of light is not known but there are hypotheses about its function. It has been hypothesized that the antidepressant effect of light therapy may be due to suppression of melatonin production, photon over exposure, activation of serotonergic system, or circadian phase shifting.

Melatonin is an essential hormone involved in the regulation of circadian rhythms. It is secreted by the pineal gland mainly at night [28]. Melatonin is secreted from the pineal gland which is connected to the retina via a multi-synaptic pathway originating in the retinal ganglion cells and involving the SCN, the PVN and superior cervical ganglion (See

figure 3.2). The circadian rhythm of melatonin can be used as an indicator of circadian phase position as it is a well-defined, high amplitude rhythm controlled by the SCN [68]. The melatonin synthesis is low during the day and high during the night (reviewed in Kennaway and Wright, 2002). Melatonin rhythm may be manipulated by light exposure [14] and can be detected via either serial blood sampling or serial salivary sampling [14, 28]. Light exposure has two main effects on melatonin rhythm, the acute inhibition of synthesis and the phase shifting of the rhythm via the SCN [69, 70]. Light pulses presented early at night inhibit melatonin production immediately but if the animals remain in darkness, the synthesis resumes. Light pulses applied later at night also suppress melatonin synthesis but there is no successive recovery [69]. The melatonin hypothesis posits that depression occurs as a result of an increase in the melatonin secretion and as light suppresses melatonin production, it produces an antidepressant effect [23]. However, there is no study that shows a direct effect of melatonin suppression in reducing depressive symptoms. In contrast, recent studies reported that administration of melatonin or its agonists have a protective effect on behavioral despair [71-73]. An indirect method by which melatonin may be involved in depression might be through circadian phase shifting [28].

Another proposal known as photon counting hypothesis assumes that depression may result from insufficient light exposure at any time of the day [16, 20, 21, and 28]. The photon-counting hypothesis is supported by observations that patients with SAD are likely to be depressive during winter when the amount of light decreases as the period of daytime shortens. More evidence for photon-counting hypothesis comes from the fact that there is a dose-response relationship for bright light therapy. Additionally, there is an inverse correlation between the intensity of light and duration of light exposure needed for the antidepressant effect. For example, durations of 30min of 10000 lux or 2 hours of 2000 to 3000 lux are satisfactory to produce antidepressant effect [21, 28, and 59].

The third potential mechanism for the ameliorative action of light is via serotonin, a chemical that serves both as a neurotransmitter and neuromodulator within the mammalian central nervous system [5]. Serotonin functions in the regulation of circadian rhythms [30]. SCN receives direct serotonergic inputs from the raphe nuclei which enables entrainment of circadian rhythms by non-photic stimuli [11, 29, and 30]. Serotonin is able to produce

phase advances during the subjective day and can inhibit light-induced phase shifts during the night [30]. This finding indicates a possible interaction between photic and nonphotic pathways of the SCN which may result in light induced regulatory effect on serotonergic system. In other words, the serotonin hypothesis assumes that light therapy may exert its antidepressant effect via regulation of serotonergic system. Additionally, it was reported that non-photic stimuli which activate serotonergic input to SCN can reduce depressive symptoms in animal models of depression [58].

Another proposed mechanism for the involvement of light therapy in depression considers that depression and other mood disorders are caused by shifts in circadian rhythms [14, 18, 23, 28, 31, and 32]. Based on the observation that bright light given in the morning leads to phase advances and bright light given in the evening leads to phase delays, it was speculated that light may exert its effect via circadian phase shifting. Light therapy given in the morning might be superior in terms of alleviating the depressive symptoms compared to the light therapy given in the evening in patients with delayed rhythms [3, 18, and 74].

4.3 The Histological Evidence of Light Based Circadian Shifting

Analysis of the photic induction of c-Fos immunoreactivity within the SCN has proven to be a powerful tool to study the central mechanisms involved in phase shifting in the circadian clock [33]. Following light exposure, immediate early genes (IEGs), especially c-fos, is transcriptially activated in the SCN immediately [7]. These genes function in coupling short-term signals to long term changes by mediating later changes in gene expression. c-Fos regulates the transcription of late response genes as it is a transcription factor [34]. Transcription of late response genes induces several transduction pathways. Thus, the presence of c-Fos may serve as a marker of neuronal activation [7]. Light induces c-Fos in the SCN only in mid-subjective night when it also phase shifts circadian rhythmicity [34].

c-Fos is expressed in two different regions within the SCN (see figure 4.1). Light induced c-Fos expression is located in the ventrolateral core region and it is temporally and

functionally related to clock resetting and entrainment of circadian rhythms. Additionally, a spontaneous free rhythm is located in the dorsomedial shell region [70].

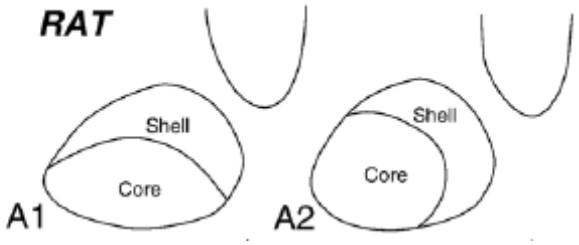

Figure 4.1 Suggested organization of the SCN of a rat: the "core" and "shell" nomenclature [55].

c-Fos expression is high during the subjective day when photic sensitivity of the core is minimal and low in the subjective night, when photic sensitivity of the core is maximal [75]. It was demonstrated that in constant darkness, 30 minutes exposure to 250lux light in the early subjective day (CT 2) or night (CT 14) induced c-Fos in the core region while suppressing the levels of c-Fos in the shell region [75]. Furthermore, light exposure at night (CT 20) increased c-Fos expression in the core, but had no effect on c-Fos expression in the shell region. The finding that c-Fos expression in the shell region of the SCN is suppressed by light at dawn and dusk suggests a critical role for the shell in photic entrainment of circadian rhythms in nocturnal rodents [75].

There are other studies that indicate the compartmentalization of SCN based on photic stimulation (see figure 4.2). The endogenous rhythm in c-Fos immunoreactivity in the whole SCN was mostly due to the marked rhythm in the dorsomedial shell region of the SCN [70, 76]. Additionally, it was reported that there is a significant correlation between c-Fos induction and the exposure time of light. When light is applied at ZT15, it induces c-*fos* expression and elevates c-Fos in the ventrolateral SCN and also induces phase shifts in the circadian rhythms [70]. These studies indicate two different rhythms of c-Fos expression in the SCN. One depends on photic input, expressed highly in the ventrolateral SCN and restricted to the subjective night. The other depends on spontaneous circadian rhythm, highly expressed in dorsomedial SCN and limited to the early subjective day [76].

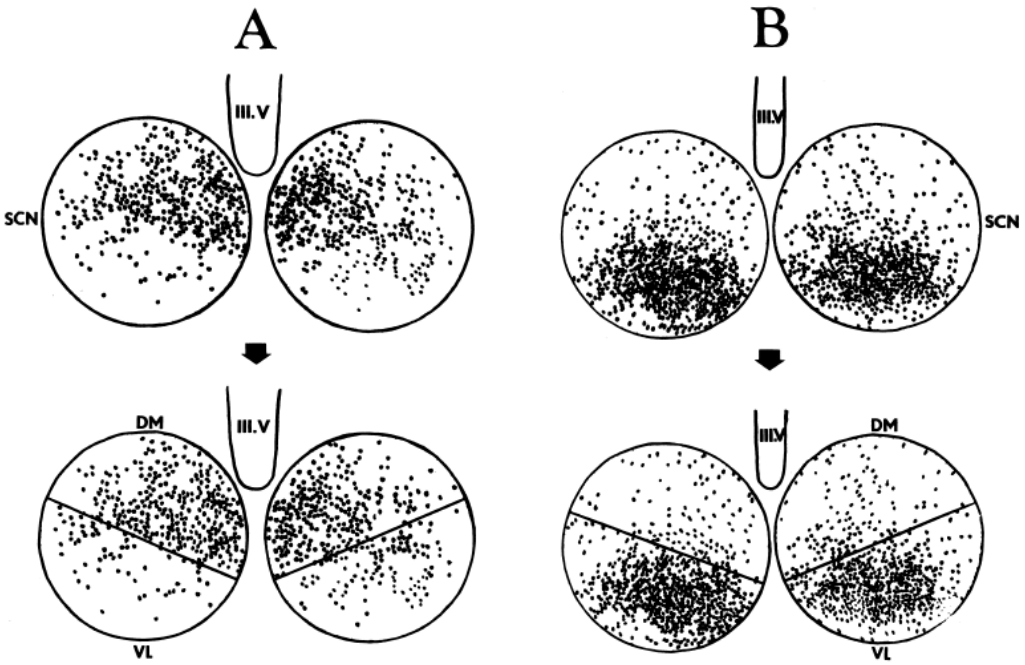

Figure 4.2 The induction of c-fos in darkness at ZT4 (A), and the induction of c-fos when 30 min light presented at ZT15 at night (B). The SCN was divided in two as dorsomedial and ventrolateral regions. When light is applied at ZT15, c-Fos expression increases in the VL region and when light is applied at ZT4, c-fos expression increases in the DM region. DM dorsomedial, VL ventrolateral, III.V third ventricle [70].

Moreover, the effects of ultra-brief, intense, light flashes were detectable via both phase shifts in free running activity rhythms and Fos expression in the SCN [77]. This study showed that very brief light pulses may induce behavioral and cellular effects via modulating phase shifts detectable by c-Fos induction.

