CHAPTER 1

INTRODUCTION

A primary system that moderates circadian rhythms in mammals is the hypothalamic suprachiasmatic nuclei (SCN), the master pacemaker of several biological rhythms (Esseveldt et al., 2000). Initially, it was thought that SCN has only oscillatory functions, but recent studies indicate that it is also involved in cognitive functions and mood (Arushanyan et al., 2000; Buijs et al., 1997; McClung, 2007). There is a mutual relationship between circadian rhythms and depression: It is well documented that depressive disorders impair normal biological rhythms. In turn, disruption of biological rhythms aggravates depression itself (Turek et al., 2001). This relationship between circadian rhythms and depression has led researchers to explore the underlying mechanism of depression by illuminating the parameters of circadian rhythms.

One line of research focuses on phototherapy: Studies have shown that light therapy is an effective treatment for both nonseasonal (Avery et al., 1998; Even et al., 2008) and Seasonal Affective Disorder (SAD) (Eastman et al., 1998; Kripke, 1998; Lewy et al., 1998; Wirz-Justice et al., 1993). Early studies focused on bright light treatment in broad-band wave length; recently, differential effects of light of different wavelengths in relation to treatment of depression have become a central topic (Brainard et al., 2001; Brainard & Hanifin 2005; Glickman et al., 2005; Hanifin & Brainard, 2007; Lockley et al., 2003; Newman et al., 2003) in research and clinical application.

There are a limited number of animal studies that focus on the parameters of light treatment (Molina-Hernandez & Tellez-Alcantara 2000; Schulz, Aksoy &

Canbeyli, 2008; Yılmaz et al., 2004). Additionally studies that assess the direct role of suprachiasmatic nucleus on depression are even rarer (Arushanyan et al., 1998; Tataroğlu, 2004).

The effectiveness of light treatment is well documented; however studies in humans generally focus on the treatment outcome because of ethical reasons. In addition, the limited numbers of animal studies are not sufficient for illuminating parameters of light treatment. Consequently, the present study aimed to investigate the underlying parameters of the ameliorative effect of photic stimulation both at the behavioral level and in terms of its neuroanatomical consequences.

An Animal Model of Depression: Forced Swim Test

Pharmacological and anatomical studies on animal models of depression suggest the potential that animal models of depression are homologous to depression in humans. Thus they are widely used to investigate the circuitry and mechanism of depression. The Forced Swim Test (FST) is a commonly used animal model of depression (Porsolt et al., 1977), in which animals are forced to swim in two consecutive test sessions separated by 24 h, in 15 minutes for the first test and 5 minutes for the second. The total duration of immobility in the second day compared to the first day serves as an index of behavioral despair. Porsolt et al. (1978) have reported that different kinds of antidepressants (tricycylics, the monoamine oxide inhibitor nialamide and electroconvulsive shock) reduce immobility scores on the second day of testing compared to vehicle administrated controls. Additionally, the forced swim test paradigm is sensitive to various types of behavioral, seasonal, pharmacological and anatomical manipulations (Aksoy, Schulz, Yılmaz, Canbeyli, 2004; Bulduk & Canbeyli, 2004; Connor, Kelly & Leonard, 1997; Kelliher, Connor, Harkin, Sanchez, Kelly, Leonard, 2000; Tataroğlu, Yılmaz, Aksoy & Canbeyli, 2004).

Moreover, it is important to note that light treatment reduces immobility scores in FST (Molina-Hernandez and Tellez-Alcantra, 2000; Prendergast and Nelson, 2005; Yılmaz et al., 2004; Schulz, Aksoy and Canbeyli, 2008).

Sex Differences in Behavioral Despair

Studies on depression indicate a clear sex difference in the epidemiology and prevalence of the phenomena for humans (Kessler, 2003). Furthermore, sex differences are reported in animal models of depression and anxiety (Palanza, 2001). Consoli et al. (2005) have reported that different stressors produce different effects in forced swim test and plasma corticosterone levels in response to antidepressants for male and female rats.

One line of evidence suggests that male rats have lower immobility scores compared to the female rats (Dalla et al., 2008; Drossopoulou, 2004). In contrast, it has also been reported that female rats have reduced immobility scores compared to male rats in forced swim test (Alonso et al., 1991; Brotto et al., 2000). In addition, the dopaminergic activity in the prefrontal cortex and the hippocampus is elevated relative to the female rats (Dalla et al., 2008). Moreover, female rats exhibit reduced serotonergic activity in the hippocampus and hypothalamus, whereas, male rats show elevated serotonergic activity in hypothalamus (Drossopoulou, 2004).

In sum, male and female rats have diverse behavioral (Alonso et al., 1991; Brotto et al., 2000; Barros & Ferrigolo, 1998; Brotto, Gorzalka, & Barr, 2001; Drossopoullou et al., 2004) and neural (Dalla et al., 2008; Drossopoulou et al., 2004) responses to the forced swim test paradigm.

Suprachiasmatic Nucleus and Depression

Originally it was assumed that SCN has only oscillatory functions; however recent research indicates that the SCN can modulate cognitive function (Arushanyan et al., 2000) and mood (Buijs et al., 1997) as well. A previous study in our laboratory revealed that SCN lesion in male rats have an ameliorative effect on behavioral despair as measured by forced swimming tests (FST) (Tataroğlu et al., 2004). An unpublished research that aimed to reevaluate the previous study by Tataroğlu et al. (2004) in a different model of depression, namely Open Space Swim Test (OSST), demonstrated that destruction of the SCN has a protective effect in the OSST for male but not for female rats.

The role of the SCN is also critical in phototherapy studies, because photic stimulations mediate circadian rhythms and other behavioral and physiological outcomes primarily thorough the SCN (Beaule, Barry-Shaw & Amir, 2003; Earnest & Olschowka, 1993; Abe, Rusak & Robertson, 1991; Beaule, Arvanitogiannis & Amir, 2001; Benloucif, Margarita & Dubocovich, 1997; Barakat et al., 2005; Schumann et al., 2006).

Structure of the Suprachiasmatic Nucleus

The SCN is located just above the optic chiasm, in the anterior ventral hypothalamus (Moore-Ede, Sulzman & Fuller, 1982). The rat SCN has approximately ten thousand neurons in a volume of approximately 0.3 mm^3 (Morin and Allen, 2005). There are different neuron groups within the suprachiasmatic nucleus. There is a general agreement about SCN having two major divisions, namely, the core and the shell based on chemoarchitecture and connections of the SCN (Abrahamson & Moore, 2001). Abrahamson and Moore (2001) using cholera toxin injections based on the distribution of neurons demonstrated two divisions: the shell neurons of SCN contain

GABA, CALB, AVP, AII and the core neurons of SCN contain GABA, CALB, VIP, CALR, GRP and NT (Abrahamson & Moore, 2001). Miller et al. (2005) have reported on the diverse effects light in the core and shell regions of the SCN (see appendix 1). Sumova, Travnicova and Illnerova (1998) specified the ventrolateral and dorsomedial regions of the core in relation to light stimulation (see appendix 2).

Afferent and Efferent Anatomy of Suprachiasmatic Nucleus

SCN is highly connected to other structures in the brain, receiving and dispersing information among numerous regions in the brain. SCN projects to the ventral lateral septum, bed nucleus of the stira terminals (BNST), paraventricular thalamus, medial preoptic region, subparaventricular zone, premammillary area, paraventricular, ventromedal, and dorsomedial nuclei, precommissural and olivary pretectal nucleus (Morin and Allen, 2005). Krout (2002) used retrograde transneuronal trackers to identify afferent projections to SCN; the study illustrated that more than 40 regions directly project to the SCN (see Krout et al., 2002 for a list). Pickard et al., (2002) also found 100-140 regions that project indirectly to the SCN (cited in Morin and Allen, 2005).

In general, there are three main afferent pathways of the SCN: The retinohypothalamic, the geniculohypothalamic and serotonergic pathways (Moore-Ede, Sulzman and Fuller, 1982; Morin and Allen, 2005; Morin, Shivers and Blanchard, 2005). Retinal projections comprise the ventrolateral part of the SCN , the serotonergic pathway projects dominantly to dorsolateral regions of the SCN (Morin and Allen, 2005).

Light arrives in the SCN through the retinohypothalamic tract using glutamate as the primary neurotransmitter; other inputs, namely the geniculohypothalamic (through IGL) and serotonergic pathways (through the DRN)

use light as an indirect modulator (Yannielli and Harrington, 2004). The non-photic pathways are influenced by factors other than light input such as social interaction, handling, conditioning, food deprivation etc. (Knoch et al., 2004; Yannielli & Harrington , 2004).

The afferent and efferent connections of the SCN with other brain regions may be involved in modulating depression. One major input of the SCN is from the dorsal raphe nucleus (DRN). This serotonergic input modulates SCN by two different pathways; a direct projection and an indirect one via the intergeniculate leaflet (IGL) (Morin and Allen, 2005). DRN plays a critical role on in depression circuitry, primarily based on its serotonergic projections. Possible interplay between the SCN and the DRN in depression circuitry is suggested by several studies. First, a direct path is found from retina to the DRN (Fite et al., 1999, 2005; Kawano et al., 1996). Fite et al. (2005) showed that a 60 min photic stimulation significantly increases the activity of the DRN, as measured by c-Fos expression. In addition, electrical stimulation of the DRN generates phase advances (Glass et al., 2000). There are other brain regions that should also be considered in relation to the SCN and depression: For instance, the indirect projection of SCN to the locus coeruleus (LC), is essential for the brains arousal system (cited in McClung, 2007). Reciprocal connections between the SCN and the striatum may also be critical for the relationship between locomotor activity and depression (Arushanian & Popov, 1994).

Light, Phototransduction, Circadian Rhythms and Depression

The cycle of day and night synchronizes the internal rhythms of sleeping, awaking, eating as well as many other rhythms (Turek et al., 2001; Vitaterna et al., 2001). The internal rhythms exist even in the absence of external signals, and can be re-adjusted by external cues, particularly by light. As mentioned above, light input generates

physiological changes via the SCN. The response of the SCN to Zeitgeber (an exogenous signal that alters the internal time keeping mechanism) is not absolute (Vitaterna et al., 2001); the responses to Zeitgebers depends on it's timing. Zeitgebers presented at different times forms a phase response curve (PRC) (see appendix). Normally for rats maintained on a 12h:12h light:dark cycle on a Zeitgeber time scheduled with light period starting at ZT0 (0700) and to end at ZT12 (1900). Studies with humans and animals have shown that Zeitgeber (i.e. photic stimulation) given in early aspects of the subjective night causes phase delays. While, Zeitgeber in the late phase of the subjective night causes phase advances (van Esseveldt et al., 2000; Vitaterna et l., 2001). This differential effect on behavior and physiology also occurs in phototherapy; studies show that late onset photic stimulation in the subjective night is more effective than early onset stimulation (Kripke., 1998; Lewy et al., 1998; Shulz et al., 2008).

Phototransduction

Phototransduction is the process of light induced physiological changes and is mediated by chromophores. The chromophores operate differently in response to wavelengths in the visible spectrum (Brainard &Hanifin 2005; Hanifin & Brainard, 2007). After transformation of light to electrical signals, the retinohypothalamic tract conveys the information from photoreceptors to the SCN. This information is transformed by release of glutamate and pituitary adenylate cyclase-activating peptide (PACAP) (Beaule et al., 2003). Lucas et al. (1999) have reported that even transgenic mice that lack rods and cones have normal melatonin suppression when appropriate amount of light is presented. These results suggest the existence of additional photoreceptors other than the known visual photoreceptors. In addition, a

number of action spectrum studies support this possibility (Hanifin and Brainard, 2007).

Provencio et al. (1998) discovered melanopsin in frog melanophore cells which are sensitive to light. Panda et al. (2005) subsequently showed that intrinsically photosensitive retinal ganglion cells (ipRGCs) produce melanopsin (a vitamin A photopigment) in mammals, and concluded that this photopigment is necessary for non-visual photic responses (i.e. behavioral responses to light).

These findings form the physiological basis for the differentiated effects of light of different wavelengths on the biological clock. Before focusing on the action spectra for melatonin, phase shifts and antidepressant effects of light, studies on broad-band wavelengths will be discussed.

Effects of light in broad-band wavelengths in relation to depression

A systematic review of nonseasonal depression and meta-analytical studies on light therapy (Even et al., 2008; Golden et al., 2005) reveals that bright light therapy is an effective treatment. In addition, there are a number of studies that specifically focus on phototherapy for Seasonal Affective Disorder (SAD) (Lewy et al., 1998; Eastman et al., 1998; Wirz-Justice et al., 1993). Similar findings have also been shown in animals.

Exposure to long photoperiod (14L/10D) for 30 days compared to short photoperiod (5L/19D) prevents behavioral despair as measured with FSTs (Molina-Hernandez and Tellez-Alcantra, 2000). A previous study in our laboratory showed that a single day of constant light (12L/12L) instead of the usual 12L:12D lighting regimen reduces immobility scores in the FST (Yılmaz et al., 2004). Moreover, Schulz et al. (2008) have reported that a 30 min light pulse at late onset reduced immobility scores in FST compared to the control groups. Furthermore, an ongoing

study in our laboratory indicates that a 10 minute light pulse at ZT21 (04:00 am) may have a protective effect on FST whereas a 10 min light pulse at ZT15 (21:00 pm) have null results.

On the other hand, a small number of studies have found negative effects for prolonged light exposure. For instance chronic constant light for 3 weeks impairs spatial memory and affects long-term depression (Ma et al., 2007). These findings suggest that experimental manipulations, timing and length of light are critical for the effectiveness of photic treatment.

Effects of Light of Different Wavelengths on the SCN

As mentioned earlier, different wavelengths of visible light produce different physiological responses in the organism and the term action spectrum is used for these differentiated effects (Brainard and Hanifin 2005). Newman et al. (2003), showed that photosensitive retinal ganglion cells express melanopsin sensitive to light with wavelengths between 420-440 nm. Studies with rodents and humans show that the most effective spectral wavelengths are between 450 and 550 nm for eliciting phase changes and melatonin suppression (Hanifin and Brainard, 2007). There are, however, studies showing that long-wavelengths (700 nm) can also produce similar effects, however, at high intensities and with relatively weaker effects (Hanifin et al., 2006).

