The Effects of Short Light Pulse at ZT21 on Behavioral Despair in Old and Young Male Wistar Rats

Thesis submitted to the

Institute for Graduate Studies in Social Sciences

in partial satisfaction of the requirements for the degree of

Master of Arts

in

Psychology

by

Elif Tunç Özcan

Boğaziçi University

2006

Abstract

The Effects of Short Light Pulse at ZT21 on Behavioral Despair in Old and Young Male Wistar Rats

by Elif Tunç Özcan

The present study investigated potentially ameliorative and preventive effects of short light pulse exposure late at night (0400 h, ZT21) in both juvenile and adult rats. Animals were maintained on a 12L/12D cycle (lights on at 0700 h) except for the days of light treatment in an insulated chamber. Light was provided by a 100W tungsten lamp, approximately 50 cm above the cage housing the subjects. There were two control groups; the first one was treated similarly except for photic stimulation and the second one was not put in the chamber but was instead taken directly from the vivarium for forced swimming tests. Our findings indicated that although light pulse exposure leads to a shorter duration of immobility and longer duration of swimming when employed before first forced swimming test (ZT21-B group), it yields no significant results. Furthermore, juvenile and adult rats differ from each other in terms of the responses to the same stressors, namely the former displays more aggravated behavioral despair compared to the latter. However, the short light pulse exposure late at night has no ameliorative or preventive effects on behavioral despair as measured by the forced swimming test on either of the groups.

Özet

ZT21'de Verilen Kısa Süreli Işık Pülsünün Yaşlı ve Genç Erkek Wistar Sıçanlarında Davranışsal Çaresizlik Üzerinde Etkileri

Elif Tunç Özcan

Bu çalışmada gece geç saatte verilen ışık pülsünün (04:00, ZT21) davranışsal çaresizlik olgusunda koruyucu ve tedavi edici etkisi olup olmadığı genç ve yaşlı sıçanlarda incelenmiştir. Laboratuar denekleri normal koşullarda 12 saat ışık ve 12 saat karanlık olmak üzere, ışık manipülasyonun yapıldığı günler hariç, 24 saatlik ışık:karanlık döngüsüyle yaşamaktadırlar. Işık, 100 wattlık bir lamba ile denek kafeslerinin 50 cm. yukarısından özel bir kutuda verilmiştir. Deneyde 2 kontrol grubu kullanılmıştır; birincisi deney gruplarıyla aynı muameleyi görmüş, ışık verilen kutuya konulmuş ancak ışık almamıştır; ikincisi ışık kutusuna konulmamış, zorunlu yüzme testleri için direk vivaryumdan alınmıştır. Sonuçlarda ışık püslü birinci zorunlu yüzme testinden önce verildiği zaman daha kısa süre durağanlık ve daha uzun süre yüzmeye neden olduğu görülmesine rağmen, istatistiksel olarak olumlu bir sonuç bulunmamıştır. Bunun yanında, ergen ve yetişkin sıçanlar aynı strese farklı tepkiler vermiş, ergenler yetişkinlerle karşılaştırıldığında daha çok davranışsal çaresizlik göstermişlerdir. Ancak, gece geç saatte verilen kısa süreli ışık pülsü, Zorunlu Yüzme Testi (ZYT) ile ölçüldüğü şekliyle davranışsal çaresizlik üzerinde her iki yaş grubunda da önleyici ve iyileştirici bir etki göstermemiştir.

Acknowledgements

Many persons contributed to this study in various ways. I want to thank everybody who helped and supported me in its preparation.

I would like to express my deepest gratitude to my thesis advisor, Prof. Dr. Reşit Canbeyli, for his encouraging support and trust in me during all of my years at university. Without his encouraging attitude, this study could have not been accomplished. Also, sharing the pleasure and excitement of doing research was a great value for me, especially for this study. I was lucky that I could work with him.