In this study, the effects of timing and intensity of light exposure were assessed on behavioral despair model of depression. It is reported that 30min exposure of light early at night did not have an antidepressant effect while light application late at night has an antidepressant effect [27]. Based on these findings, the effect of brief exposure of light was evaluated early at night (ZT15) and late at night (ZT21). These time points were chosen as light applied at ZT15 is known to induce phase delays while light applied at ZT21 is known to induce phase advances in rodents [78]. Following light stimulus, c-fos is transcriptially activated in the SCN [7, 78]. It was reported that light stimulated c-Fos induction occurs when light is administered at circadian times at which the pulse cause a phase shift (34, 78). Circadian times ZT15 and ZT21 were evaluated for c-Fos expression in order to discuss the mechanism underlying the antidepressant effect of light exposure.

5. METHOD

5.1 Subjects

Fifty- two male Wistar rats raised in the breeding colony of Psychobiology Laboratory of Bogazici University, weighing between 250-300 g at the beginning of the experiment, were randomly assigned to experimental and control groups. All animals were maintained in a temperature controlled room (22±2 °C) on a 12h light/12h dark cycle with light onset at 06:00h. Animals were allowed food and water ad libitum. All behavioural testing was conducted between 14:00 and 16:30h and videotaped.

5.2 Experimental Protocol

Forty adult rats were randomly assigned to one of five groups (n = 8 each) for behavioral testing. Groups were administered either 900lux or 1350lux light either at ZT21 or ZT15. The control group remained in the chamber without light exposure. Light treatment was administered in a sound and light insulated ventilated chamber. Light was provided for 10 min either by a 100W (900lux) or a 200W (1350lux) tungsten lamp, approximately 50 cm above the housing cage.

The first experimental group received light pulse at ZT21 with amplitude of 900lux, the second one received light pulse at ZT21 with amplitude of 1350lux; the third one received light pulse at ZT15 with amplitude of 900lux, the fourth one received light pulse at ZT15 with amplitude of 1350lux before the first forced swimming test. The fifth group was the control group which was placed in the chamber but did not receive a light pulse.

For light treatment as well as for the control group, subjects were taken from their home cages 10 minutes before the beginning of the dark period in the L/D cycle (at 1750 h-ZT12) and were introduced into the insulated chamber as seen in the photos below. Four animals in two cages were tested in a single insulated chamber. There were two insulated chambers which enabled testing of 8 subjects on a given day. Animals had free access to food and water in the cages in the insulated chamber. Timers were used to enable light

exposure at desired circadian time. Air circulation was provided by means of air pumps as seen below.

Figure 5.1 Photographs of the insulated chamber, the timer, and the air pump.

The chamber was kept dark in the 12 h dark phase of the L/D cycle (1800-0600 h) except for a 10-minute, 900lux or 1350lux light pulse exposure at either ZT21 (0300h-3 hours before lights on) or ZT15 (2100 h-3 hours after lights off) for the experimental groups. The control group was also kept in the chamber, but did not receive light pulses. For all groups housed in the chamber, light was turned on at 0600 h (ZT0) in the insulated chamber to start the light phase of the L/D cycle. Animals were kept in the insulated chamber until 1400 h (ZT8) when the first of the forced swimming test was conducted as specified below. Animals were returned to their home cages in the vivarium after the first forced swim test and kept there until the second forced swimming test conducted 24 h later as specified below. Following is the graphical representation of experimental procedure:

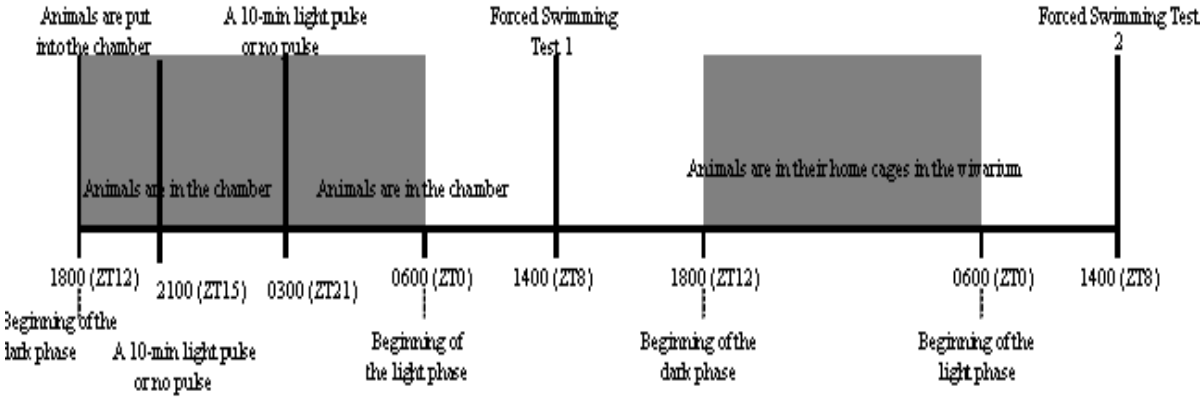

Figure 5.2 The graphical representation of experimental procedure.

Animals were tested individually in two forced swim tests (FSTs) separated by 24 h. The tests were conducted in a Plexiglas cylinder of 45 cm height and 30 cm diameter. For each animal, the cylinder was refilled with fresh water (25º) to a height of 25 cm. In the first swim test, animals were individually forced to swim for 15-minute followed 24 h later by a 5-minute second test. After each test, the animal was towel dried and placed under a lamp for 30-minutes for drying.

5.3 Histology

Twelve male Wistar rats were randomly assigned to three groups; ZT21, ZT15, and control for c-Fos immunohistochemistry. For light treatment, subjects were taken from their home cages 10 minutes before the beginning of the dark period in the L/D cycle (at 1850 h-ZT12) and were introduced into the insulated chamber. Each animal was housed in a plastic cage, two plastic cages were placed in an insulated chamber, and two animals were tested in a single insulated chamber. Since there were two insulated chambers 4 subjects were tested on a given day. Animals had free access to food and water in the chamber. The chamber was kept dark in the 12 h dark phase of the L/D cycle (1900-0700 h) except for a 10-minute, 1350 lux light pulse exposure at either ZT21 (0400h-3 hours before lights on) or ZT15 (2200 h-3 hours after lights off). The control group was also kept in the chamber, but did not receive light pulses.

c-Fos immunoreactivity was measured as an indicator of c-Fos protein level. Rats were deeply anesthetized with ketamine/xylazine (160mg/kg, intraperitoneally, Richter Pharma) 80 minutes after the beginning of light exposure, and perfused through the ascending aorta with saline followed by freshly prepared 4% paraformaldehyde in PBS 90 minutes after light exposure. Brains were removed, post-fixed for at least 2 days in 4^0 C. After fixation, coronal 50 μm thick sections were cut via a vibrotome (Campden instruments, UK, 752M Vibroslice) and processed for immunohistochemistry. Immunostaining for c-Fos was carried out on free-floating sections. Sections were treated with 0.1 M glycine solution (in PB) for 30 min and with 0.5% hydrogen peroxide for 10 min. Three washes with PB preceded and followed these treatments. After preincubation with normal goat serum (NGS; 1% NGS in 0.3% Triton X-100/PB) for 1 h for blocking, sections were incubated for approximately 48 h at 4 °C in primary antibody- rat monoclonal antibody raised against the N-terminal sequence of c-Fos (corresponding to N terminal residues 4–17 of human c-Fos) in rabbit (Calbiochem, PC38, Darmstadt, Germany). The antibody was diluted 1:10000 with a solution of 0.3% Triton X-100, in Phosphate-buffered saline with 1% normal goat serum. Following incubation in primary antibody, sections were then washed with PB, exposed for 1 h to biotinylated goat antirabbit IgG (1:200 in 0.3% Triton X-100/PB), washed again with PB and incubated for 1 h in avidin-biotin peroxidase complex (1:100 in 0.3% Triton X-100/PB; Vectastain, Vector Labs, Burlington, CA). After 3 PB washes, sections were treated with 0.5% diaminobenzidine (DAB) in the presence of 0.1% hydrogen peroxide. Sections were then mounted on slides, dehydrated and coverslipped with Permount [79].

5.4 Data Collection

All swim test sessions were recorded on videotape. Behavior in the swim tests were evaluated as follows. Immobility was defined as staying motionless or floating by keeping the head above the water surface without leaning against the wall of the cylinder. Comparison of duration of immobility in the first and second swim tests reveals behavioral despair. In addition to immobility, duration of swimming, number of head shakes, jumps and dives were coded. Jumps were counted when the animal lifted itself up and revealed at least two thirds of its body length. Dives were recorded when the rat's body was totally

immersed in water. Moreover, to enhance the sensitivity of traditional FST to measure serotonergic activation, the data was recoded by a time sampling technique to rate the predominant behavior over a 5-s interval. This sampling technique enabled to distinguish specific behavioral components of active behaviors. Active behaviors measured in this sampling method are as follows (see figure 5.3). Climbing behavior is defined as upward-directed movements of the forepaws along the side of the swim chamber; swimming behavior is defined as the horizontal movement throughout the swim chamber that also includes crossing into another quadrant; and immobility is defined as remain motionless other than that required to keep the rat's head above the water [24, 66, 80].