Action Spectrum for Melatonin Regulation

Brainard et al. (2001) investigated the effect of different wavelengths on the melatonin suppression in humans; they tested wavelengths between 420-600 nm and found those between 446 to 477 nm are the most effective in melatonin suppression. Based on these findings the authors argued that a photopigment plays a critical role for eliciting this effect which is not among rod and cone photoreceptors for vision

(Brainard et al., 2001). Lockley et al. (2003) demonstrated that photic stimulation at 460 nm for 6.5 hours produce greater phase shift and melatonin suppression compared to the photic stimulation at 555 nm with equal photon density.

Action Spectrum for Phase Shifts

Takahashi et al. (1984) showed that the largest phase shift effect was observed between 500-511 nm (cited in, Brainard and Hanifin 2005). It has been hypothesized that the difference between action spectrum for melanopsin expression and phase shifts points to the possibility that classical photoreceptors are also involved in phase shifting as well as the melanopsin releasing ipRGCs (Brainard and Hanifin 2005). Additionally, Yoshimura (1996) showed that mice lacking functional visual photoreceptors have the greatest phase changes at a 480 nm photic stimulation; on the other hand, for mice with visual photoreceptors, the greatest action spectrum for phase shifts is at 500 nm (cited in, Brainard and Hanifin 2005).

Action Spectrum for Antidepressant Effects of Light

An initial meta-analytical study on spectral characteristic of the light on photo therapy of SAD showed that medium wavelengths (blue, green, yellow) are more effective then long wavelengths (Lee et al., 1997). Glickman et al. (2005), comparing the antidepressant effects of blue light at 468 nm and red light at 654 nm in humans, demonstrated that light at the blue spectrum was much more effective. One critical problem of the study is the photon densities of different wavelengths were not equivalent (7.30 x 1015 for blue spectrum and 1.13 x 1014 for red spectrum); thus result of this study is not convincing.

As noted above, the underlying mechanism of light therapy is unknown; however, studies of action spectrum analysis indicate that the effectiveness of light therapy is highest in the short wavelength region of the visible spectrum between 450

and 550 nm eliciting strongest outcomes in phase advance and melatonin suppression. Moreover, ameliorative effects of light correlate with changes in the phase shifts (Lam, 1998). Lewy et al. (1987) have reported that bright light stimulation early in the morning has an antidepressant effect and also produces phase advances in night melatonin rhythms. Thus, these studies suggest a possible explanation for the underlying mechanism for the effectiveness of light.

c-Fos Immunohistochemistry

c-Fos is an early immediate gene, identified as a proto-oncogene; during neural activation Fos protein is activated and binds to chromosomes, (Correa-Lacarcel, 2000). Immunohistochemistry techniques stain the activated cells (see the method section). Thus, this technique is widely used to investigate the activated brain structures in response to diverse experimental manipulations.

Sumova et al. (1998) have reported that there are spontaneous c-Fos rhythms in the SCN and such that expression of c-Fos is mainly observed in the dorsomedial part of the SCN. Miller et al. (2005) extended the Sumova et al.'s (1998) study and proposed that these spontaneous rhythms of c-Fos were mainly detected in the core regions of the SCN (see the appendix).

The spontaneous c-Fos rhythms in the SCN are altered by external entrainments due to both photic (Abe, Rusak & Robertson, 1991; Barakat et al., 2005; Beaule, Arvanitogiannis & Amir, 2001; Beaule, Barry-shaw & Amir, 2003; Benloucif, Margarita & Dubocovich, 1997; Earnest & Olschowka, 1993; Schumann et al., 2006) and nonphotic stimulation (Mikkelsen, Vrang & Mrosovsky, 1998). Sumova and Illnerova (2005) have reported that c-Fos expression rhythm in the rat SCN is affected by the changes in the photoperiod (a 9h light pulse after midnight). A number of studies have shown that light entertainment increases c-Fos expression

in the rat SCN. A fifteen minute bright photic stimulation (2200 lux) at middark phase (ZT21) exhibits a significant increase in FOS-immunoreactivity, especially in the ventrolateral parts of SCN (Rea, 1989). Moreover, 30 min photic stimulation increased the c-Fos activation is observed in the ventrolateral parts of the SCN (Sumova et al., 1998) (see appendix). On the other hand photic stimulation in early subjective day or night decreases the c-Fos expression in the shell region of the SCN (Beaule, Arvanitogiannis & Amir, 2001; Beaule, Shaw & Amir, 2003.)

Furthermore, an association between c-FOS immunoreactivity and its behavioral outcomes has been reported. For instance, in Benloucif et al. (1997), short light pulses at ZT14 produce phase advances in young rats but not in older rats. In addition to this behavioral outcome, the study reported that the old rats had reduced c-Fos expression in response to light stimulation.

Light entrainment causes c-Fos expression in other brain structures as well. For example, a 30 min photic stimulation induces c-Fos imunoreactivity in DRN (Fite, Wu & Bellemer, 2005). In addition, the intensity of light has diverse effects in serotonergic and non-serotonergic neurons in the DRN (Bouwkencht et al., 2007). DRN play a critical role in depression circuitry (Greenwood et al., 2003), and possibly has pivotal role in the circadian aspects of depression. For instance, electrical stimulation of the DRN causes 5-HT release in the SCN and induces circadian phase-shifts (Brenstein & Morin, 1999; Glass, DiNardo & Ehlen, 2000). Consequently light induced c-Fos expression in the SCN and DRN, and their differential effects on different wavelengths is examined in the present study.

Present Study

While there is ample evidence in both humans and animals that light treatment has an ameliorative effect on depression and behavioral despair, few studies have studied the parameters of phototherapy. A previous study in our laboratory revealed that short light pulses in the late potion of the night decrease immobility scores in forced swim test (Schulz et al., 2008). Also, prolonged light exposure has similar consequences (Molina-Hernandez & Tellez-Alcantara 2000; Yılmaz et al., 2004). Moreover, action spectrum studies that investigate the phase response (Brainard and Hanifin 2005) and antidepressant (Lee et al., 1997; Glickman et al., 2005), effects of light suggest that short wavelength light is far more effective as an antidepressant treatment than long wavelength. In line with these findings the present study aimed to investigate the effects of short light pulses in different wavelengths on behavioral despair in male and female rats.

There are a number of studies that shows sexually dimorphic responses of male and female rats to experimental manipulations of behavioral despair (Alonso et al., 1991; Brotto et al., 2000; Brotto, Gorzalka, & Barr, 2001; Dalla et al., 2008; Drossopoullou et al., 2004). Thus, the present study investigated the ameliorative effects of light for both male and female rats.

Finally, it has been shown that light entertainment increases c-Fos expression in the rat SCN, and other structures that activated during manipulation in the brain. To assess the structures involved in the process, c-Fos immunohistochemistry was carried out.

CHAPTER 2

METHOD

Subjects

A total of 48 experimentally naïve adult (5-6 month old) male and female (24 each) adult Wistar rats were obtained from the Boğaziçi University Psychobiology Laboratory vivarium. At the beginning of the experiment, the weight range of the rats was between 180-210 gram for females and 220-250 gram for males. Subjects were maintained on a standard 12L/12D (lights on at 0700) cycle at approximately 22°C, except on the days of experimental manipulation as noted below. Three groups of rats were randomly assigned to one control and three experimental groups, with 8 subjects each. Animals were group-housed with food and water available ad libitum, with 4 rats in each cage.

Apparatus

Photic stimulation was administrated in an insulated chamber. The daily light schedule of the chamber was identical to vivarium (12L/12D and 250 lux) except when light stimulation was administrated. Photic stimulation was provided by six 15W fluorescent lamps, approximately 50 cm above the subjects. The wavelengths of light were manipulated by light filters: Bright blue ((LP) 118); Dark Amber ((LP) 022) which can be seen in appendix 1. Inntensity of light was kept at approximately 1300 lux, regardless of the filter used.

Spectral Analysis

Spectral assessments were performed by a spectrometer, 10 different measurements being made for each filter (To obtain the mean spectral power distributions of two different light filters see appendix 2). The bright blue filter peaks at 540 nm with half

peaks at 490 and 430 nm. The dark amber filter peaks at 680 nm with a half peak at the 620 nm (see the Appendix).

Forced Swim Test

Animals were tested between 1400 (ZT7) and 1630 h (ZT16,5). The FSTs consisted of two consecutive days of testing in a 30 cm diameter Plexiglas cylinder filled with water (25 °C) to a height of 25 cm. Before each swim test, animals were placed in individual cages for 10 minutes earlier in order to habituate them to the experimental room. In this paradigm, animals were allowed to swim for 15 minutes on the first day of the experiment, followed 24h later by a second swim test for lasting 5 min. Immobility is defined as staying motionless or floating without leaning against the wall of cylinder. Total duration of immobility in the second swim test is analyzed compared to the first five minutes of first forced swim test. Increased immobility in the second test compared to the first day of swimming assessed as an indicator of behavioral despair.

Experimental Procedure

This study was conducted in three phases: In the first experiment, the behavioral despair is tested in male rats in response to red or blue light stimulation. One week later, the group that had received red light first was administrated blue light and the group exposed to blue light was switched to red stimulation. In the second experiment, female rats underwent the same experimental procedure as males, except for the manipulations in the second week. And in the third experiment c-Fos immunohistochemistry of male rats in response to red or blue light stimulation is examined.

Experiment 1: Effects of photic stimulation with different wavelengths on behavioral despair in male rats.

A total of twenty four experimentally naïve rats were randomly assigned to two experimental and a control group, with 8 subjects each. Rats were maintained on a 12L:12D lighting cycle except for the experimental manipulations specified below, and were housed in fours in their homecage. Ten minutes prior to placement in the light chamber, rats were moved to cage of twos. And the two groups of twos from each cage were assigned to different experimental groups (i.e. two rats were assigned to group (A) and two rats were assigned to group (C) from same cage).

All groups were placed in the experimental room ten minutes prior to lights off ZT12 (1900). And at ZT12 rats were placed in to the light chamber. There were four rats in one chamber, in two different cages; each group came from a different homecage. Light stimulation was administrated to the two experimental groups (group (B) and (R)) at ZT21 for 10 minutes with different wavelengths (either in blue or in red spectrum). The lux of light was held constant for all experimental groups at Approximately 1300 lux. In the first week of the experiment, group (B) received 10 minutes of photic stimulation at the blue spectrum, on the other hand group (R) received the same stimulation at the red spectrum. The daily lights were on at ZT0 (0700) as in regular housing conditions. The animals were kept in the light chamber until approximately 1400 (ZT7), when they were transferred to individual cages for 10 minutes for habituation to the experimental room. Then the first swim test was conducted for 15 minutes. Following the swim test, animals were placed in individual cages for 30-minutes for drying. Following this, rats were returned in their home cages in the vivarium, with their regular lighting schedule. Next day, a second swim test was conducted for five minutes. Afterwards the animals were kept in the

vivarium for five days, when the same experimental procedure was performed except that group (B) that had received blue stimulation received photic stimulation at the red spectrum (B-(R)) and group (R), that had received red light stimulation received photic stimulation at the blue spectrum (R-(B)). The same experimental manipulations were administrated to the control group (C) except for light stimulation (see the Appendix to obtain the schematic timeline of the experiment).

Experiment 2: Effects of photic stimulation with different wavelengths on behavioral despair in male rats

The same experimental procedure was administrated to twenty four experimentally naïve female rats, with the exception of experimental manipulations in the second week of the experiment. Light stimulation was administrated to the two experimental groups at ZT21 (0400) for 10 minute either in the blue or red region of the visible spectrum, this manipulation is followed by FST procedure starting from the very next day.

Experiment 3: Effects of photic stimulation with different wavelengths on c-Fos expression

Eleven adult male Wistar rats were obtained for immunohistochemistry, 8 for the two experimental conditions and 3 for the control group. A photic stimulation at ZT21 for 10 minutes was administrated at either red (peaks at 680 nm) or blue (peaks at 540 nm) spectrum (n=4 for each). Three rats underwent the same experimental procedure except that they were not exposed to light stimulation.

Ninety minutes after photic stimulation, each animal was anesthetized with ketamine/xylazine (160mg/kg, intraperitoneally, Richter Pharma), and then were perfused intracradially with 0.9% saline followed by 4% paraformaldehyde in phosphate buffer (PB). Brains were removed and post fixed in paraformadehyde

solution for two days. Fifty micron thick brain slices were cut using a vibratome (Campden instruments, UK, 752M Vibroslice).

All staining procedures were carried out with free-floating sections. On the first day, sections were rinsed three times in phosphate buffer for ten minutes. Subsequently, sections were incubated in 0.1 M Glycine (1.5 g glycine/200ml PB) for 30 minutes. Slices were then rinsed again three times, followed by a 10 minute wash in 0.5% H2O2 (3.3 ml 30% H2O2 / 200ml distilled H2O). Following the three rinsing, slices were preincubated in 1%NGS/PBT (Normal Goat Serum / 300µ Triton-x in 100 ml PB) for blocking. Then slices were incubated in the 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) for 48 hours in 4^0C. After three times of rinsing, the secondary antibody (biotinylated goat antirabbit IgG (1:200 in 0,3% Triton X-100/PB)) was administrated for an hour. Following this procedure, sections were incubated in avidin-biotin peroxidase complex (1:100 in 0.3% Triton X-100/PB; Vectastain, Vector Labs, Burlington, CA). Slices were prepared by DAB protocol on ice for three minutes (in 5 ml distilled water to which 2 drops of Buffer solution, 4 drops of Dab stock and 2 drops of hydrogen peroxide and 1 drop of nickel were added). Afterwards, sections were mounted on slides.

The borders and subdivisions of the SCN were assessed by using nearby anatomical markers particularly the optic chiasm and the third ventricle based on the studies of Sumova et al. (1998) and Miller et al., (2005).

CHAPTER THREE

RESULTS

Forced Swim Test

The mean durations of immobility for the three groups (blue, red and control) of male and female rats in the two forced swim tests (FST1 and FST2) are presented in Table 1 and Figure 1.