I would like to thank Assist. Prof. Serra Müderrisoğlu for all her patience through my undergraduate and graduate years, her enthusiastic support, guidance, responsiveness and being there whenever I need her help.

I would also like to thank Assoc. Prof. İ. Ercan Alp for going over a great amount of materials within a very short time, his invaluable assistance and his encouragement in the preparation of my thesis.

I thank my laboratory and assistant friends for their help and support during my experimental process. Additionally, I thank all my friends for their belief in me and being with me all the time.

I thank my beloved family members whose memories or lively existence have been of great support during all my life.

Finally, I dedicate this work to my husband, Murat Özcan, for his endless love, support, and encouragements that make everything possible.

Contents

Figures

Page

Tables

Page

CHAPTER ONE

INTRODUCTION

Depression is a serious disorder that has a wide demographic and geographic base. It is a heterogeneous disorder that often is manifested with symptoms at the psychological, behavioral and physiological levels (American Psychiatric Association Diagnostic and Statistical Manual of Mental Disorders, 1994). Phototherapy, the treatment of depressed patients with bright light exposure, has been used successfully to improve symptoms of both Seasonal Affective Disorder (SAD) (Eastman et al., 1998; Rosenthal et al., 1982; Wirz-Justice et al., 1993) and non-seasonal depression (Kripke, 1998; Kripke et al., 1992). SAD is a type of cyclic depression that occurs during autumn and winter months, while improving in spring and summer months (Rosenthal et al., 1984). Abnormal circadian rhythms are found in SAD (Sher, 2003); SAD patients display disturbances in the regulation of circadian rhythms based on the length of the day and season of the year (Wehr et al., 2001).

Phototherapy is also an effective treatment method for non-seasonal depression. A week-long treatment with bright light in patients with major depression has ameliorative effects observed as early as within the first two days of treatment (Kripke et al., 1992). In another study, Kripke (1998) compared studies that used light treatment in SAD, light treated non-seasonal depression and antidepressant treated patients. Results revealed that light treatment has a rapid recovery effect on SAD and non-seasonal depression compared with antidepressants. Although phototherapy is used widely in the treatment of depression, the mechanisms by which light improves depressive symptoms are not understood fully (Rosenthal et al., 1984; Wehr & Wirz-Justice, 1982).

Because of ethical restrictions in conducting certain studies in humans, animal models of depression provide the basis for elucidating the underlying mechanisms of the ameliorative effect of light treatment on depression. There are, however, only a few studies that report on

the effects of light exposure on animal models of depression (Molina-Hernandez & Tellez-Alcantra, 2000; Prendergast & Nelson, 2005; Schulz, Aksoy, & Canbeyli, submitted; Yilmaz, Aksoy, & Canbeyli, 2004).

The Forced Swimming Test (FST) is a behavioral paradigm that was developed by Porsolt, Le Pichon and Jalfre (1977) in rats and generally is used to assess various antidepressant activities in rodents (Porsolt et al., 1978). The FSTs has a very widespread applicability due to its ease of use, a highly relevant reliability across many laboratories and its ability to detect many different spectra of antidepressant agents and neurochemical, endocrine and immune changes in rodents (Connor et al., 2000; Connor, Kelly, & Leonard, 1997; Porsolt et al., 1977, 1978). In the FST, animals are forced to swim in a water-filled cylinder tank at 25 ^{o}C for 15 minutes on the first day of the test. Escape is impossible from this cylinder tank and the animals eventually develop an immobile posture from their initially struggling behaviors. On the second day of testing, 24-hours after the first one, the animals resume the immobile posture quickly within a five minutes testing session and stay immobile for longer durations in comparison to the first five minutes in the first swimming test. This increased immobility is accepted as a sign of depression and is known as “behavioral despair”. Immobility levels in the behavioral despair can be reduced by the administration of various antidepressants that are used to treat depression in humans (Porsolt et al., 1977, 1978). Behavioral despair is related to many physiological, endocrine and immune changes (Connor et al., 2000, 1997). Additionally the ameliorative or debilitating effects of various treatments such as prolonged light treatment (Molina-Hernandez & Tellez-Alcantara, 2000; Prendergast & Nelson, 2005; Yilmaz et al., 2004) and loud tone exposure (Bulduk & Canbeyli, 2004) have been assessed by using the behavioral despair paradigm.