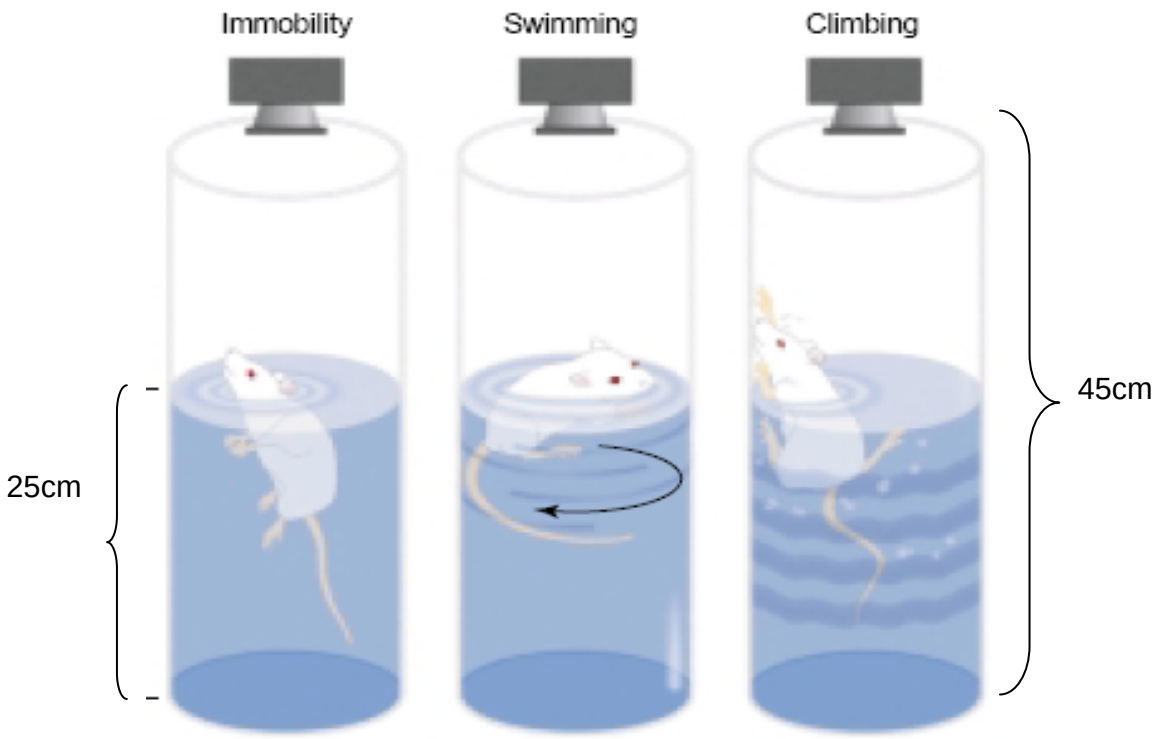

Figure 5.3 Schematic representations of behavioral parameters that were measured during the FST. In this study, durations of immobility and swimming were measured [24].

5.5 Statistical Analysis

Statistical significance between groups in terms of duration of immobility and swimming, and number of head shakes was tested by analysis of variance (ANOVA), repeated measures of ANOVA. Statistical significance between groups in terms of counts of immobility, swimming, and climbing was tested by one way ANOVA. The statistically significant level of difference was considered to be at $p < 0.05$. The region between $0.1 < p < 0.05$ was considered as marginally significant. Finally, the mean values of the groups which are statistically significant and marginal significant, were calculated to plot their bar graphs.

6. RESULTS

6.1 Behavioural Tests

The mean durations of immobility (mean ± SEM) of the five groups of male Wistar rats in two forced swim tests (FST1 and FST2) are shown in Figure 6.1.

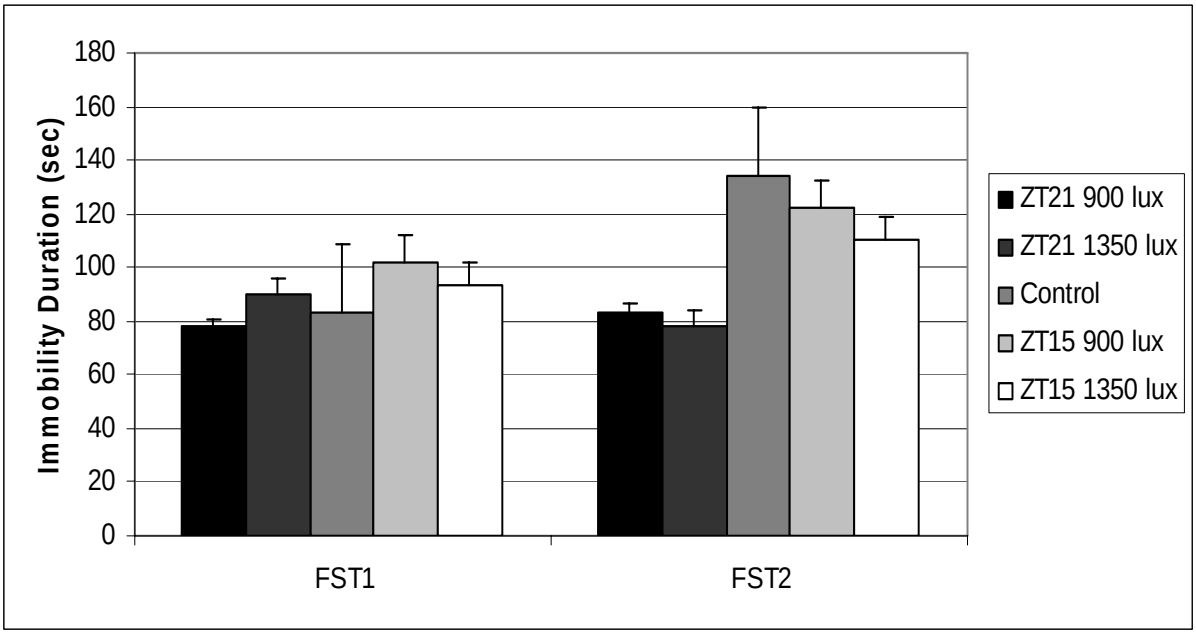

Figure 6.1 Durations of immobility (mean ± SEM) in two forced swim tests (for the first 5 min of FST1 and FST2) conducted 24 h apart. For five experimental groups, animals were exposed to a 10-minute, 900lux or 1350lux light pulse either at ZT21 or at ZT15. The control group was kept in the insulated chamber without light exposure.

Figure 6.2 shows the mean number of head shakes (mean ± SEM) for the five groups in the first 5 min of FST1 and FST2.

Total durations of immobility and swimming, and the number of diving, jumping and head shake behaviors were measured for the first five minutes of the first and the second swim tests for each subject. The means and the standard deviations for these

parameters are presented in Table 6.1. Since there was no jumping in any of the groups, this variable was excluded from statistical analysis.

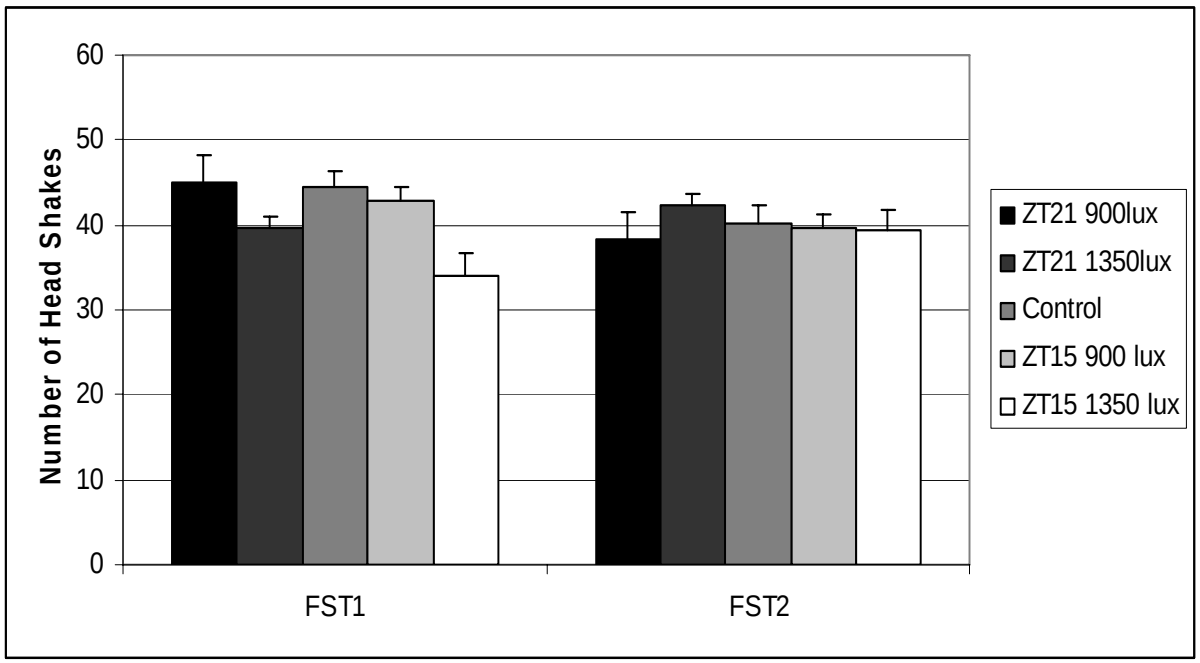

Figure 6.2 Number of head shakes (mean ± SEM) in two forced swim tests (for the first 5 min of FST1 and FST2) conducted 24 h apart. For five experimental groups, animals were exposed to a 10-minute, 900lux or 1350lux light pulse either at ZT21 or at ZT15. The control group was kept in the insulated chamber without light exposure.

Table 6.1

The means and the standard deviations for total durations of immobility and swimming, and the number of diving, jumping and head shake behaviors.