Table1. Mean and standard deviations of immobility (sec) in two forced swim tests for experimental and control groups (n=8, for each group)

	Male				Female			
	Mean		Standard Deviation		Mean		Standard Deviation	
	FST1	FST2	FST1	FST2	FST1	FST2	FST1	FST2
Blue	75,38	55,25	17,72	11,42	91,88	217,87	20,84	83,91
Red	64,63	82,37	24,7	48,63	104,01	148,5	26,47	62,64
Control	72,88	89,37	14,88	36,22	94,25	157,75	16,27	84,56

An analysis of variance (ANOVA) with repeated measures comparing the immobility scores of first and second days of forced swim test showed significant difference [$F(1, 42) = 30.57$, $p < 0.001$]. In addition, test day X gender interaction was significant [$F(1, 42) = 24.01$, $p < 0.001$]. There were no significant test days X group interaction for the immobility scores ($F<1$). On the other hand, test day X sex X group interaction was significant [$F(2. 42) = 6,08$, $p < 0.05$]. Because, male and female rats respond differently in the experimental manipulations, data for male and female rats were subsequently analyzed separately.

Male Rats

An analysis of variance (ANOVA) for immobility scores with repeated measures indicated no significant difference on test day [F (2. 21) = 0.75, p >0.05, η_p^2=0,034]. Swim test X group interaction was significant [F (2. 21) = 5.21, p < 0.05, η_p^2=0,332]. Post-hoc test (LSD) showed that there were no significant differences among groups, but the difference between control and group (B) (blue light stimulated group) was in marginal rage (p=0,069). Because the blue group showed considerably reduced immobility compared to the other groups, group-wise comparisons were calculated. A paired sample t-test indicated a significant decrease for the blue group $t(7) = 3.7$, p<.05 (Bonfferoni Corrected), But there were no significant change for red and control groups.

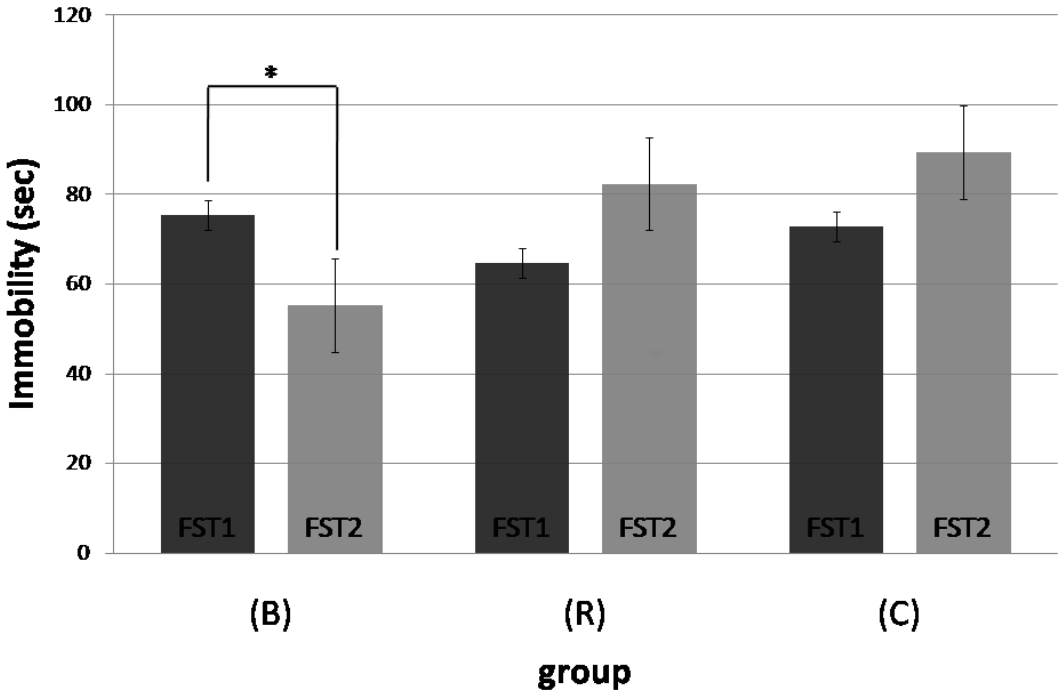


Fig 1 . Mean duration (sec) of immobility in two forced swim tests (FST1 and FST2) in males exposed to blue or red light stimulation (1300 lux) prior to FST1 or were kept in the light chamber with no photic stimulation (Controls)

An analysis of variance (ANOVA) comparing the immobility difference of FST2 and FST1 (FST2-FST1) indicate a significant difference among groups [F (2, 21) = 5.21, $p < 0.05$, η_p^2=0,332]. Post-hoc tests (LSD) showed that blue group was significantly different than both the red ($p = 0.010$) and control ($p = 0.012$) groups, while the control and red groups not differ significantly ($p = 0.926$), (Fig. 2)

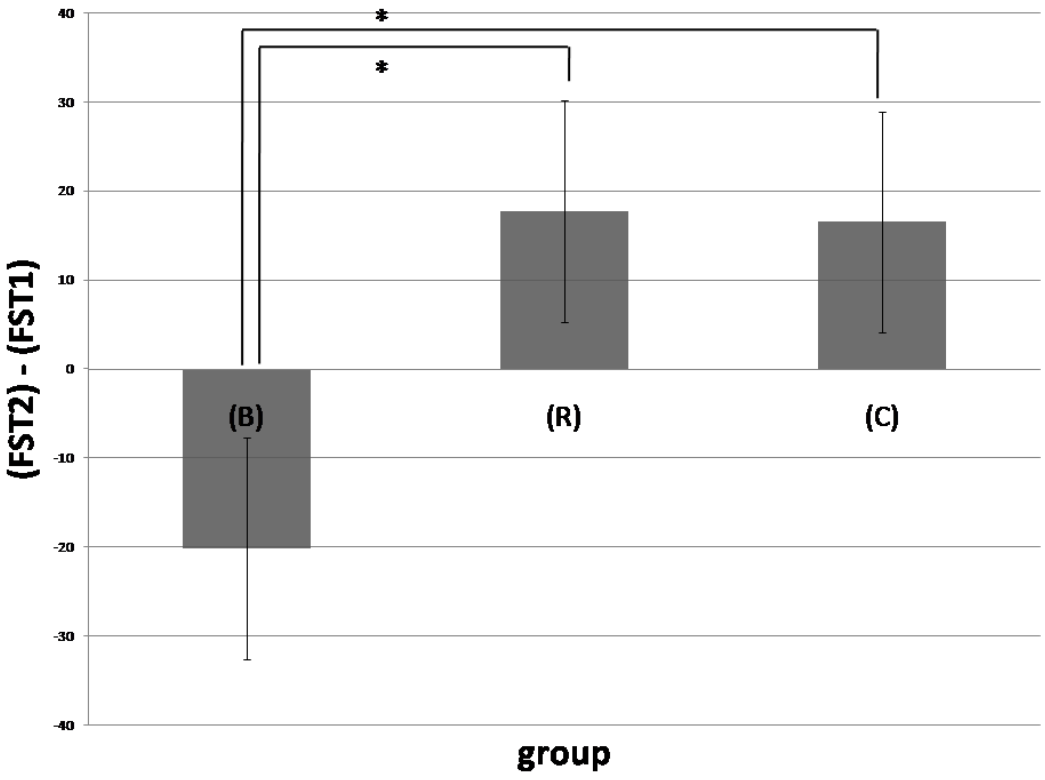


Fig 2. The mean differences between FST2 and FST1 immobility scores in males exposed to blue (B) or red (C) light stimulation (1300 lux) prior to FST1 or were kept in the light chamber with no photic stimulation (C).

Reversal of Wavelengths of Photic Stimulation

The mean durations of immobility for the three groups [(B-(R)), (R-(B)) and (C-(C))] of male rats in two forced swim tests of second week (FST3 and FST4) are presented in Table 2.

Table 2. Mean and standard deviations of immobility (sec) in two forced swim tests for experimental and control groups (n=8, for each group)

	Immobility			
	Mean		Standard Deviation	
	FST3	FST4	FST3	FST4
R-(B)	100,87	124,25	74,07	105,19
B-(R)	82,25	83,75	69,03	77,87
C-(C)	122,37	103,62	78,01	57,79

An analysis of variance (ANOVA) with repeated measures indicated no significant main effect of test day [F (1. 21) = 0.38, p >0.05, η_p^2=0,002]. or swim test X group interaction [F (2. 21) = 1.359, p >0.05, η_p^2=0,115].

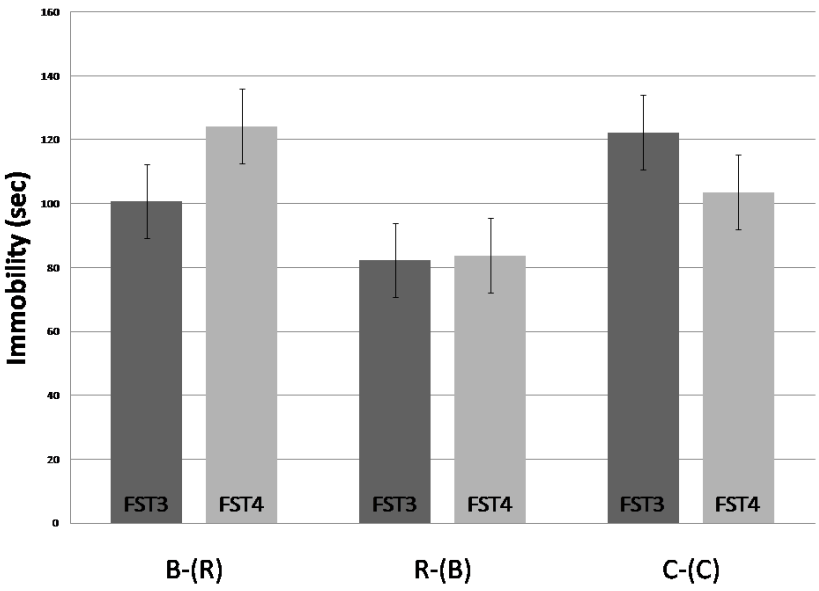


Fig 3. Mean duration (sec) of immobility in two forced swim tests (FST3 and FST4) in males exposed to blue or red light stimulation (1300 lux) prior to FST3 or were kept in the light chamber with no photic stimulation (Controls)

An analysis of variance (ANOVA) comparing the immobility difference of FST4 and FST3 (FST4-FST3) indicate no significant difference among groups [F (2, 21) = 1.359, p > 0.05, η_p^2=0,115].

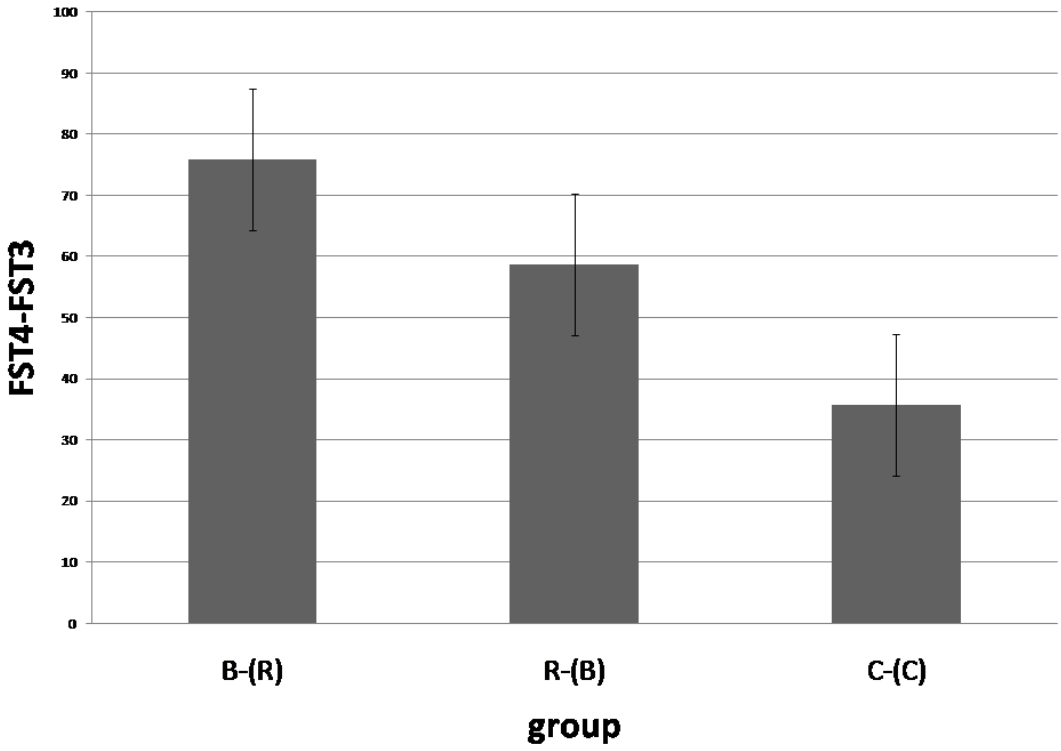


Fig 4. The mean differences between FST3 and FST4 immobility scores in males exposed to blue (B) or red (C) light stimulation (1300 lux) prior to FST1 or were kept in the light chamber with no photic stimulation (C).

Female Rats

Figure 4 shows the mean duration of immobility scores in two forced swim tests

An analysis of variance (ANOVA) with repeated measures indicated that the immobility scores of female rats increased significantly on the second day of experiment compared to first day [F (1, 21) = 31.33, p < 0.001, η_p^2= 0.599]. However, test day X group interaction was not significant [F (2, 21) = 3.13, p > 0.05, η_p^2= 0,229]. (But it was in a marginal range p = 0.065=) (see figure 4).