Laboratory animals are generally kept under 12-hour ligth/12-hour dark (12L/12D) cycle (in general lights on at 0700 h and lights off at 1900 h). This L/D cycle can be manipulated by illuminating the vivarium at different time points or by using different light

onset or offset times. Molina-Hernandez and Tellez-Alcantra (2000) used Wistar rats to assess the effects of exposure to long- or short-photoperiod on behavioral despair compared to antidepressant treatment. The animals were kept under either a long (14L/10D) or short photoperiod procedure (5L/19D) for 30 days or treated with tricyclic antidepressants for 30 days under normal photoperiod (12L/12D). The results indicated an antidepressant effect of the long photoperiod, as shown by the fact that animals in the long photoperiod and in antidepressant groups displayed similar performances in the behavioral despair task compared to the short photoperiod procedure. In a similar study, Prendergast and Nelson (2005) examined in hamsters the effects of long photoperiod on behavioral despair. At the beginning of weaning, 18 days after birth, hamsters were divided into two groups, one of which was kept under a 16L/8D cycle (lights on at 2300 h and off at 1500 h), while another group was kept under a 8L/16D cycle (light on at 0700 h and off at 1500 h) for two weeks. At the end of the period, animals were subjected to different behavioral tests. Hamsters under the short day condition for two weeks were more susceptible to behavioral despair than hamsters kept under the long day condition. It was concluded that longer photoperiod has a protective effect on behavioral despair.

Molina-Hernandez and Tellez-Alcantra (2000) and Prendergast and Nelson (2005) have shown that prolonged treatment with a long photoperiod provides immunity for behavioral despair as measured by FSTs. Yilmaz et al. (2004) examined the effects of a single day of constant light on the reduction of immobility in the FST where animals were exposed to light in the dark phase of a 12L/12D daily lighting schedule. Results showed that a single day of 12-hour light exposure in the dark phase of the normal lighting schedule has a protective effect on behavioral despair. Animals in the 12-hour light treatment group displayed shorter immobility in the second day of the testing. Thus, there is no need for days of longer light periods to obtain ameliorative effect of light on behavioral despair.

Arvanitogiannis and Amir (1999) showed that the circadian clock can be entrained by short light pulses; accordingly the suprachiasmatic nucleus (SCN) can be modified by a short light pulse to prevent depression. Schulz et al. (submitted) examined this possibility and explored the dark phase of the L/D cycle with short light pulses, varying in the length and timing, by assessing behavioral despair. Results revealed that a 30-minute light pulse delivered 8¼ h after dark onset on a 12 h L/D cycle reduced immobility on the second day of the testing. This finding shows that the timing and the duration of the light pulse is important in preventing depression. Accordingly, a shorter light pulse can prevent depression, if it is given at the right time point.

An unpublished pilot study in our laboratory revealed that a 10-min, 100 watt light pulse at ZT21 (0400 h), 9 hours after the lights off in the vivarium, reduced immobility in behavioral despair on the second day of the testing. The same light pulse had no effect at ZT15 (2200 h). "ZT" means zeitgeber time, which means the timing of external entrainment cues of endogenous biological clock; generally the L/D cycle. ZT12 (1900h) is the time of dark onset and ZT0 (0700h) is the time of light onset in the vivarium. These results point out that protective effect of light stimulation on behavioral despair depends on the timing and duration and possibly the intensity of light exposure.