		FST1				FST2			
		Immobility	Swimming	Head Shake	Diving	Immobility	Swimming	Head Shake	Diving
ZT21_900lux	Mean	77.75	125.25	44.88	0.63	83.38	130.13	38.25	0.25
	SD	31.604	52.186	8.59	1.768	37.864	70.101	9.83	0.707
ZT21_1350lux	Mean	89.75	100.88	39.63	3.38	77.75	106	42.25	1.25
	SD	24.283	18.161	10.99	2.387	46.095	42.702	7.85	1.832
ZT15_900 lux	Mean	101.88	99.38	44.38	1.13	122.13	90.25	40.28	0.86
	SD	16.488	17.196	10.99	1.808	41.478	34.977	9.74	2.268
ZT15_1350 lux	Mean	93.38	104.38	42.88	3.38	110.63	107.38	39.71	0.71
	SD	29.428	26.533	12.66	3.852	60.83	43.365	12.51	1.254
Control	Mean	83.38	113	34.13	2.5	134.25	92.25	39.25	1.5
	SD	28.869	41.203	10.11	3.505	55.849	45.862	9.42	2.97

3 way ANOVA was used to evaluate the results of duration of immobility. Full model was used to see both main effects of all factors and main effects plus two or three factor interactions. First of all, mean values of the control group were subtracted from the light exposure groups to find delta values. After that n- way ANOVA was run in MATLAB for the four groups that were exposed to light. Results showed that there is a significant difference among groups in the first and second swimming tests in terms of immobility duration [$F(1,56)=8.82$, $p<0.05$]. There are three factors; FST day (X1) as FST1 and FST2, time of light exposure (X2) as ZT21 and ZT15, and intensity of the light (X3) as 900lux and 1350lux. The results of the n-way ANOVA are given in Table 6.2.

Table 6.2

The main effects of factors X1, X2, and X3 and the interaction effects among them.

Source	Sum Sq.	d.f.	Mean Sq.	F	Prob>F
X1	14762.2	1	14762.2	8.82	0.0044
X2	2352.3	1	2352.3	1.41	0.2408
X3	4160.2	1	4160.2	2.49	0.1205
X1*X2	49	1	49	0.03	0.8648
X1*X3	5112.3	1	5112.3	3.05	0.086
X2*X3	600.2	1	600.2	0.36	0.5517
X1*X2*X3	4290.2	1	4290.2	2.56	0.115
Error	93735.5	56	1673.8		
Total	125062	63			

Figures 6.3, 6.4, and 6.5 show the post-hoc tests on the factors and their significance levels.

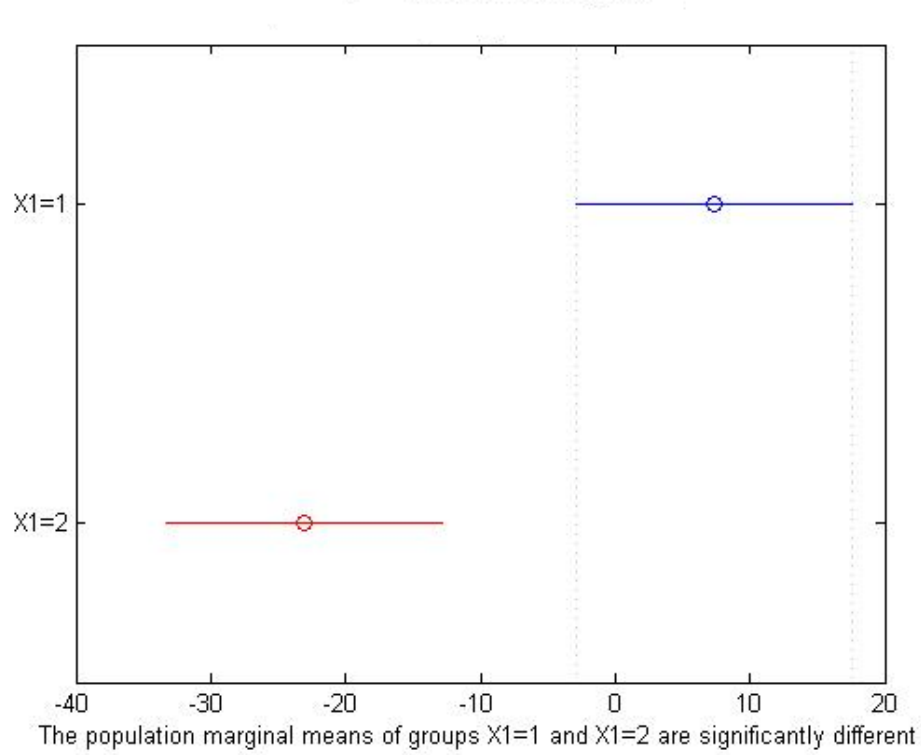

Figure 6.3 The difference between the mean durations of immobility in terms of FST day 1 (X1=1) and FST day 2 (X1=2).

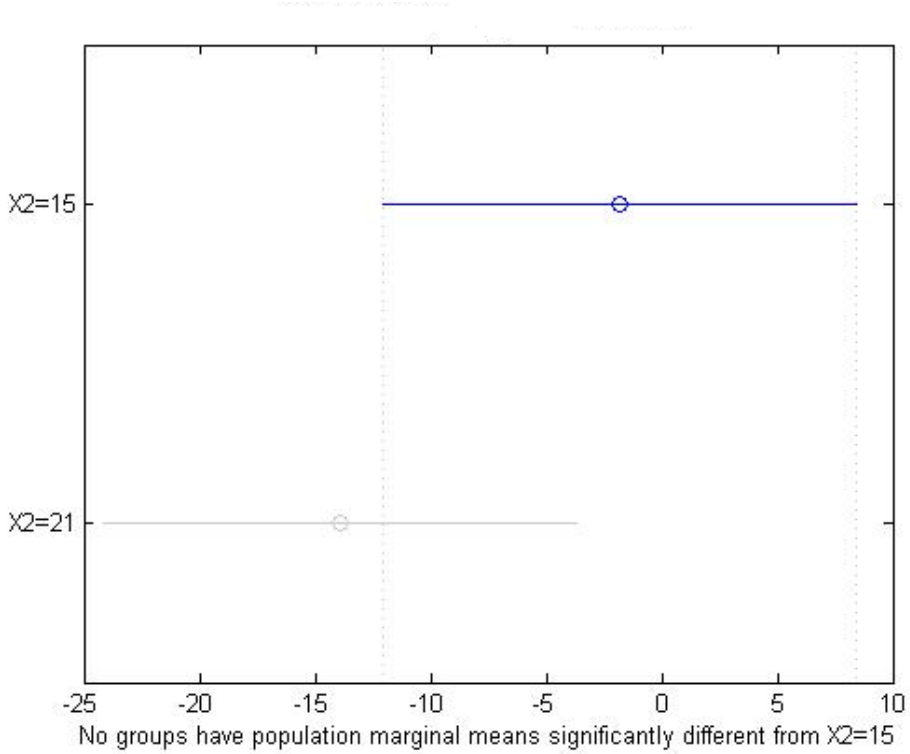

Figure 6.4 The difference between the mean durations of immobility in terms of light exposure time ZT21 (X2=21) and ZT15 (X2=15).

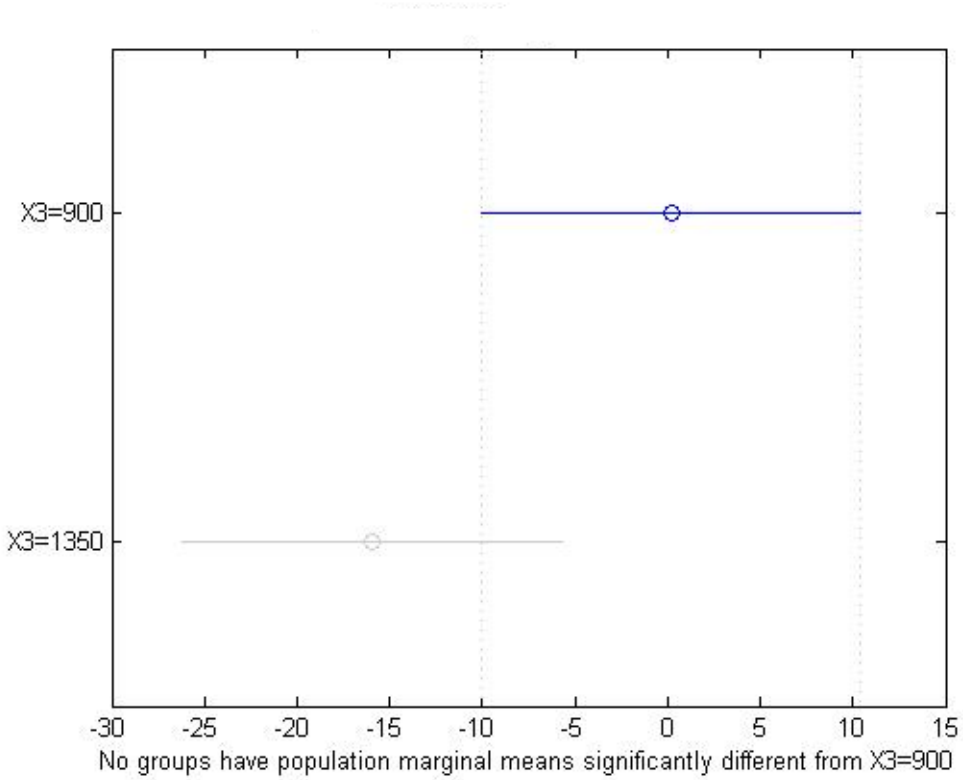

Figure 6.5 The difference between the mean durations of immobility in terms of the intensity of light, 900lux (X3=900) and 1350lux (X3=1350).