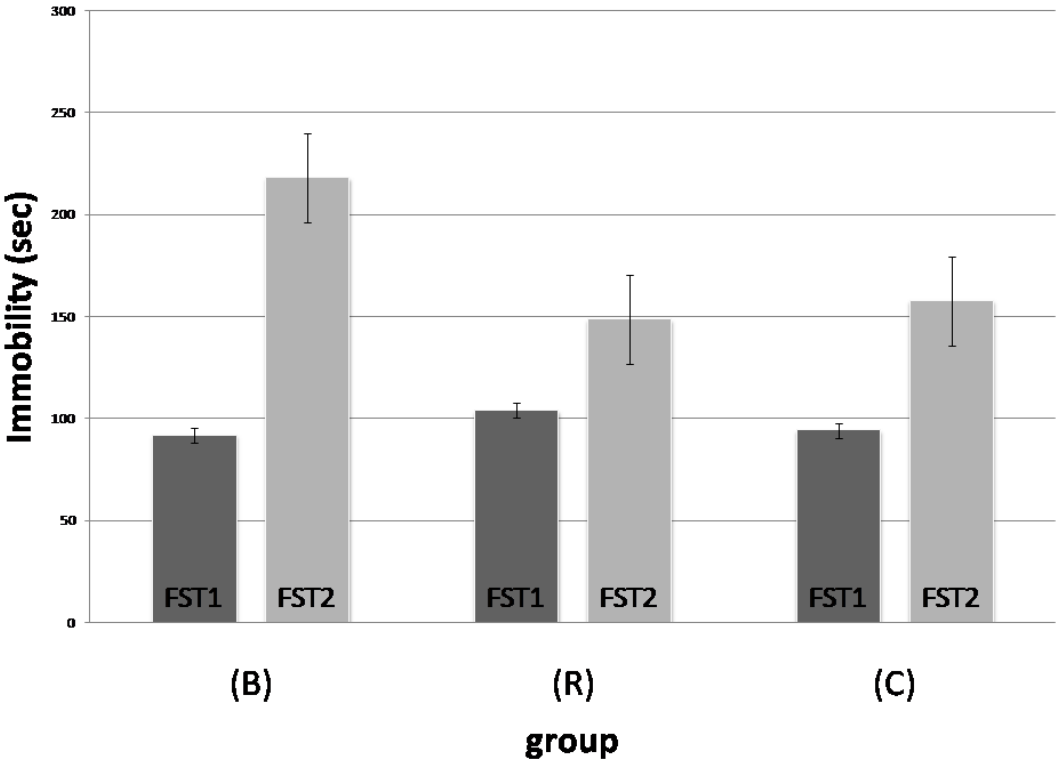


Fig 5 . Mean duration (sec) of immobility in two forced swim tests (FST1 and FST2) in females exposed to blue or red light stimulation (1300 lux) prior to FST1 or were kept in the light chamber with no photic stimulation (Controls).

An analysis of variance (ANOVA) comparing the immobility difference of FST2 and FST1 (FST2-FST1) indicate no significant difference among groups [F (2, 21) = 3.12, $p > 0.05$, η_p^2=0,229]. Post-hoc tests (LSD) showed that blue group was significantly different than the red ($p = 0.026$), while the control group not differ significantly from red ($p = 0.584$) or blue ($p = 0.081$) (Fig. 5)

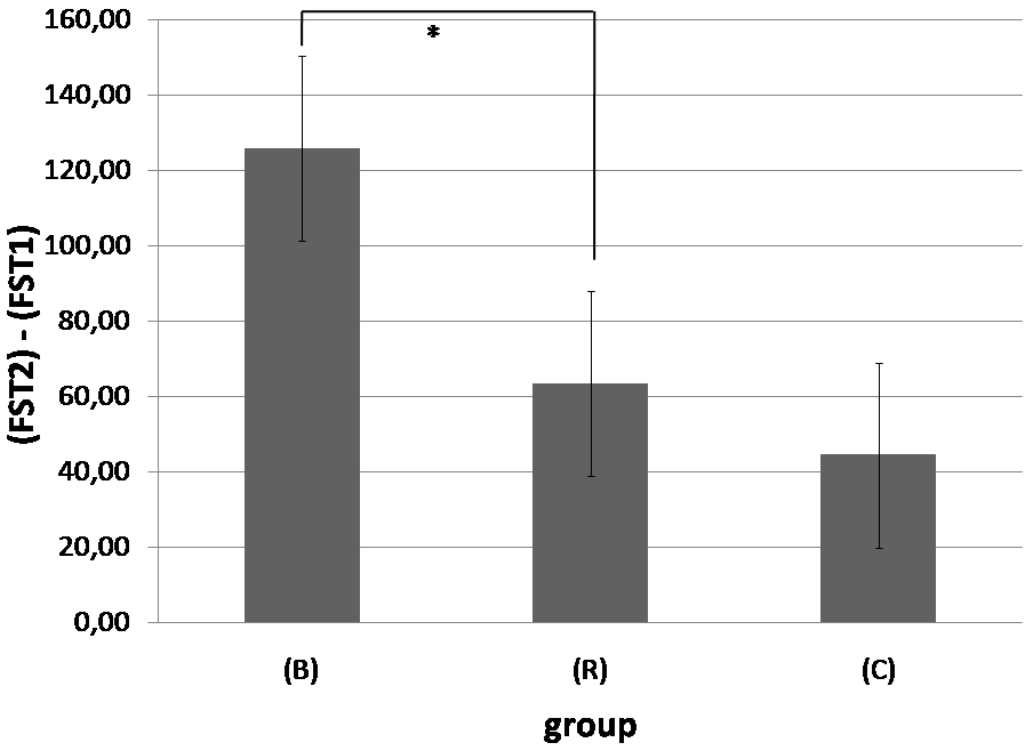


Fig 6 . The mean differences between FST2 and FST1 immobility scores in females exposed to blue (B) or red (C) light stimulation (1300 lux) prior to FST1 or were kept in the light chamber with no photic stimulation (C).

Results of the c-Fos Immunohistochemistry

The mean numbers and standard deviations of the c-Fos labeled cells for the three groups (blue, red and control) in the SCN are presented in table 3.

Table 3. Means and standard deviations of c-Fos labeled cells in the SCN

	c-Fos Labeled cells	
	Mean	Standard Deviation
Blue (N=7)	655,43	89,65
Red (N=13)	564,00	210,44
Control (N=10)	356,90	111,98

An analysis of variance (ANOVA) indicated a significant difference among groups [F (2, 27) = 8.171, p < 0.005, η_p^2=0,377]. (see figure 6). Post-hoc tests (LSD) showed that control group was significantly different than both the red (p = 0.005) and the blue (p = 0.001) stimulated groups, while the blue and red groups not differ significantly (p = 0.234).

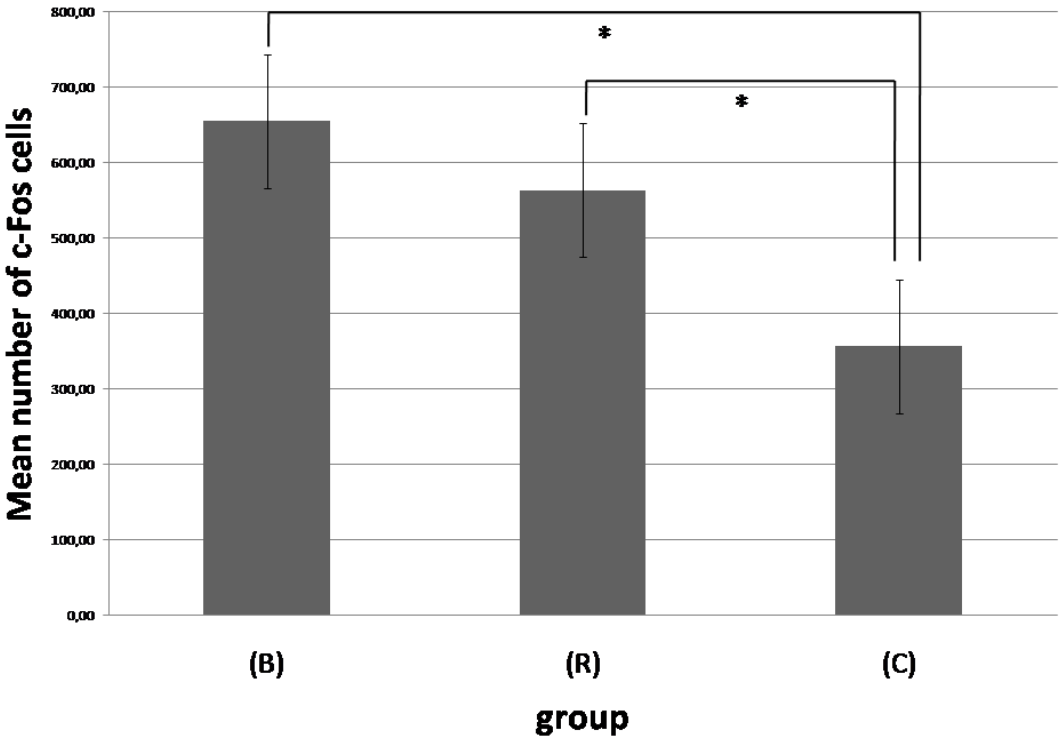


Fig 7 Mean number of c-Fos labeled cells in the SCN

The mean numbers and standard deviations of the c-Fos labeled cells for the three groups (blue (B), red (R) and control (C)) in two subdivisions (core and shell) of the SCN are presented in table 4.

	Core		Shell	
	Mean	Std.	Mean	Std.
Blue	467,14	73,99	188,29	20,11
Red	426,23	144,46	137,77	73,19
Control	235,10	73,59	121,80	43,52

Table 4. Means and standard deviations of c-Fos labeled cells in Core and Shell regions of the SCN

A multivariate analysis of variance (MANOVA) comparing the number of c-Fos labeled cells in two subdivisions (core and shell) of the SCN showed a significant difference [$F(4, 54) = 8.349$, $p < 0{,}000$, $\eta_p^2 = 0{,}382$]. Post-hoc tests (LSD) showed that for the shell the region blue light administrated group (B) was significantly different than control group ($p = 0.022$) but not from red light administrated group (R) ($p = 0.064$), also control (C) and red (R) group did not differ significantly ($p = 0.501$). For the core region, control group (C) was significantly different than red (R) ($p = 0.000$) and blue (B) ($p = 0.000$), and red and blue light administrated groups did not differ significantly ($p = 0.438$).

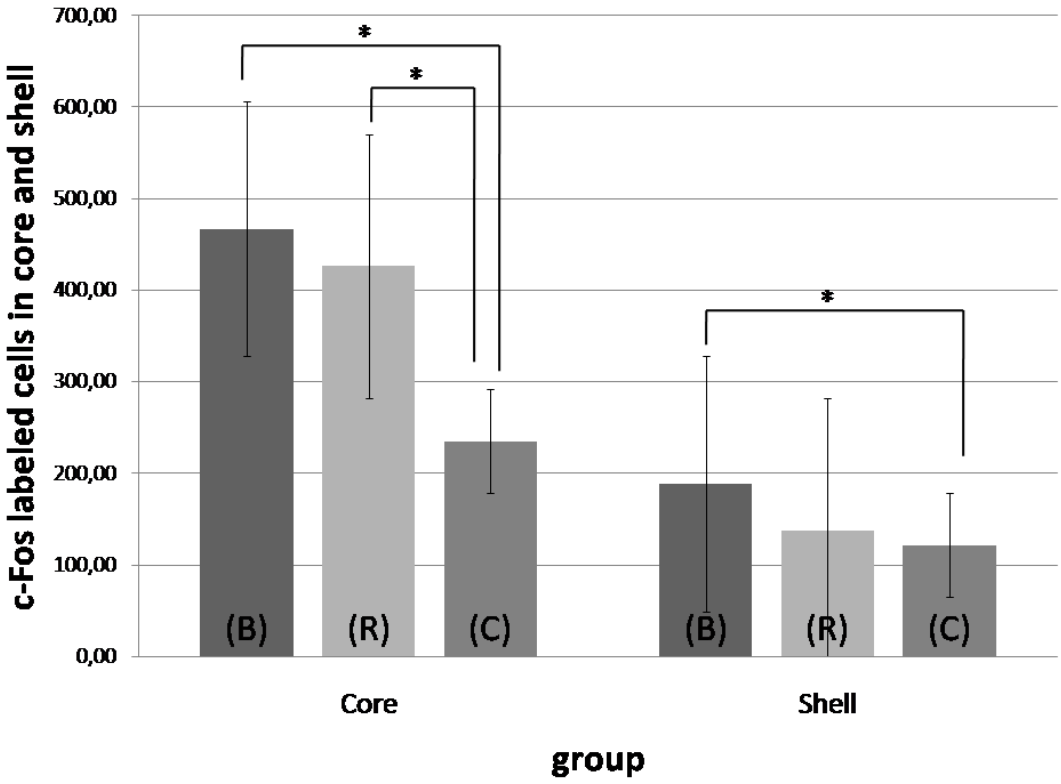


Fig 8 Mean number of c-Fos labeled cells in the Core and Shell subdivisions of SCN.

The mean numbers and standard deviations of the c-Fos labeled cells in subdivisions of SCN are presented in Table 5.

Table 5. Means and standard deviations of c-Fos labeled cells in different subdivisions of SCN

	Dorsomedial		Ventrolateral		Shell	
	Mean	Std.	Mean	Std.	Mean	Std.
Blue	177,71	31,45	305,14	84,07	188,29	20,11
Red	162,54	68,52	263,69	94,18	137,77	73,19
Control	127,50	48,32	107,60	51,10	121,80	43,52

To examine the distribution of the c-Fos labeled cells in the subdivisions of Core of the SCN, a multivariate analysis of variance (MANOVA) is used. Comparing the number of c-Fos labeled cells in the subdivisions (dorsamedial and ventrolateral) of the Core region of the SCN showed a significant difference [$F(4, 54) = 5.06$, $p < 0.005$, $\eta_p^2 = 0{,}272$]. Post-hoc tests (LSD) showed that the control group was significantly different than red ($p = 0.000$) and blue ($p = 0.000$) light administrated groups in the ventrolateral division of the SCN. For the dorsomedial region, Post-Hoc results (LSD) did not demonstrate a significant difference.