The mechanism activated by light in the modulation of depression in humans or in behavioral despair in rats is not understood fully. A reasonable explanation for the different effects of light stimulation at different times in the dark phases of the L/D cycle might be that these differentially affected the suprachiasmatic nucleus (SCN), the central endogenous biological clock (Yilmaz et al., 2004). Light exposure during the early parts of the subjective night causes a phase delay in the regulation of behavioral and biological rhythms, whereas light exposure at the end of the subjective night causes phase advances (Rosenberg, Zee, & Turek, 1991; van Esseveldt et al., 2000; Vitaterna et al., 2001). It is possible that both the timing and the duration of the light pulses might differentially affect phase shifts in the SCN

depending on the exact phase of the endogenous biological clock as described below (van Esseveldt, 2000).

The Suprachiasmatic Nucleus (SCN) and Circadian Rhythms

In general, all humans have regular, rhythmic lives where sleeping, awakening, eating etc are regulated by an approximately 24-hour period that is dictated by the earth's spin and its revolution around the sun (Turek, Dugovic, & Zee, 2001). In fact, internal physiological organizations of living organisms are also controlled according to these 24-hour periods (Vitaterna, Takahashi, & Turek, 2001). The SCN is the central circadian clock that is located in the anterior hypothalamus in mammals and is entrained mainly by the L/D cycle in the external environment (Saitoh, Nihonmatsu, & Kawamura, 1987; van Esseveldt, Lehman, & Boer, 2000). The SCN is known as the main pacemaker of mammalian circadian rhythms because it regulates nearly all 24-hour rhythms and strikes a balance between internal and external environment, sleep-wake cycle, and endocrine and physiological rhythms (Saper et al., 2000; Vitaterna et al., 2001). The daily L/D cycle is not the only mechanism that entrains the SCN; the biological clock can be modulated by feeding and activity cycles, temperature changes and social interactions (Vitaterna et al., 2001). In fact, the SCN organizes the biological and the physiological rhythms, and is in turn modulated by these rhythms.

Circadian rhythms that are observed in the absence of any external signal are said to "free run", indicating the self-sustained and endogenous nature of the biological clock. Therefore, a 24-hour period of free running circadian rhythms persists under constant laboratory conditions, such as in constant light or constant darkness (van Esseveldt et al., 2000). A widely used method to assess the effects of L/D cycle on the entrainment of the circadian system is light pulse exposure in animals kept under constant darkness. The exogenous signal in the entrainment of circadian rhythms is known as the Zeitgeber and the Phase Response Curve (PRC) is plotted by using the relationship between the zeitgeber and

phase shift in the circadian system (Vitaterna et al., 2001). If a light pulse is received during free running period as a zeitgeber, circadian rhythms are entrained according to this new cue (van Esseveldt et al., 2000). The endogenous clock responds to light pulses differently according to its position on the PRC at the time of light exposure. If the light pulse is presented during the subjective day, circadian time ZT0 (0700 h-lights on) to ZT12 (1900 h-lights off), there is almost no effect on the PRC. If the light pulse is exposed during the early parts of the subjective night, ZT12 (1900 h) to ZT14 (2100 h), there is a phase delay in the regulation of behavioral and biological rhythms that are completed at a later time. Light exposure at the end of the subjective night, ZT18 (0100 h) to ZT24 (0700 h-lights on), causes phase advanced in the regulation of behavioral and biological rhythms that begin earlier than enduring rhythms (Rosenberg, Zee, & Turek, 1991; van Esseveldt et al., 2000; Vitaterna et al., 2001).

Neuroanatomically, the SCN has three main input and three main output pathways to regulate body temperature, locomotor activity, sleep, estrous cycle, water and food intake, corticosterone production and melatonin release (van Esseveldt et al., 2000). The main input pathways of the SCN are the retinohypothalamic tract (RHT), the geniculohypothalamic tract (GHT) and the raphe nuclei projections of the midbrain (Rosenwasser, 2001; van Esseveldt et al., 2000). The SCN gets entrainment cues from its input pathways and after decoding these cues sends messages to different regions of the brain to regulate rhythmic behaviors and physiological functions of the body (Séron-Ferré et al., 2002). The three main output pathways of the SCN are dorsally directed fibers to the ventral and dorsal subparaventricular zone, to the dorsomedial and the ventromedial nuclei of the hypothalamus and to the paraventricular nucleus of the thalamus (Saper et al., 2005; van Esseveldt et al., 2000).