An analysis of variance (ANOVA) run in SPSS 16.0 with repeated measures comparing durations of immobility among five groups also indicated a significant difference between FST days [$F(1,35)=5.99$, $p<0.05$]. However, Post- hoc tests showed no significant difference among groups. As there was no significant effect of intensity of light, the groups were combined based on the light exposure times and one way ANOVA was conducted with three groups as ZT21, ZT15 and control groups. The findings of this analysis showed that there is a significant difference among groups in the second swim test in terms of mean durations of immobility [$F(2,39)=4.00$, $p<0.05$]. Post-hoc tests revealed that the ZT21 group is significantly different in the mean duration of immobility in the second swim test from the control and the ZT15 groups ($p<0.05$).

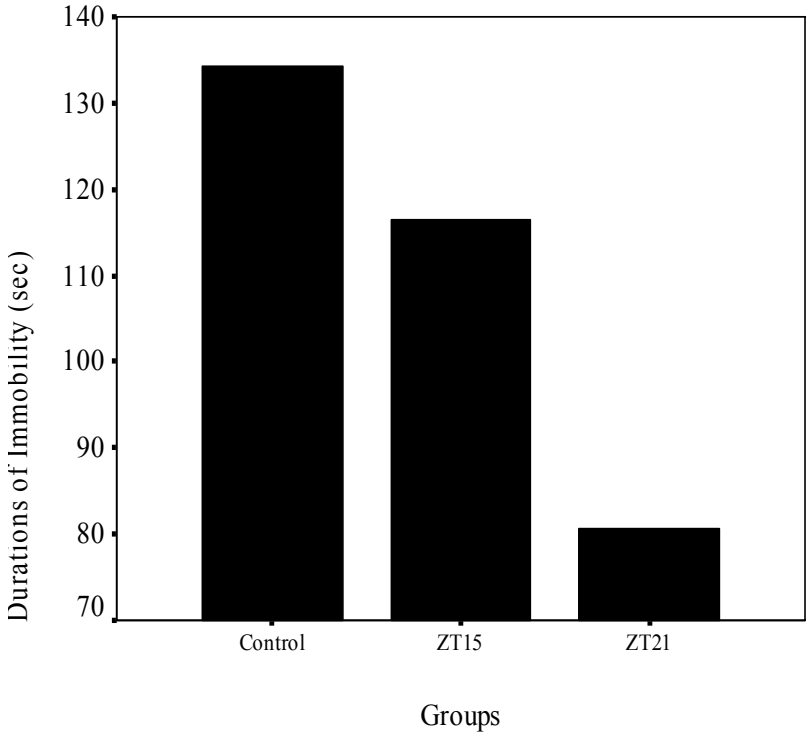

Figure 6.6 The bar graph of durations of immobility. * indicates that ZT21 group is significantly different from the control and the ZT15 groups ($p<0.05$).

An analysis of variance (ANOVA) comparing durations of swimming showed no significant results either for FST1 or FST2 respectively [$F(2,39)=0.518$, $F(2,39)=0.986$, $p>0.05$]. An analysis of variance (ANOVA) comparing the number of head shakes showed no significant results either for FST1 or FST2 respectively [$F(2,39)=0.902$, $F(2,37)=0.03$, $p>0.05$].

An analysis of variance (ANOVA) comparing durations of immobility in the modified FST showed significant results for FST2 [$F(2,39)=4.234$, $p<0.05$]. Post Hoc tests revealed that the ZT21 group is significantly different in the mean duration of swimming in the second swim test from the control and the ZT15 groups ($p<0.05$).

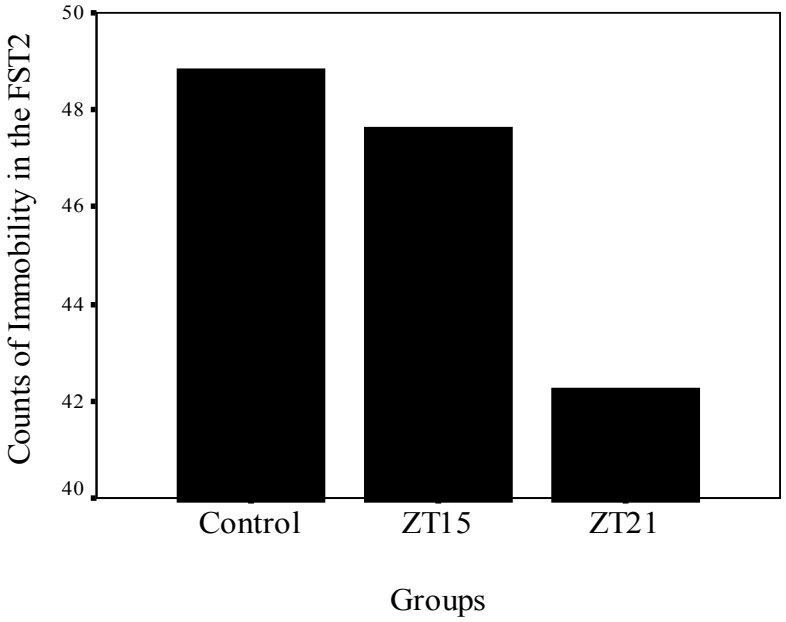

Figure 6.7 The bar graph of counts of immobility versus groups. * indicates that ZT21 group is significantly different from the control and the ZT15 groups ($p<0.05$).

6.2 Histology

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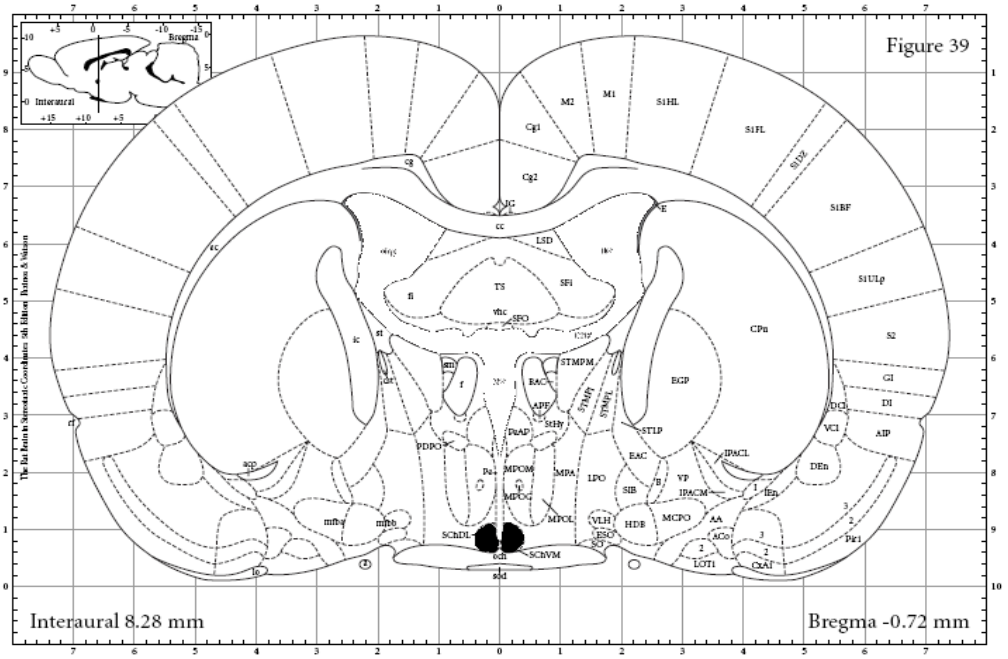

Figure 6.8 The SCN region was labeled as black [81].

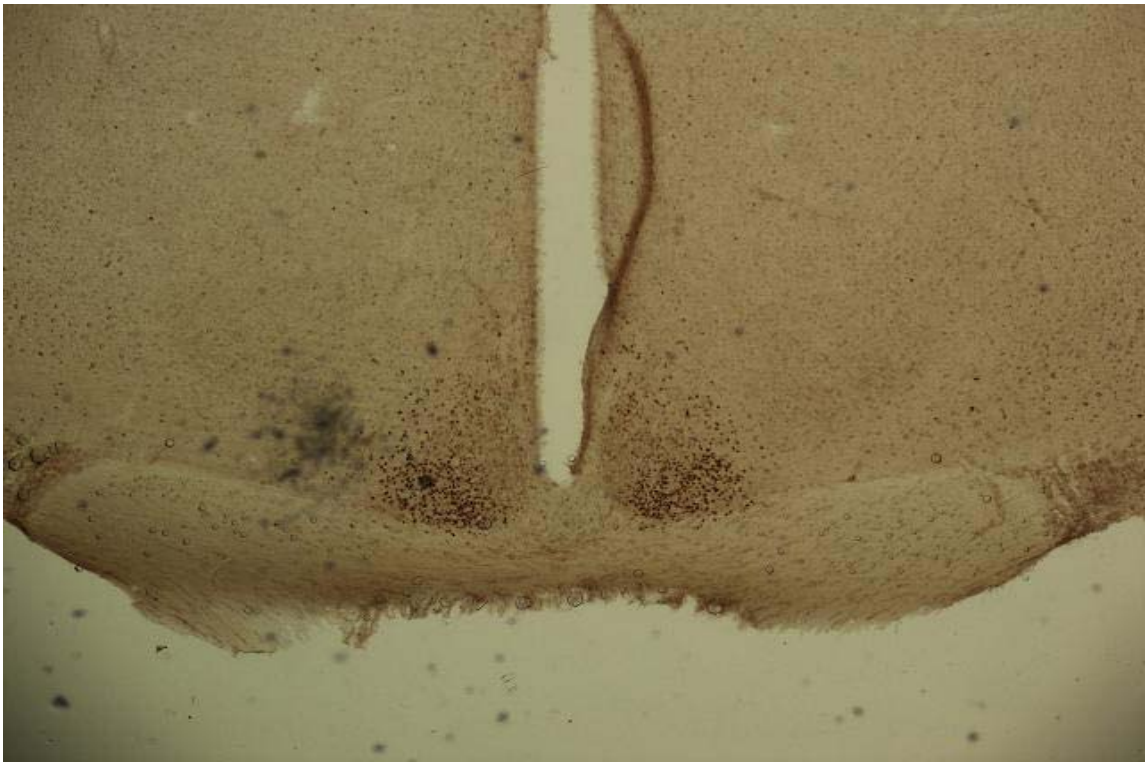

Figure 6.9 Photograph of a coronal section through the SCN region of a rat brain stained for c-Fos immunoreactivity with a rabbit antibody at a dilution of 1:10000. The rat was exposed to light at ZT21 and perfused 90 minutes after photic stimulation.