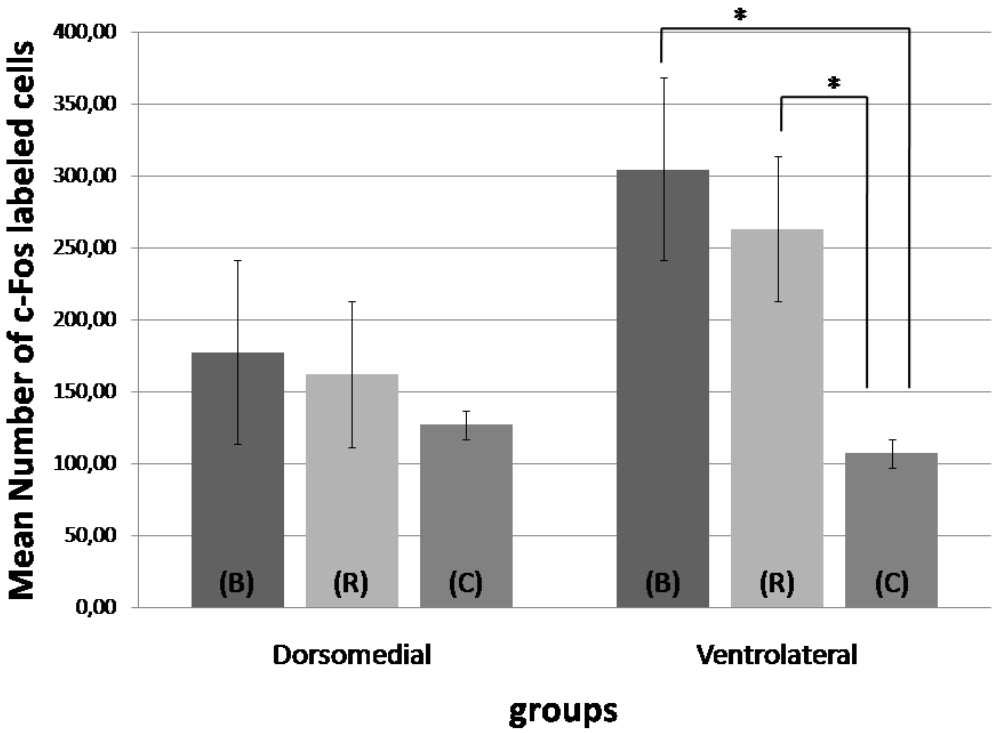


Fig 9 Mean number of c-Fos labeled cells in the Ventrolateral and Dorsomedial subdivisions of SCN.

CHAPTER FOUR

Discussion

Analysis of variance indicated that exposure to only ten minutes of blue but not red light significantly reduced behavioral despair compared to controls. Rats exposed to red light behaved similar to controls. Recently Shulz et al. (2008) reported that a brief (30 min) exposure to a broad band light in late portion of the night has an antidepressant effect in female Wistar rats. The results of the present study refined the previous study and showed that only light of short wavelength has ameliorative effect on behavioral despair. Thus the effectiveness of light pulses in the late portion of night is depended on the spectral composition of light.

Interestingly, the present study did not demonstrate any ameliorative effect of blue light for female rats, an unexpected result in light of the findings by Schulz et al. (2008). There are possible explanations for the discrepancy between female and male rats. Consoli et al. (2005) have reported that different stressors produce different effects for male and female rats in forced swim test in response to antidepressants. In addition, a number of studies in the literature on the sex difference of behavioral despair suggest that female rats are more vulnerable to FST paradigm. For example, Dalla et al.'s (2008) study showed that female rats are more immobile in the Forced Swim Test compared to males. On the other hand, it is important to note that contrary findings exist (Alonso et al., 1991; Brotto et al., 2000). Furthermore Barros and Ferrigolo (1998) have reported that female rats are resistant to antidepressant effects of imipramine. Thus, our results may suggest that the effectiveness of light treatment with the present parameters is not sufficient to

alleviate behavioral despair in females. Secondly, change in the housing conditions may have disparate effects on the behavior of male and female rats. In the present experiment, rats were placed in light chamber in pairs, but normally they were housed four to a cage. Studies show that if the male rats are removed from large groups to smaller group housing, an increase in their explorative behavior is observed, whereas the same procedure reduces the explorative behavior in female rats (Palanza, 2001.). In the present study male and female rats were group housed in the vivarium conditions (four rats in each cage), but during the experimental manipulations, only two rats were placed in the light chamber. In the present study female rats may became more anxious because of a change in housing conditions whereas the same procedure may have positive effect for male rats. Thus, the experimental may produce a difference in addition to the experimental manipulation. Furthermore, Consoli et al. (2005) showed that "low intensity physical stressors" have an antidepressant effect on male rats, but this effect is not observed in female rats. Thus, it's possible to argue that light stimulation at night may cause a mild stress which in turn has an antidepressant effect for male rats.

A previous study in our laboratory has found that destruction of SCN has an ameliorative effect on behavioral despair in a different animal model of depression (Open Space Swim Test) for male rats. On the other hand, for females, destruction of SCN had no protective effect on behavioral despair (manuscript in preparation). Along with the present experiment, these evidence suggests that the sexually dimorphic structure of SCN may be a potential explanation for the incompatibility.

An important result of the present study is that despite the similar c-Fos immunohistoreativity observed in whole SCN due to red or blue light pulse, there is a significant difference inthe behavioral outcome. A detailed analysis of c-Fos staining in the subdivisions of the SCN, showed that: c-Fos results of controls animals of the present study confirmed the study by Miller et al. (2005) which reported that the spontaneous c-FOS rhythms of SCN is mainly located in the shell region of the SCN. Moreover, c-Fos expression in the present study is seen mainly in the ventrolateral parts of the SCN after blue and red light stimulation; this part of the SCN is considered the input site of photic and nonphotic stimulation in the SCN (Sumova, Travnickova & Helena 2000). In c-Fos staining, the only difference found in the present study between blue and red light stimulated groups is in the shell region. The red light treated group did not differ from control animals. The shell region of the SCN is considered as having a major role in the intrinsic rhythms of the SCN, whereas core regions have a role in the regulation external entrainments (Miller et al., 2005). The present study demonstrates that both red and blue light pulses alter c-Fos expression in the ventrolateral subdivision. However, only the blue light can modulate the c-Fos expression in the shell region, indicating to a zeitgeber effect on SCN. The behavioral results of the present study also support this view. These results suggest that along with SCN, other brain regions should also play a role in alleviating behavioral despair.

The DRN may have a pivotal role in the effect observed in the present study; as mentioned above, a 60 min light stimulation significantly increases c-Fos expression in the DRN (Fite et al., 2005). Stimulation of DRN generates phase advances (Glass et al., 2005). However, the present study did not find any significant difference in c-Fos activity in the anterior parts of DRN. The present study,

however, does not rule out the possible role of DRN. First, c-Fos data is missing for the posterior part of the DRN due to limitations of the slicing technique. In addition, animals were perfused 90 minutes after the photic stimulation, in order to assess the activity on the stimulation time. If the activation in DRN occurred after this point on or considerably earlier, the present procedure would not detect it. Thus, the serotonergic inputs and phase advance capability of DRN may still be critical in the ameliorative effect of light observed in the present study.

It has been reported that melanopsin-containing ganglion cells are sensitive to short wavelength light, and has an essential role in circadian regulation (Newman et al., (2003). On the other hand potential additive role of rods and cones are indicated by many studies (Hanifin & Brainard, 2007). Examination of c-Fos labeled cells in the present study supported this view, because both red and blue light stimulation increased the c-Fos expression in the ventrolateral part of the SCN.

Results of the present study do not seem to support the potential role of the effects of light on melatonin rhythms in relation to behavioral despair. Light suppresses melatonin secretion via the SCN (Morrin et al., 2005), and a potential relation between this suppression and depression had been proposed. Recent studies have reported that both short (Lockley et al., 2008) and long (Hanifin et al., 2006) wavelengths suppress melatonin. These studies are in line with the c-Fos results of the present study, in that both short and long wavelengths activate the SCN. Even though, the different wavelengths have similar effects on the melatonin secretion and in the stimulation of SCN, their behavioral consequences are different.

Red illumination is widely used in circadian studies for control conditions and considered to have no effect on albino rats in rhythm and many other studies. There are a small number of studies that points to the possible shortcomings of red light

illumination in such studies. McCormack and Sontags (1980) have shown that red light (>600 nm) influences phase curves in albino rats. Larcarcel et al. (2000) have reported that red light stimulation affects c-Fos expression in different centers of the visual pathway such as the geniculate lateral complex, superior colliculus and primary visual cortex. The present study is the first to show that red light stimulation for ten minutes significantly alters the c-Fos expression in SCN compared to control, thus the experimental procedures that use red light in control conditions or in other experimental manipulations may be biased.

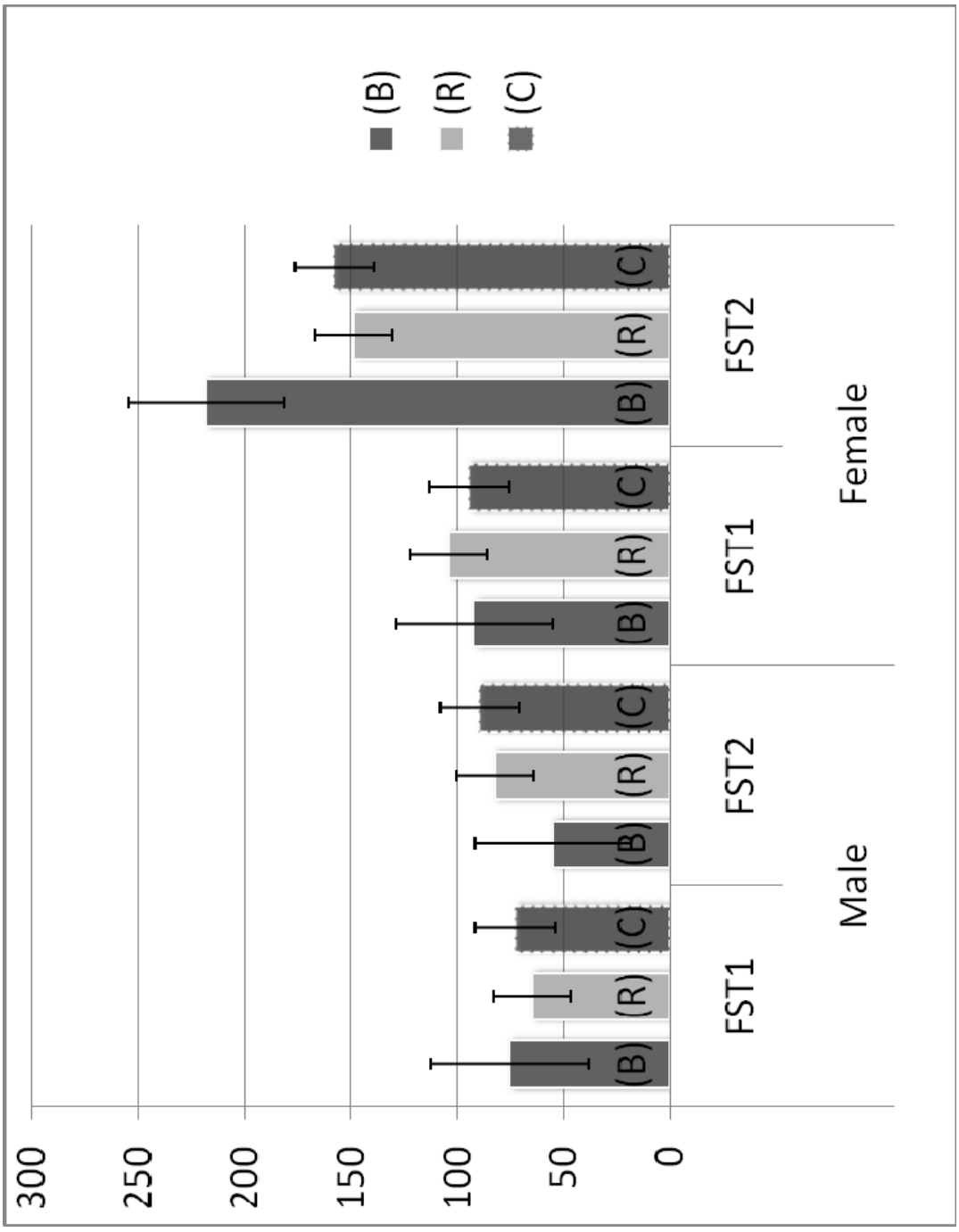
Chapter Five

Conclusion

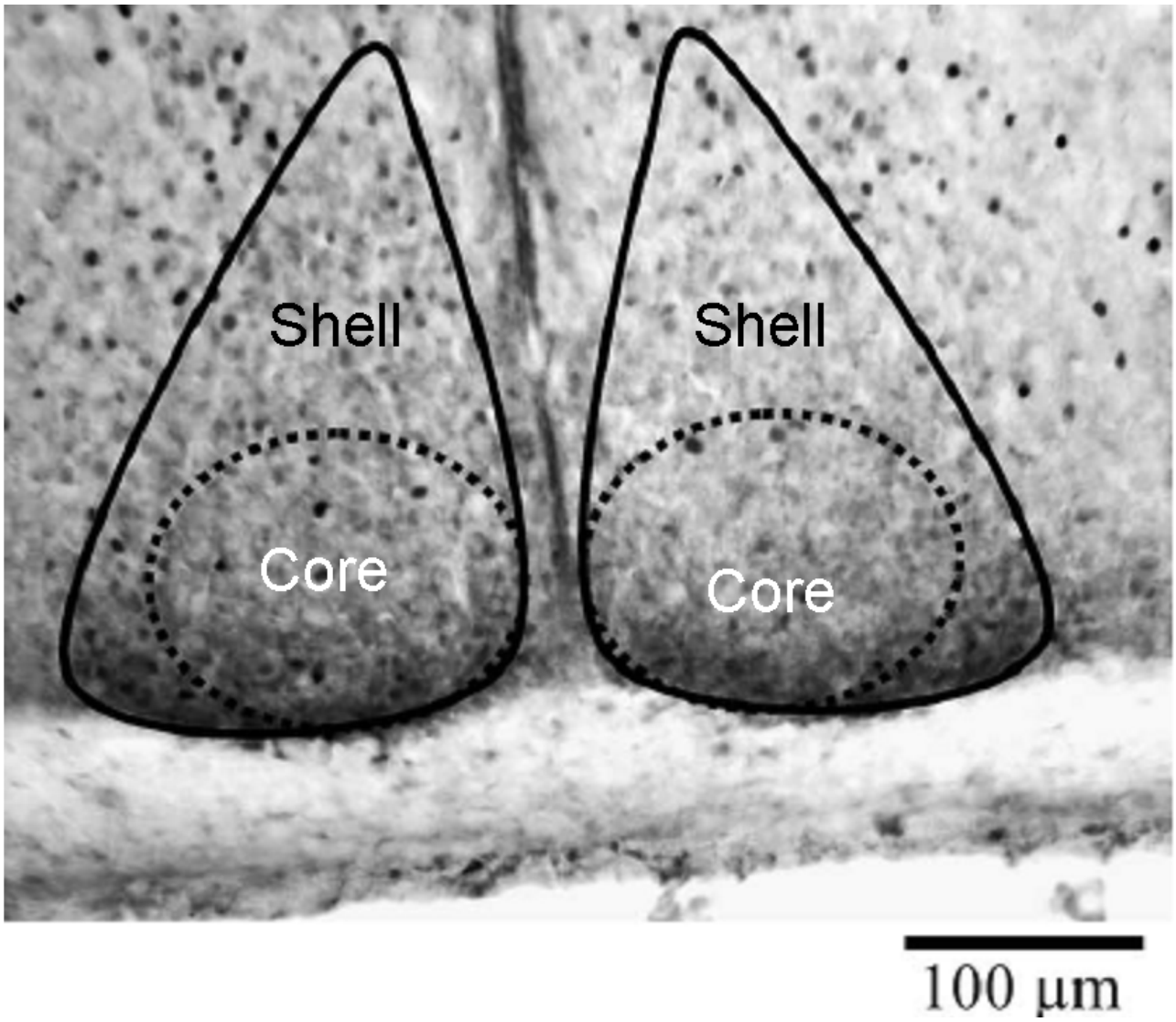
The present findings indicate that a short pulse of blue light has antidepressant effect in an animal model of depression for male but nor for female rats. Future studies should consider the effects of light pulse with different spectral distributions on phase response curves. In addition, multiple c-Fos analysis with different timing should be held in order to assess the chain of activities in the brain. Lesion studies have potential to illuminate the mechanisms behind the ameliorative effect of light treatment.