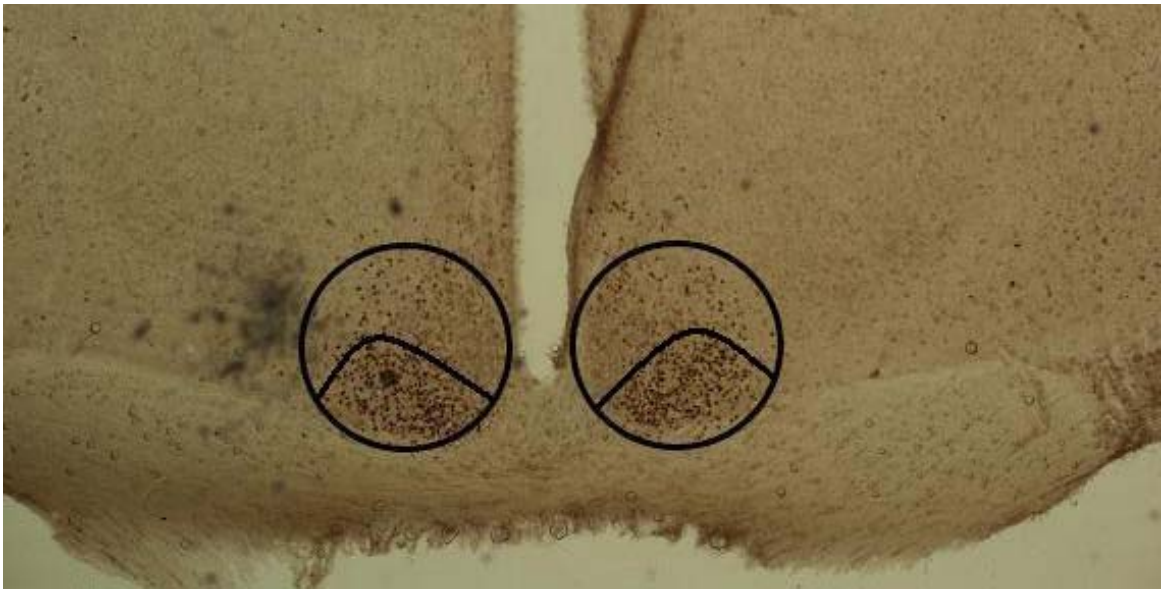

Figure 6.10 The same photograph in figure 6.9. The core and shell regions of the SCN were drawn. The lower part of the ellipse is the core region while the upper part is the shell region. Photic stimulation at ZT21 induces expression of c-Fos in the core part of the SCN.

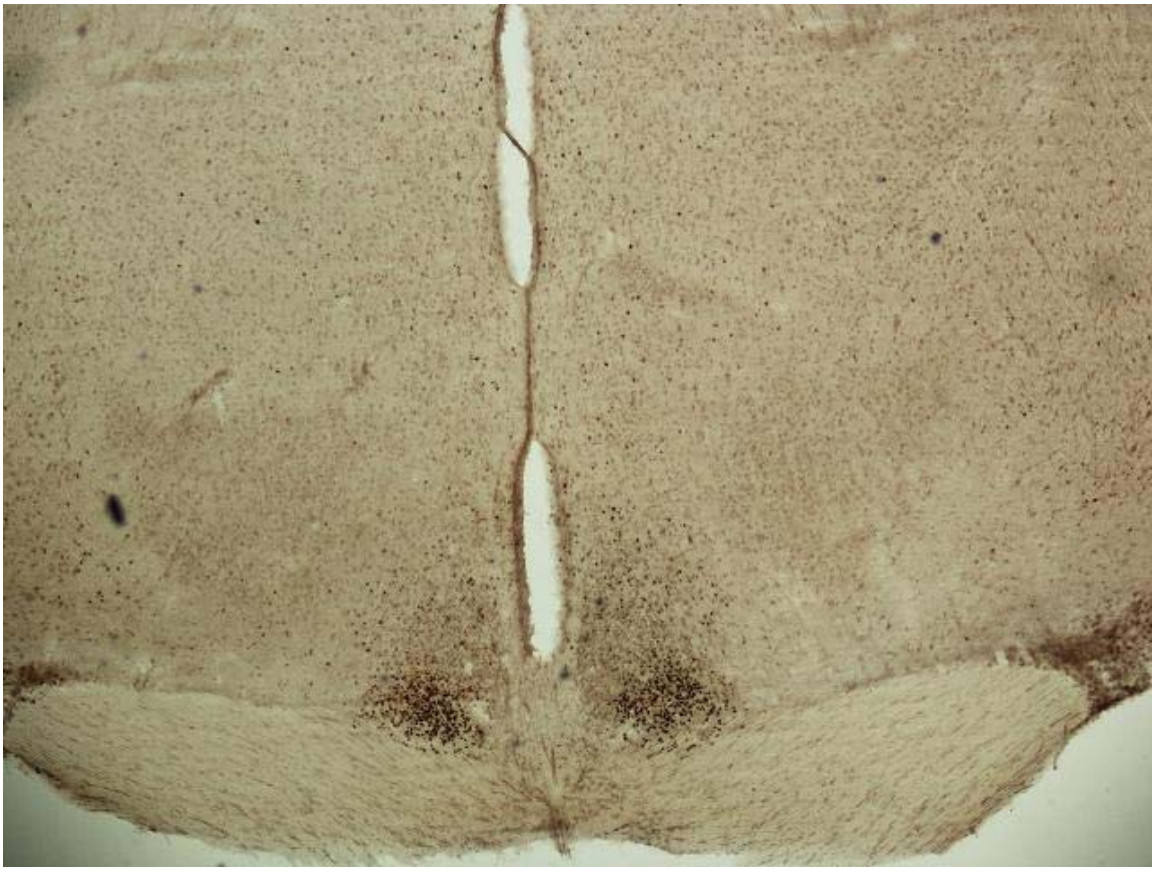

Figure 6.11 Photograph of a coronal section through the SCN region of a rat brain stained for c-Fos immunoreactivity with a rabbit antibody at a dilution of 1:10000. The rat was exposed to light at ZT15 and perfused 90 minutes after photic stimulation.

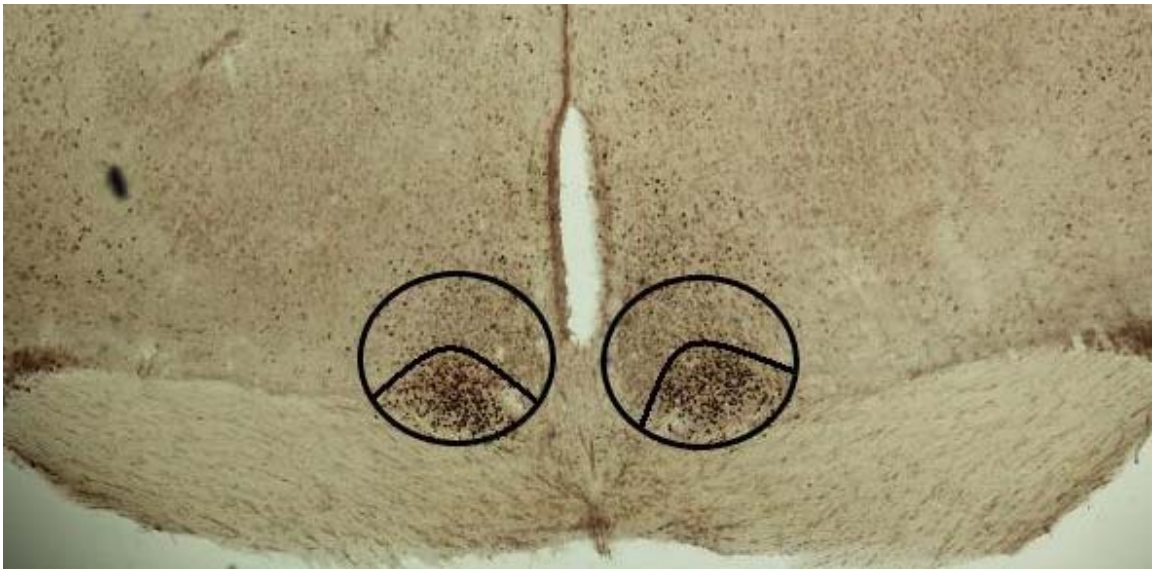

Figure 6.12 The same photograph in figure 6.11. The core and shell regions of the SCN were drawn. The lower part of the ellipse is the core region while the upper part is the shell region. Photic stimulation at ZT15 induces expression of c-Fos in the core part of the SCN.

Figure 6.13 Photograph of a coronal section through the SCN region of a rat brain stained for c-Fos immunoreactivity with a rabbit antibody at a dilution of 1:10000. The rat was a control rat which was not exposed to light.