APPENDICES

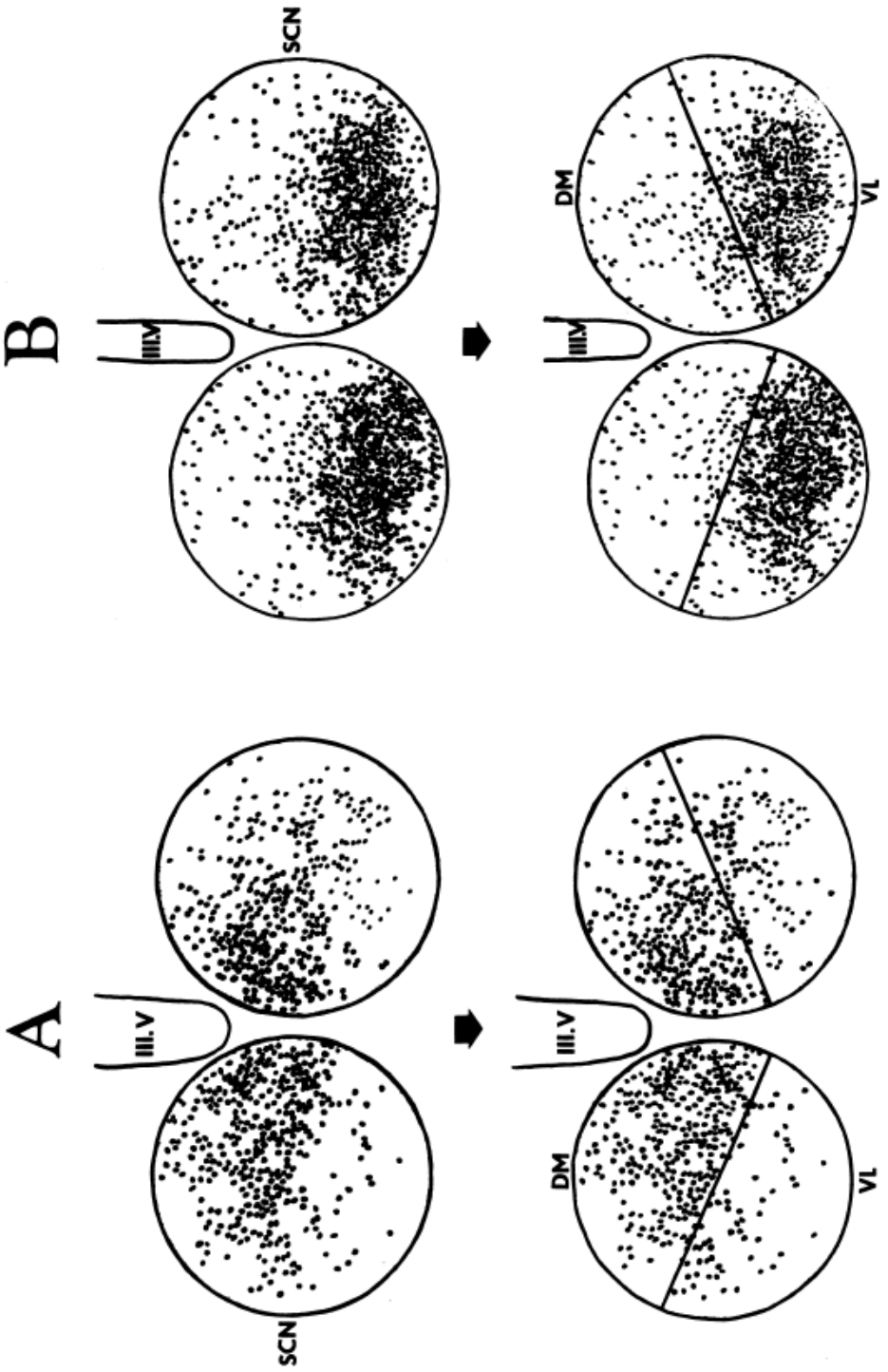
APPENDIX A: COMPARISONS OF MALE AND FEMALE RATS FORCED SWIM TEST SCORES


(B)
(R)
(C)
300
250
200
150
100
50
0
Female
Male
FST1
FST2
FST1
FST2
(B)
(R)
(C)
(B)
(R)
(C)
(B)
(R)
(C)
(B)
(R)
(C)

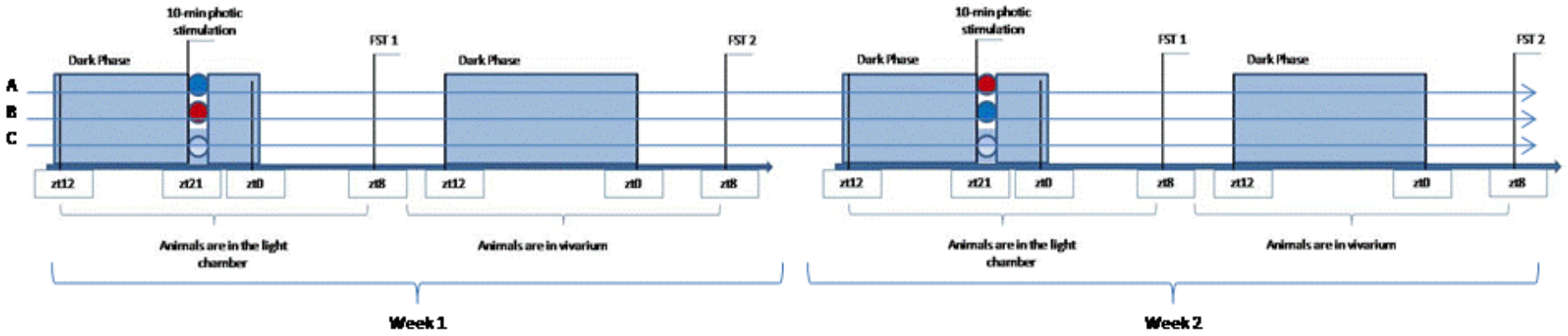
APPENDIX B. ANATOMICAL BORDERS OF THE CORE AND SHELL REGIONS OF SCN (Miller et al., 2005, p.1110)


Shell
Shell
Core
Core
100 µm

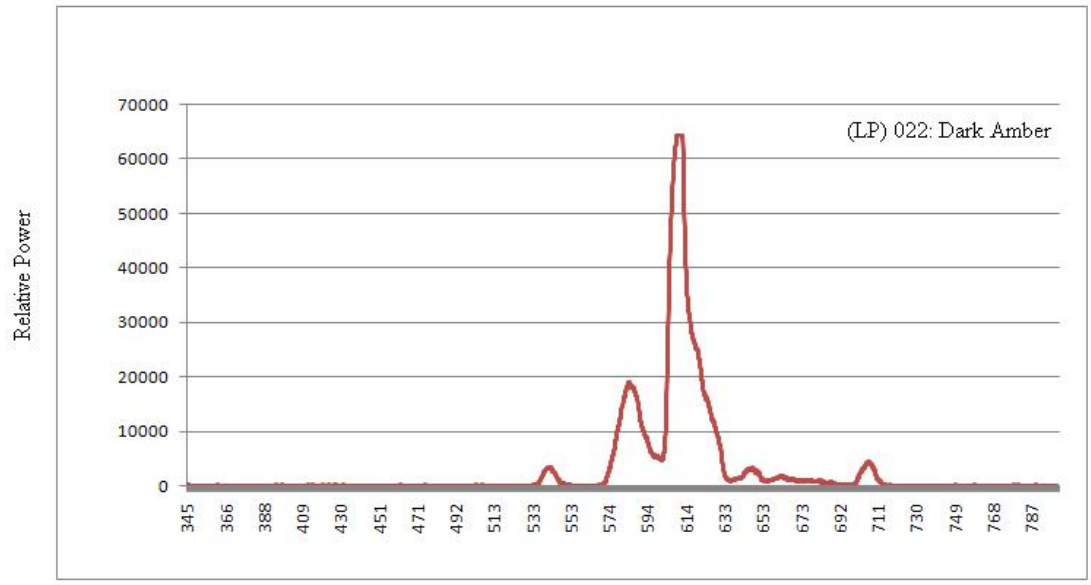
APPENDIX C. ANATOMICAL BORDERS OF THE DORSOMEDIAL (DM) AND VENTROLATERAL (VL) REGIONS OF SCN (Sumova et al., 1998, p.256)

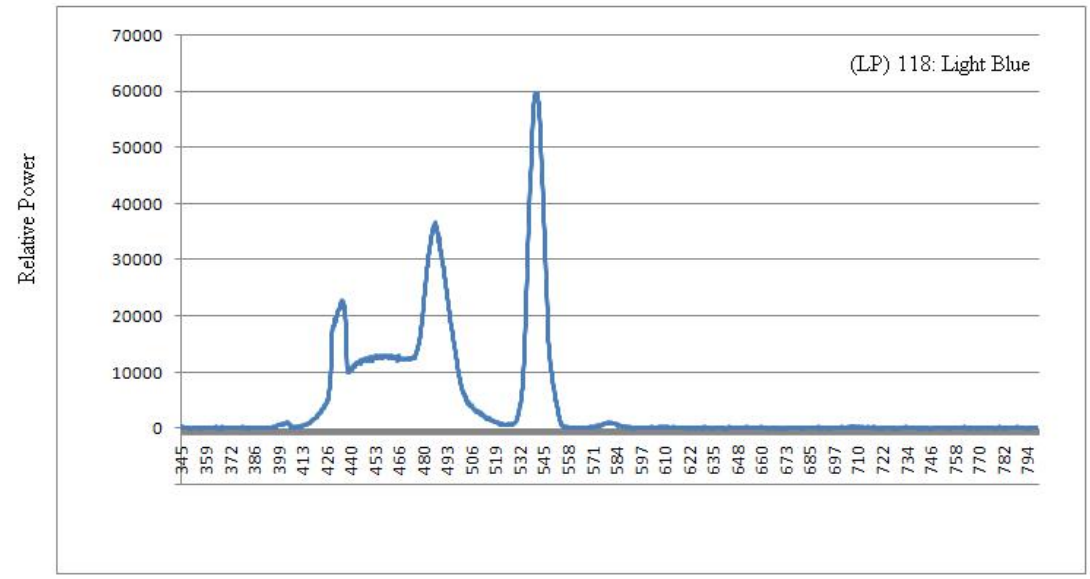

A
III.V
SCN
DM
VL
B
IIIM
SCN
DM
VL

APPANDIX D. A SCHEMATIC ILLUSTRATION OF EXPERIMENTAL PROCEDURES


10-min photic stimulation
FST 1
FST 2
Dark Phase
Dark Phase
10-min photic stimulation
FST 1
FST 2
Dark Phase
Dark Phase
A
B
C
zt12
zt21
zt0
zt8
zt12
zt0
zt8
zt12
zt21
zt0
zt8
zt12
zt0
zt8
Animals are in the light chamber
Animals are in vivarium
Animals are in the light chamber
Animals are in vivarium
Week 1
Week 2

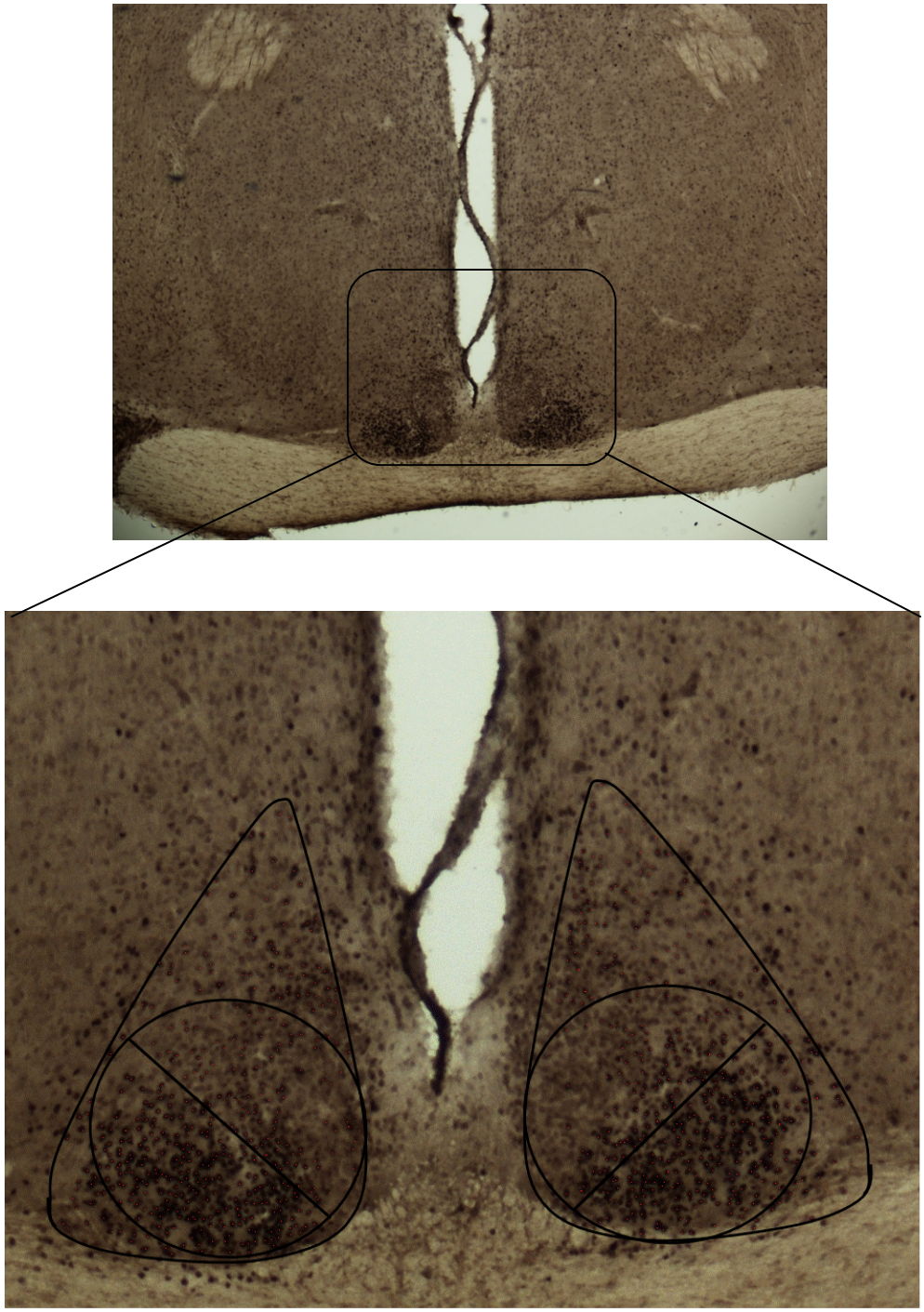
APPENDIX E. MEAN SPECTRAL POWER DISTRIBUTIONS OF TWO DIFFERENT LIGHT FILTERS



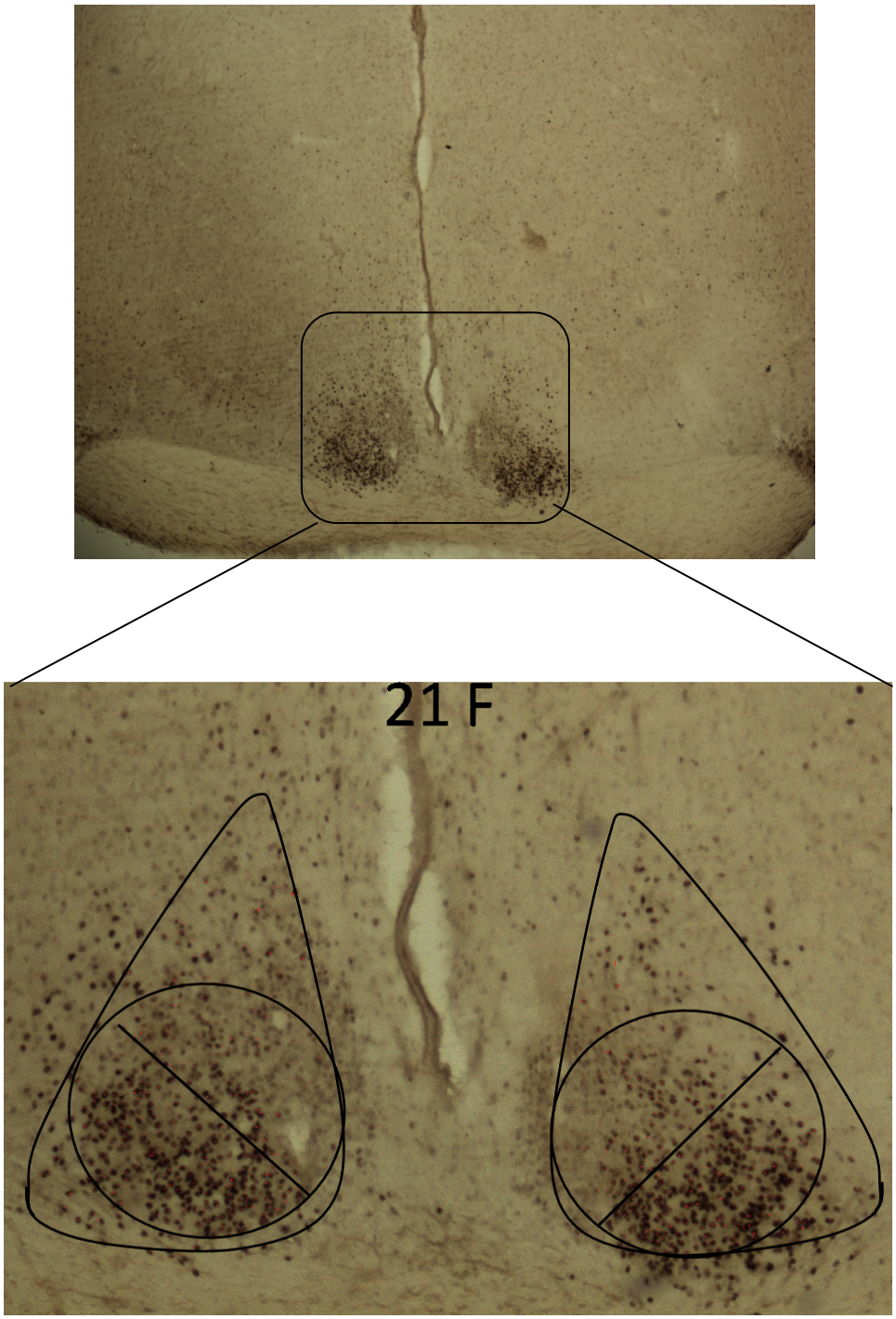


Wavelength (nm)

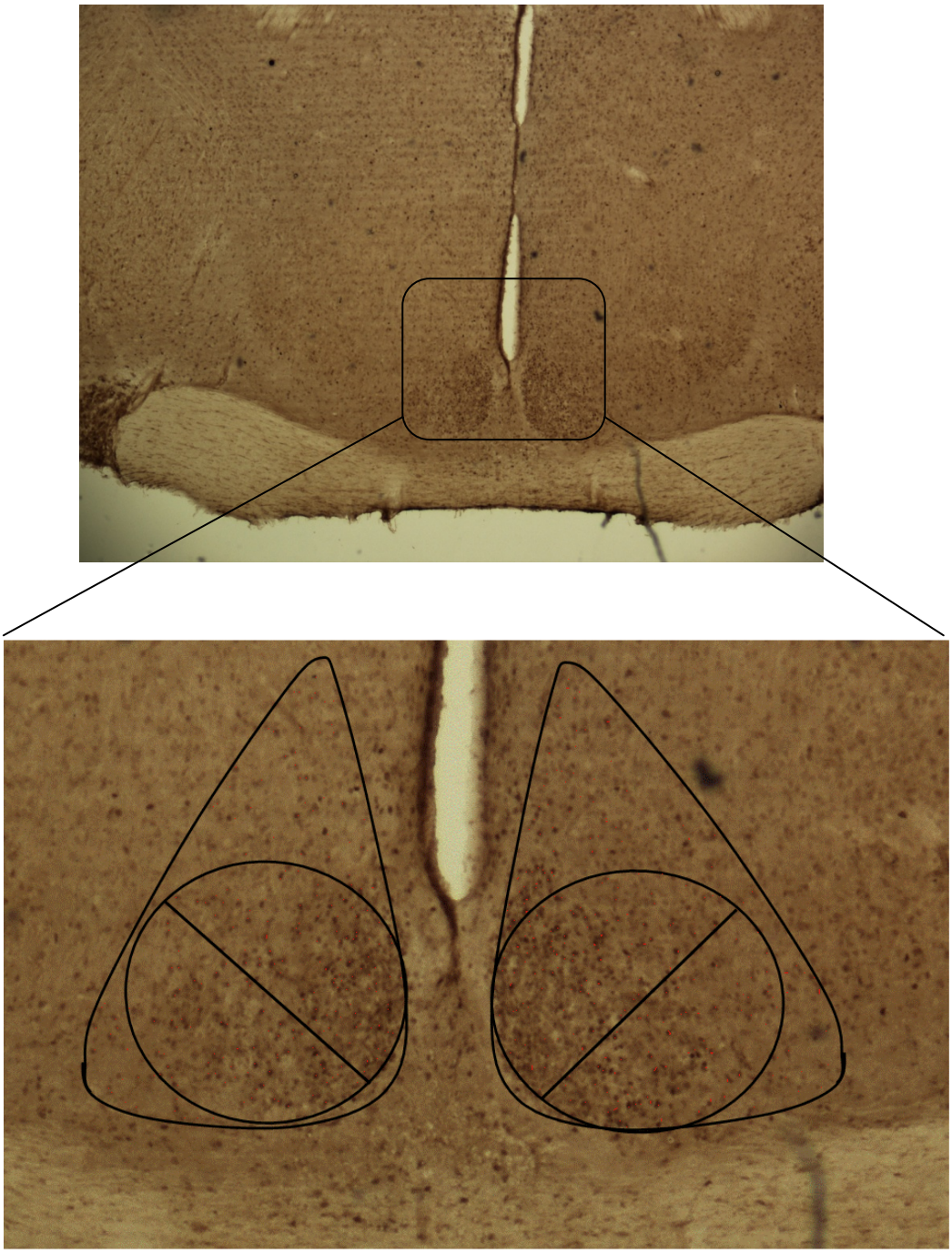
APPENDIX F. A C-FOS STAINED SECTION OF A BLUE LIGHT STIMULATED RAT, FROM SCN REGION



APPENDIX G. A C-FOS STAINED SECTION OF A RED LIGHT STIMULATED RAT, FROM SCN REGION


21 F

APPENDİX H. A C-FOS STAINED SECTION OF A CONTROL RAT, FROM SCN REGION



References

Abrahamson, E. E., & Moore, R. Y. (2001). Suprachiasmatic nucleus in the mouse: Retinal innervation, intrinsic organization and efferent projections. *Brain Research, 916,* 172- 191.

Abel, E. L. (1993). Physiological correlates of the forced swim test in rats. *Physiology and Behavior, 54*, 309-317.

Abel, E. L. (1995). Circannual changes in the duration of the immobility response of rats in the forced swim test. *Physiology and Behavior, 58,* 591-593.

Aksoy, A., Schulz, D., Yilmaz, A., & Canbeyli, R. (2004). Seasonal variability in behavioral despair in female rats. *International Journal of Neuroscience, 114*, 1513-1520.

Alonso, S.J., Castellano, M.A., Alfonso D., & Rodriguez, M. (1991). Sex differences in behavioral despair: relationship between behavioral despair and open field and open field activity. *Physiology and Behavior, 49*, 69-72

Amir, S., & Stewart, J. (1999). Effectiveness of light on the circadian clock is linked to its emotional value. *Neuroscience, 88,* 339-345.

Arushanyan, E. B., Vodolazhskaya, M. G., & Popov, A. V.(2000). Acceleration of the acquisition of a conditioned reflex to time in rats with disruption of the suprachiasmatic nuclei of the hypothalamus. *Neuroscience, Behavior and Physiology, 30*, 649–651.

Arvanitogiannis, A., & Amir, S. (1999). Resetting the rat circadian clock by ultra-short light flashes. *Neuroscience Letters, 261,* 159-162.

Avery, D.H. (1998). A turning point for seasonal affective disorder and light therapy research? *Archives in General Psychiatry. 55*, 863-864.

Benloucif S., Masana M.I., Dubocovich M.L. (1997). Light-induced phase shifts of circadian activity rhythms and immediate early gene expression in the suprachiasmatic nucleus are attenuated in old C3H/HeN mice. *Brain Research 747,* 34-42

Barros, H.M.T. & Ferigolo, M. (1998). Ethopharmacology of immipramine in the forced swim test: gender differences. *Neuroscience & Biobehavioral reviews, 23*, 279-286

Beaulé, C., Robinson, B., Lamont, E. W., & Amir, S.(2003). Melanopsin in the Circadian timing system. *Journal of Molecular Neuroscience, 21(1),* 73-89.

Beaule C., Arvanitogiannis A., and Amir S. (2001). Light suppresses FOS expression in the shell region of the suprachiasmatic nucleus at dusk and dawn: implications for photic entrainment of circadian rhythms. *Neuroscience 106,* 249-254.

Brainard, G. C., Hanifin, J.P., Greeson, J.M., Byrne, B., Glickman, G., Gerner, E., & Rollag, M. D. (2001). Action spectrum for melatonin regulation in humans: Evidence for a novel circadian ph otoreceptor. *Journal of Neuroscience, 21(16),* 6405-12.

Brainard, G.C., & Hanifin, J.P. (2005). Photons, clocks, and consciousness. *Journal of Biological Rhythms, 20(4),* 314-25.

Brotto, L. A., Gorzalka, B.B. & Barr, A.M. (2000). Sex differences in forced swim and open field test behaviours after chronic administration of melatonin. *European journal of Pharmacology 402*, 87-93

Bouwknecht J. A., Spiga F., Staub D. Rr, Hale M. W., Shekhar A., Lowr C. A. (2007). Differential effects of exposure to low-light or high-light open-field on anxiety-related behaviors: Relationship to c-Fos expression in serotonergic and non-serotonergic neurons in the dorsal raphe nucleus. *Brain Research Bulletin, 72,* 32-43

Buijs, R. M., Markman, M., Nunes-Cardoso, B., Hou, Y.X., & Shinn, S. (1993). Projections of the suprachiasmatic nucleus to stress-related areas in the rat hypothalamus: a light and electronmicroscopic study. *Journal of Comparative. Neurology , 335,* 42–54.