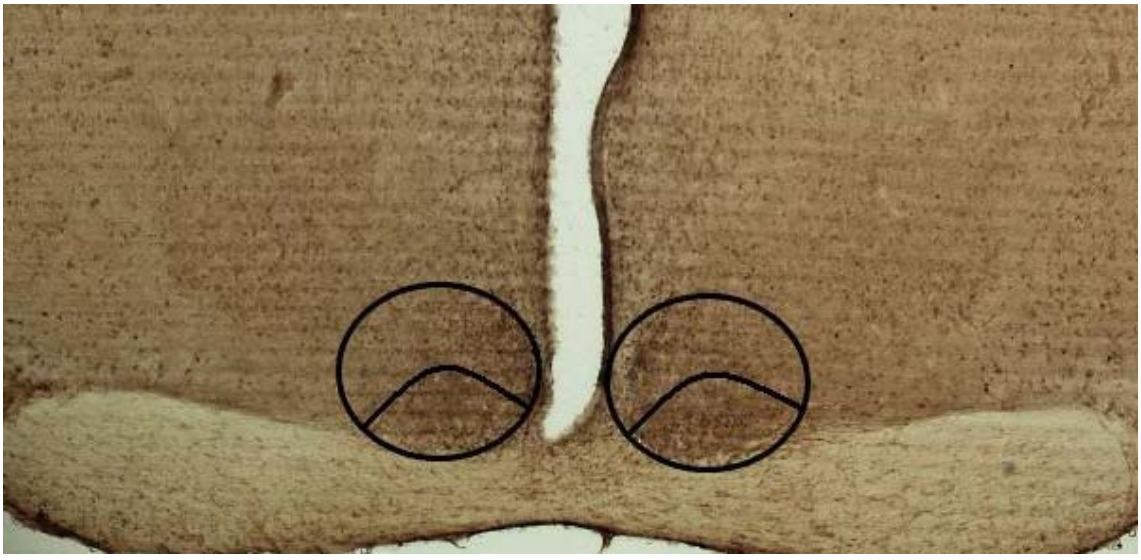

Figure 6.14 The same photograph in figure 6.13. The core and shell regions of the SCN were drawn. The lower part of the ellipse is the core region while the upper part is the shell region. Photic stimulation at ZT15 induces expression of c-Fos in the core part of the SCN.

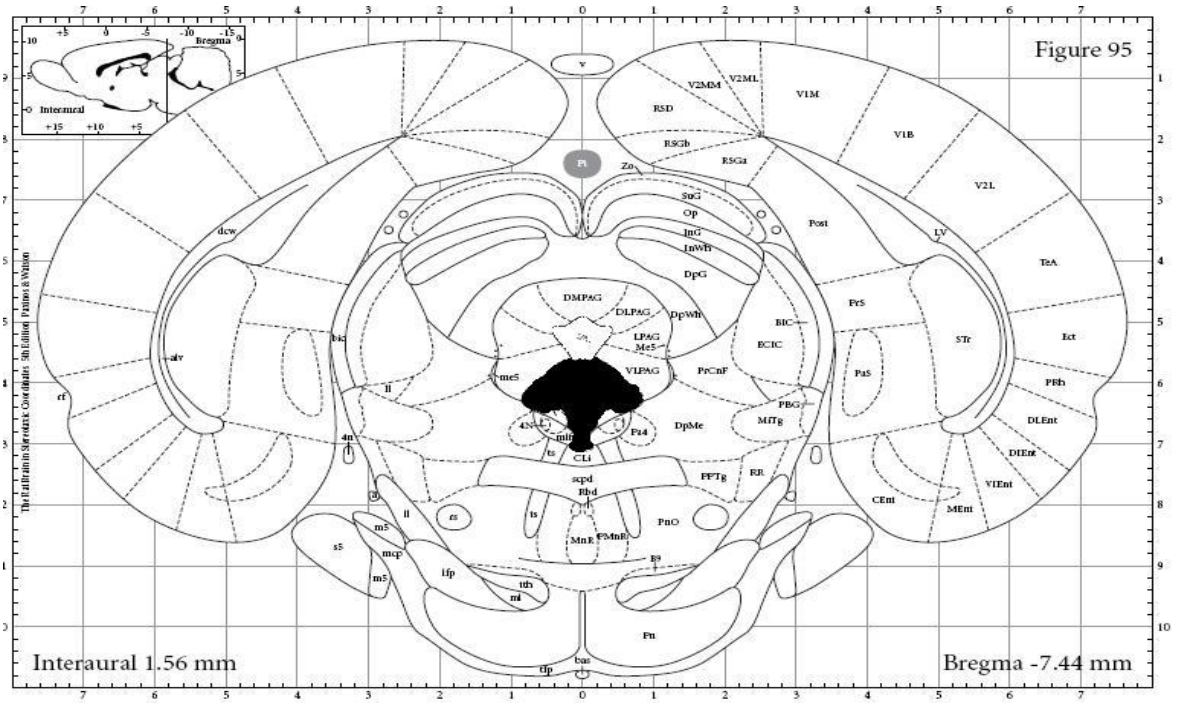

Figure 6.15 The DRN region was labeled as black [81].

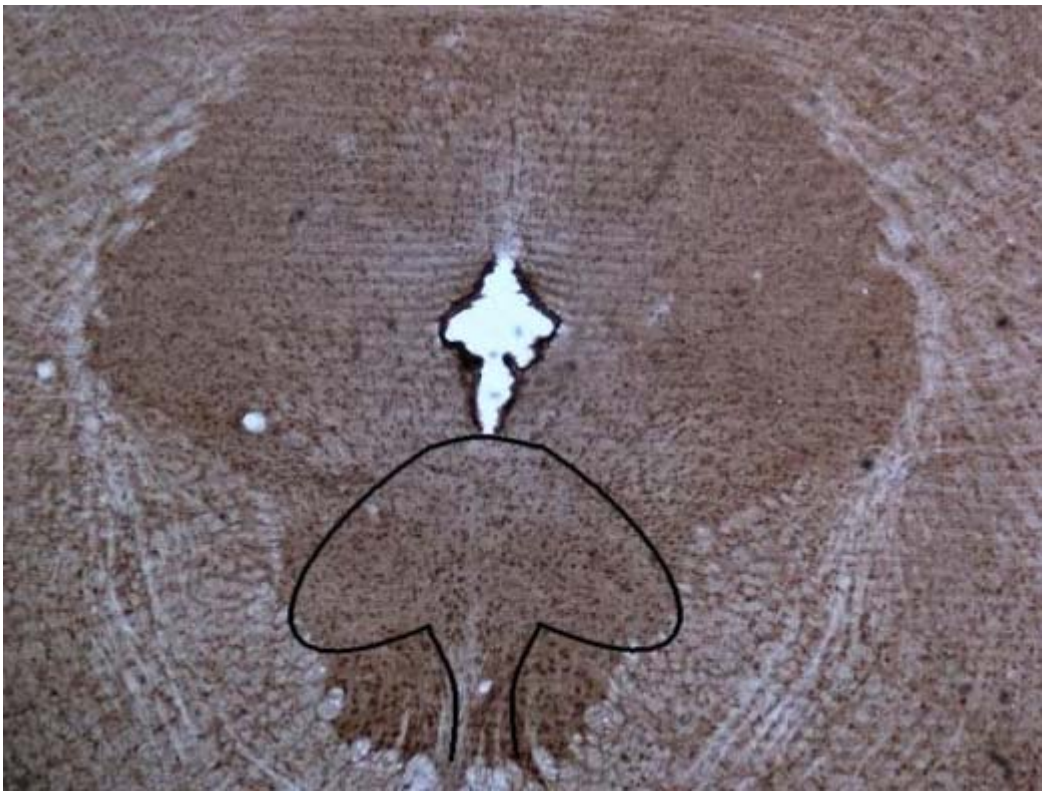

Figure 6.16 Photograph of a coronal section through the DRN region of a rat brain stained for c-Fos immunoreactivity with a rabbit antibody at a dilution of 1:10000. The rat was exposed to light at ZT21 and perfused 90 minutes after photic stimulation.

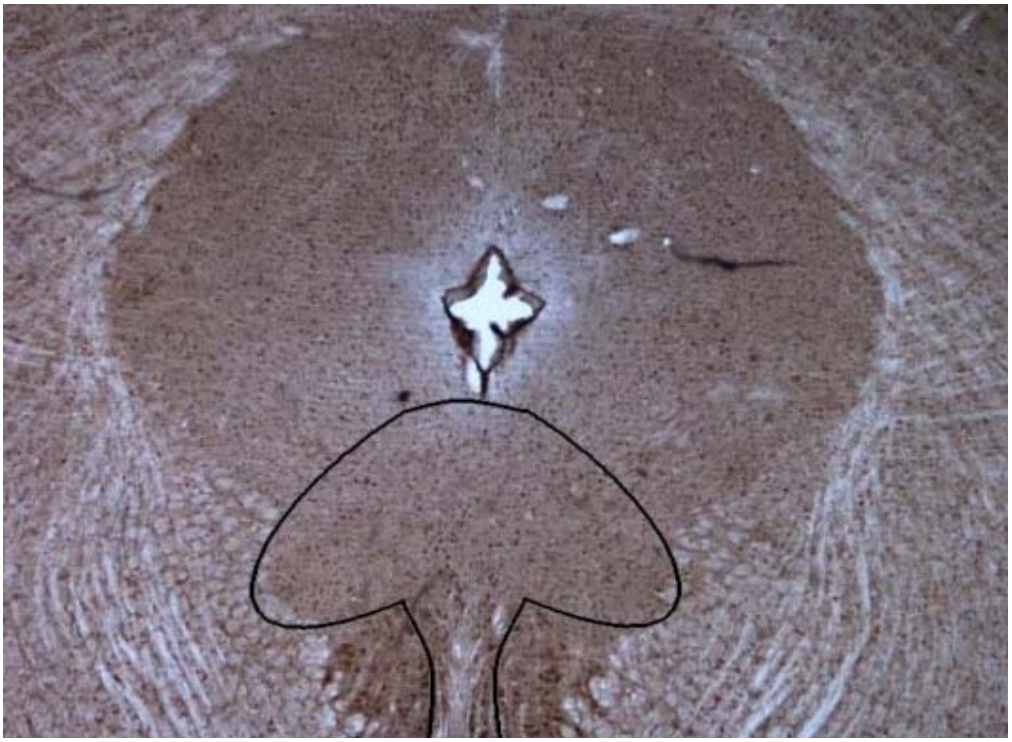

Figure 6.17 Photograph of a coronal section through the DRN region of a rat brain stained for c-Fos immunoreactivity with a rabbit antibody at a dilution of 1:10000. The rat was exposed to light at ZT15 and perfused 90 minutes after photic stimulation.

Figure 6.18 Photograph of a coronal section through the DRN region of a rat brain stained for c-Fos immunoreactivity with a rabbit antibody at a dilution of 1:10000. The rat was a control rat which was not exposed to light.

7. DISCUSSION AND CONCLUSION

The present findings indicated that a single, 10 minutes exposure to light treatment in the late hours of the dark phase (ZT21) of a 12:12 L/D cycle can have an antidepressant effect on behavioral despair. It was demonstrated that rats that were exposed to light late at night had a decreased duration of immobility in the test swim of forced swim tests compared to the control animals. Previously, Yilmaz et al (2004) reported that a single 12-hr exposure to light in the dark phase of an L/D cycle has an ameliorative effect on behavioral despair. More recently, Schulz et al reported (2008) antidepressant effect of a single 30 min exposure of light in the late part of the dark phase on behavioral despair. Our results support previous findings and showed that light exposure as brief as 10 minutes are sufficient to produce ameliorative effects on behavioral despair model of depression.

In this study, the effects of timing and intensity of light exposure were assessed on behavioral despair. Alterations in the timing of circadian rhythms are important in the therapeutic effects of light [28]. It is known that the timing of circadian rhythms is delayed in depressed SAD patients compared with control subjects [14]. Therefore it was speculated that morning phototherapy is superior to evening therapy as it induces a corrective phase advance [14, 18, and 20]. Based on these findings, the effect of brief exposure of light was evaluated early at night (ZT15) and late at night (ZT21). These time points were chosen as light applied at ZT15 is known to induce phase delays while light applied at ZT21 is known to induce phase advances in rodents [82]. Our results support previous findings that light treatment that produces phase advances is superior to light exposure that produces phase delays in terms of alleviating the symptoms of depression. Light applied at ZT15 did not have any protective effect on behavioral despair. As light is the primary cue for the entrainment of the SCN, a phase shift by the light may induce several signal transduction pathways which results in different biological functions [8].

The fact that there is no effect of light on the first day of FST indicates that the present results are not due to a nonspecific effect of light such as arousal or increased mobility but it is due to a specific antidepressant effect of light that becomes evident on the second day of the testing. The increased immobility during the test swim of the FST

demonstrates emotional and behavioral abnormality related with depression as antidepressants rather than anxiolytic treatments alleviate the immobility behavior [22-26].

Although groups differed in terms of durations of immobility in the second swim test, one-way ANOVA results indicate that there is no significant difference between groups in terms of durations of swimming in FST1 and FST2. Moreover, there is not a significant difference between groups in terms of number of head shakes. It was demonstrated that selective serotonin reuptake inhibitors (SSRIs) such as fluoxetine increase durations of swimming in the FST without affecting climbing behavior while norepinephrine (NE) reuptake inhibitors such as desipramine increase climbing without altering swimming (Page et al., 1999). These findings indicate that different antidepressant drugs results in different patterns of behavior in the FST [24, 66, and 80] In order to measure different pattern of behaviors rather than immobility, climbing behavior and durations of swimming were analyzed by using sampling method used in several studies [24, 66, and 80]. This sampling technique enables to distinguish specific behavioral components of active behaviors and enhances the sensitivity of traditional FST to measure serotonergic activation. Behavioral measurements in the swim tests were evaluated by counting frequencies of immobility (floating without struggling), swimming (making active swimming motions) and climbing (upward forepaw movements directed against the wall of the cylinder). Specifically, the second swim test was divided into 5-min blocks in which each 5-s bin was counted either as immobility, swimming or climbing depending on the predominant behavior. This method of counting frequencies has been shown to be as reliable as timing the absolute duration of behaviors. The data indicates that groups are lack of displaying climbing behaviors. Moreover, one way ANOVA indicates that there is a significant difference between groups in terms of duration of swimming and immobility in the data recoded via sampling method. Post-hoc tests revealed that the ZT21 group was significantly different in the mean duration of swimming and mean duration of immobility in the second swim test from the control and the ZT15 groups ($p<0.05$). The significance between groups in terms of duration of immobility supports the results of the original coding. The significance between groups in terms of duration of swimming may result from the activation of the serotonergic system.

Analysis of the photic induction of c-Fos immunoreactivity within the SCN has proven to be a powerful tool to study the neurochemical mechanisms involved in phase shifting in the circadian clock [33, 78]. Following light stimulus, c-fos is transcriptially activated in the SCN [7, 78]. After its activation, c-fos regulates the activation of other genes which may critically involve in the antidepressant effect of light. Moreover, it was reported that light stimulated c-Fos induction occurs when light is administered at circadian times at which the pulse cause a phase shift [34, 82]. In order to evaluate light induced phase shifts, c-fos immunohistochemistry were performed in this study. The perfusions were done 90 minutes after the onset of light stimulation. This time point was selected as it was reported that c-Fos reached its peak level between 1-2 hours [83].It was known that c-fos expression exhibits biphasic decay in a way that the first phase has a half-life of 45 min and the second phase has a half-life of 90-120 min. c-Fos binds to c-Jun and forms a heterodimer in order to activate AP1 transcription factor which induce several signal transduction pathways. During the first phase, 20-30% of c-Fos is bound with c-Jun molecules and during the second phase c-Fos synthesis is stopped and 90% of c-Fos is coupled with c-Jun proteins [84].

The mechanism underlying the ameliorative effect of light is not fully known but there are hypotheses about its function. Melatonin hypothesis is not enough to explain present results. Light exposure has two main effects on melatonin rhythm, the acute inhibition of synthesis and the phase shifting of the rhythm via the SCN [69, 70]. The melatonin hypothesis posits that depression occurs as a result of an increase in the melatonin secretion and as light suppresses melatonin production, it produces an antidepressant effect [23]. However, recent studies reported that administration of melatonin or its agonists have a protective effect on behavioral despair [71-73] which is contradictory to light induced antidepressant effects. An indirect method by which melatonin may be involved in depression might be through circadian phase shifting [28].

The photon counting hypothesis which assumes that depression may result from insufficient light exposure at any time of the day [16, 28] was evaluated by using different intensities of light. As the intensity of light increases the number of photons increases also. In the present study rats were exposed to either 900 lux or 1350 lux. These intensities were chosen based on previous studies [27]. Our results showed that both 900lux and 1350lux

light have protective effects on behavioral despair if they are applied at ZT21. Evaluation of t-test results indicated that 1350lux group was less immobile in the test swim compared to 900lux group, yet, the difference is not significant.

It was indicated that the SCN receives direct serotonergic inputs from both medial and dorsal raphe nuclei, which enabled entrainment of circadian rhythms for non-photic stimuli [11, 29, and 30]. Several studies indicate a possible interaction between photic and nonphotic pathways of the SCN which may results in light induced regulatory effect on serotonergic system [29, 85]. In the present study serotonin hypothesis which assumes that light therapy may exert its antidepressant effect via regulation of serotonergic system was evaluated via c-Fos immunohistochemistry. Presence of c-Fos immunoreactivity in the dorsal raphe nucleus (DRN) would be indicative of neuronal activation which may support the serotonin hypothesis. However, the present results did not find a significant difference between light exposed and control groups in terms of c-Fos expression in the DRN. It was demonstrated that exposure to a low frequency visual stimulus (2 Hz, 600lux), early at night (ZT13) results in suppression of c-Fos expression in the DRN while exposure at midnight (ZT18) cause enhanced c-Fos expression in the DRN compared to controls [85]. These differences may depend on the experimental conditions. In their study, c-Fos measurements were done immediately after stimulation although in the present study measurements were done 90 minutes after photic stimulation [85]

Dense c-Fos immunoreactivity in the SCN regions of rats that were exposed to light either at ZT21 or ZT15 compared to controls in the present study indicates light induced phase shifts. It was known that light applied early at night induce phase delays while light applied late at night induce phase advances [78]. Although activity rhythms could not be measured in this study, we can assume that phase delay occurs at ZT15 while phase advances occur at ZT21. There is slight and distributed c-fos immunoreactivity in the controls which may be due to spontaneous c-Fos rhythm. These results support previous finding that light induced c-Fos expression located in the ventrolateral core region in the SCN [70, 75, 76, 78]. Moreover these results support previous findings that brief light exposure as short as 10 minutes is sufficient to induce behavioral and cellular effects via modulating phase shifts detectable by c-Fos induction [77].

Conclusion

The present findings indicate that 10 minutes of light pulse exposure late at night has an ameliorative effect on behavioral despair model of depression as measured by forced swimming test in male Wistar rats. Histological analyses support the phase shifting hypothesis. Further work would be necessary to investigate the effects of light pulses at different time points late at night. Additionally, light exposure of different durations and different intensities would be evaluated via different animal models of depression and histological analysis.

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