Bulduk, S. & Canbeyli, R. (2004). Effect of inescapable tones on behavioral despair in Wistar rats. *Progress in Neuropsychopharmacology and Biological Psychiatry, 28,* 471-475

Consoli D., Fedotova J., Micale V., Sapronov N.S., Drago F. (2005). Stressors affect the response of male and female rats to clomipramine in a model of behavioral despair (forced swim test). *European Journal of Pharmacology, 520,* 100-107

Connor, T. J., Kelly, J. P., & Leonard, B. E. (1997). Forced swim test-induced neurochemical, endocrine, and immune changes in the rat. *Pharmacology Biochemistry and Behavior, 58,* 961-967.

Correa-Lacarcel L., Pujante M. J., Terol F. F., Almenar-Garcia V., Purchades-Orts A., Balesta J. J., Lloler J., Robles J. A., Sacnchez-del-Campo F. (2000) Stimulus frequency affects c-fos expression in the rat visual system. *Journal of Chemical Neuroanatomy, 18,* 135-146

Dalla C., Antoniou K., Kokras N., Drossopoulou G., Papahanasiou G., Bekris S., Daskas S., Papadopoulou-Daifoti Z. (2008). Sex differences in the effects of two stress paradigms on dopaminergic neurotransmission. *Physiology & Behavior 93,* 595-605

Drossopoulou G., Antoniou K., Kokras N., Papahanasiou G., Bekris S., Daskas S., Dalla C., Papadopoulou-Daifoti (2004). Sex differences in behavioral, neurochemical and neuroendocrine effects induced by the forced swim test in rats. *Neuroscience 126,* 849-857

Eastman, C. I., Young, M. A., Fogg, L. F., Liu, L., & Meaden, P. M., (1998). Bright light treatment of winter depression. *Archives of General Psychiatry, 55*, 883-889

Esseveldt, K. E. L., Lehman, M. N., & Boer, G. J. (2000). The suprachiasmatic nucleus and the circadian time-keeping system revisited. *Brain Research Review, 33,* 34–77.

Even, C., Schröder, C.M., Friedman, S., & Rouillon, F. (2007). Efficacy of light therapy in nonseasonal depression: A systematic review. *Journal of Affective Disorders, 18*, 741-782

Fite, K.V., Janusonis, S., Foote, W. Bengston, L., (1999). Retinal afferents to the dorsal raphe nucleus in rats and mongolian gerbils, *Journal of Comparative Neurology 414,* 469-484.

Fite, K.V., Wu, P.S., Bellemer, A., (2005). Photostimulation alters c-Fos expression in the dorsal raphe nucleus, *Brain Research. 1031*, 245-252.

Frank, K.D., & Zimmerman, W. F. (1969). Action spectra for phase shifts of a circadian rhythm in drosophila. *Science, 163(868),* 688-697.

Glass, J.D., DiNardo, L.A., Ehlen, J.C., (2000). Dorsal raphe nuclear stimulation of SCN serotonin release and circadian phase-resetting, *Brain Research. 859*, 224-232

Golden, R. N., Gaynes, B. N., Ekstrom, R. D., Hamer, R. M., Jacobsen, F. M., Suppers, T., Wisner, K. L., & Nemeroff, C. B. (2005). The efficacy of light therapy in the treatment of mood disorders: a review and meta-analysis of the evidence. *American Journal of Psychiatry, 162(4),* 656-662

Golombek, D. A., Ferreyra, G. A., Katz, M. E., Marpegan, L., Pinto, F. T., Alfonso, T. F., & Yannielli, P. C. (2000). Neurochemistry of mammalian entrainment: Signal transduction pathways in the suprachiasmatic nuclei. *Biological Rhythm Research, 31(1),* 56-70.

Glickman, G., Byrne, B., Pineda, C., Hauck, W. W., & Brainard, G. C. (2006). Light therapy for seasonal affective disorder with blue narrow-band light-emitting diodes (LEDs). *Biological Psychiatry, 59(6),* 502-7.

Greenwood B. N., Foley T. E., Day H. E., Campisi J., Hammack S.H., Campeau S., Maier S. F. & Fleshner M., (2003). Freewheel running prevents learned helplessness/behavioral depression: role of dorsal raphe serotonergic neurons. *Journal of Neuroscience, 23*, 2889-2898.

Hanifin, J. P., Brainard G. C. (2007). Photoreception for circadian, neuroendocrine, and neurobehavioral regulation. *Journal of Physiological Anthropology 26*, 87-94.

Hanifin, J. P., Stewart K. T., Smith P., Tanner R., Rollag M., Brainard G. C. (2006). High-intensity red light suppresses melatonin. *Chornobiology International*, 23, 251-268.

Jesberger, J. A, & Richardson, J. S. (1985). Animal models of depression: Parallels and correlates to severe depression in humans. *Biological Psychiatry, 20,* 764-784.

Kawano, H., Decker, K., Reuss, S., (1996). Is there a direct retino-raphe-suprachiasmatic pathway in the rat? *Neuroscience Letters, 212,* 143-146.

Kesser R. C. (2003) Epidemiology of women and depression. *Journal of Affective Disorders, 74,* 5-31

Krout, K. E., Kawano, J., Mettenleiter, T. C., & Loewy, A. D. (2002). CNS inputs to the suprachiasmatic nucleus of the rat. *Neuroscience, 110 (1),* 73-92.

Kripke, D.F., (1998). Light treatment for nonseasonal depression: speed, efficacy, and combined treatment. *Journal of Affective Disorders , 49,* 109-117.

Kripke, D. F., Mullaney, D. J., Klauber, M. R., Risch, S. C., & Gillin, J. C. (1992). Controlled trial of bright light for nonseasonal major depressive disorders. *Biological Psychiatry, 31,* 119-134.

Kripke, D.F., Risch, S.C., Janowsky, D.S., (1983). Bright white light alleviates depression. *Psychiatry Research 10,* 105-112.

Knoch, M. E., Gobes, S. M., Pavlofska, I., Su, C., Mistlberger, R. E., Glass, J. D., (2004). Short-term exposure to constant light promotes circadian phase resetting responses to non-photic stimuli in Syrian hamsters. *European Journal of Neuroscience, 19,* 2779-2790

Lam R. W. (1998). Seasonal affective disorder: diagnosis and management. *Primary Care Psychiatry, 4,* 63-74

Lee, T.M., Chan, C.C., Paterson, J.G., Janzen, H.L., & Blashko, C.A. (1997). Spectral properties of phototherapy for seasonal affective disorder: a meta-analysis. *Acta Psychiatry Scand, 2,* 117-21.

Lewy, A. J., Rough, J. N., Songer, J. B., Mishra, N., Yuhas, K., & Emens, J. S. (2007). The phase shift hypothesis for the circadian component of winter depression. *Dialogues in Clinical Neuroscience, 9(3),* 291-300.

Lewy, A. J., Bauer, V. K., Cutler, N. L., Sack, R. L., Ahmed, S., Thomas, K.H., Blood, M. L., & Jackson, J. M. (1998). Morning vs evening light treatment of patients with winter depression. *Archives of General Psychiatry, 55(10),* 890-6.

Lewy, A.J.,. Sack, R.L., Miller L.S., Hoban, T.M., (1987). Antidepressant and circadian phase-shifting effects of light, *Science 235*, 352-354.

Lino-de-Oliveira, C., De Lima, T. C. M., & Carobrez, A. P. (2005). Structure of the rat behavior in the forced swimming test. *Behavioral Brain Research, 158,* 243-250.

Lockley, S.W., Brainard, G. C., & Czeisler, C. A.(2003). High sensitivity of the human circadian melatonin rhythm to resetting by short wavelength light. *Journal of Clinical Endocrinolology and Metabolism. 9,* 4502-4505.

Lucas, R. J., Freedman, M. S., Muñoz, M., Garcia-Fernández, J. M., & Foster, R. G. (1999). Regulation of the mammalian pineal by non-rod, non-cone, ocularphotoreceptors. *Science, 284(5413),* 505-507.

Mackert, A., Volz, H. P., Stieglitz, R. D., & Müller-Oerlinghausen, B. (1991). Phototherapy in nonseasonal depression*. Biological Psychiatry, 30(3),* 257-268.

Mccormack, C. E, & Sontag C. R. (1980). Entertainment by red light of running activity and ovulation rhythms of rats. *American Journal of Physiology 239,* 450-453

McClung, C.A. (2007). Circadian genes, rhythms and biology of mood disorders. *Pharmacology and Therapy. 114,* 222-232.

Miller J. P., McAluley J. D., & Pang K. C. H. (2005). Spontaneous fos expression in the suprachiasmatic nucleus of young and old mice. *Neurobiology of Aging 26*, 1107-1115

Morin, L. P., & Allen, C. N. (2005). The circadian visual system, 2005. *Brain Research Reviews, 51,* 1-60

Morin, L. P., (1994). The circadian visual system. *Brain Research Reviews, 19,* 102-127

Morin, L. P., Shivers, K. Y., Blanchard, J. H., & Muscat, L. (2005). Complex organization of mouse and rat suprachiasmatic nucleus. *Nuroscience.*

Molina-Hernandez, M., & Tellez-Alcantara, P., (2002). Long-photoperiod regimen may produce antidepressant actions in the male rat. *Progress in Neuro-Psychopharmacology and Biological Psychiatry 24,* 105-116

Newman, L. A., Walker, M. T., Brown, R. L., Cronin, T. W., & Robinson, P. R. (2003). Melanopsin forms a functional short-wavelength photopigment. *Biochemistry, 42(44),* 12734-8.

Oren, D.A., Terman, M. (1998). Tweaking the human circadian clock with light, *Science 279,* 333-334.

Palanza P. (2001). Animal models of anxiety and depression: how are females different? *Neuroscience and Biobehavioral Reviews 25*, 219-233

Panda S., Nayak S. K., Campo B., Walker J. R., Hogenesch J.B., Jegla T. (2005). Illumination of the melanopsin signaling pathway. *Science, 28, (5709),* 600-604.

Porsolt, R. D., Anton, G., Blavet N., & Jalfre, M. (1978). Behavioral despair in rats: A new model sensitive to antidepressant treatments. *European Journal of Pharmacology, 47,* 379-391.

Porsolt, R. D., LePichon, M., & Jalfre, M. (1977). Depression: A new animal model sensitive to antidepressant treatments. *Nature, 266,* 730-732.

Rea M. A., (1989). Light increases Fos-related protein immunoreactivity in the rat Suprachiasmatic nuclei. *Brain Research Bulletin, 23,* 577-581.

Rosenthal, N. E., Sack, D. A., Gillin, J. C., Lewy, A. J., Goodwin, F. K., Davenport, Y., Mueller, P. S., Newsome, D. A., & Wehr, T. A. (1984). Seasonal affective disorder: A description of the syndrome and preliminary findings with light therapy. *Archives of General Psychiatry, 41,* 72-80.

Rosenwasser, A. M. (2001). Alcohol, antidepressants, and circadian rhythms. *Alcohol Research and Health, 25,* 126-135.

Rusak, B., (2000). Chronobiology and mood disorders: background and introduction. *Journal of Psychiatry & Neuroscience, 25(5),* 443-445.

Schulz, D., Aksoy, A., & Canbeyli, R., (2008). Behavioral despair is differentially affected by the length and timing of photic stimulation in the dark phase of an L/D cycle. *Progress in Neuro-Psychopharmacology Biological Psychiatry, 32*, 1257-1262

Sumova A., & Illnerova H. (1998). Effect of photic stimuli disturbing overt circadian rhythms on the dorsomedial and ventrolateral SCN rhythmicity. *Brain Research, 1048*, 161-169

Sumova A., Travnikova Z., Mikkelsen J. D., & Illnerova H. (1998) Spontaneous rhythm in c-Fos immunoreactivity in the dorsomedial part of the rat suprachiasmatic nucleus. *Brain Research 801,* 254-258

Tataroğlu, Ö., Aksoy, A., Yılmaz, A., & Canbeyli, R., (2003). Effect of lesioning the suprachiasmatic nuclei on behavioral despair in rats. *Behavioral Brain Research 1001,* 118-124.

Thompson C. L., Selby C. P., Partch C. L., Plante T. P., Thresher R. J., Arujo F. & Saccar A. (2004). Further evidence for the role of crytochromes in retinohypothalamic Photoreception/photransduction. *Molecular Brain Research 122*, 158-166

Turek, F. W., Dugovic, C., & Zee, P. C. (2001). Current understanding of the circadian clock and the clinical implications for neurological disorders. *Archives of Neurology, 58,* 1781-1787

Van Esseveldt, E., Lehman, M. N., & Boer, G. J. (2000). The suprachiasmatic nucleus and the circadian time-keeping system revisited. *Brain Research Reviews, 33,* 34-77.

Vitaterna, M. H., Takahashi, J. S., & Turek F. W., (2001). Overview of circadian rhythms. *Alcohol Research and Health, 25,* 85-93

Wirz-justice, A., Graw, P., Krauchi, K., Gisin, B., Jochum, A., Arendt, J., Fisch, H. U., Buddeberg, C., & Pöldigner, W. (1993). Light therapy in seasonal affective disorder is independent of time day or circadian phase. *Archives of General Psychiatry, 50,* 929- 937

Yamada, N., Martin-Iverson, M. T., Daimon, K., Tsujimoto, T., & Takahashi, S. (1995). Clinical and chronobiological effects of light therapy on nonseasonal affective disorders. *Biological Psychiatry, 37,* 866-873.

Yannielli P. & Harrington M.E., (2004). Let there be "more" light: enhancement of light actions on the circadian systems through non-photic pathways. *Progress in Neurobiology, 74,* 59-76

Yılmaz, A., Aksoy, A. & Canbeyli, R., (2004). A single day of constant light (L/L) provides immunity to behavioral despair in female rats maintained on an L/D cycle. *Progress in Neuro-Psychopharmacology and Biological Psychiatry, 28,* 1261-1265