Disruptions in the regulation of circadian rhythms, whether caused by distortions in the input or output pathways of the SCN, have deleterious effects on mental and physical health of individuals (Vitaterna et al., 2001). The SCN also controls the circadian rhythms of

stress hormones, prolonged disturbance of which can cause affective disorders (van Esseveldt et al., 2000). In addition, circadian rhythm disturbances are usually found to underlie different neurological and affective disorders, such as epilepsy, dementia, movement disorders, headache and depression (Turek, Dugovic, & Zee, 2001).

The SCN, Stress and Depression

Stress and Depression

Depression is not a unitary disorder. Many symptoms are included in its description. These symptoms can be primarily psychological or physiological in nature (Cryan, Merkou, & Lucki, 2002; Jesberger & Richardson, 1985). Symptoms of major depression are considered below (American Psychiatric Association Diagnostic and Statistical Manual of Mental Disorders, 1994, p. 320):

- Depressed mood most of the day (in children and adolescents, irritability might signify a depressed mood)
- Markedly diminished interest or pleasure in all or most activities most of the day
- Large increase or decrease in appetite
- Insomnia or excessive sleeping
- Psychomotor agitation (evident by, for example, hand wringing) or slowness of movement
- Fatigue or loss of energy
- Indecisiveness or diminished ability to think or concentrate
- Feelings of worthlessness or excessive or inappropriate guilt
- Recurrent thoughts of death or suicide.

Stress is one of the most pronounced precipitating factors in the development of depression. There is a strong association between onset of depression and stressful life events just prior to the onset of depression (Leonard, 2001). Additionally, in animal models of depression, inescapable stress generally is used as a depression inducing process (Blackburn-

Munro & Blackburn-Munro, 2001; Bulduk & Canbeyli, 2004). Nevertheless, genetic vulnerability and environmental factors should be considered together in the induction of depression, because stress is not enough by itself to cause depression, as it is seen in some individuals (Nestler et al., 2002).

The brain automatically reacts to stressful stimuli by activating the stress axis, known as the hypothalamic-pituitary-adrenal (HPA) axis. Thus, the brain activates fight or flight responses (Blackburn-Munro & Blackburn-Munro, 2001). Many different stressors activate the HPA axis by activating the limbic system which projects to the Paraventricular Nucleus (PVN). The PVN produces corticosterone releasing hormone (CRH), which in turn activates the anterior pituitary where it causes adrenocorticotrophic hormone (ACTH) secretion. ACTH in turn stimulates the adrenal cortex to synthesize glucocorticoids, the main hormone in the adrenal cortex. Activation of the HPA axis and glucocorticoid synthesis leads to adaptation to the stressful environment behaviorally and physiologically. In addition, glucocorticoid synthesis activates a negative feedback system based on the hippocampal inhibition for further CRH and ACTH synthesis. By this method, the stress axis begins to prepare the organism for other incoming stressors and negative effects of excessive glucocorticoids on different brain structures are prevented (Blackburn-Munro & Blackburn-Munro, 2001; Nestler et al., 2002; van Esseveldt et al., 2000). However, functioning of the HPA axis is disrupted in some cases, especially in prolonged and inescapable stress. Under these circumstances, functioning of the HPA axis and the negative feedback system become maladaptive such that they can no longer properly regulate the circulation of stress hormones in the brain and the body (Blackburn-Munro & Blackburn-Munro, 2001). Abnormally higher activation of the HPA axis and maladaptive functioning of the related negative feedback system is also seen in many depressed patients (Nestler et al., 2002).

The SCN can be implicated in some aspects of depression, since it controls the circadian rhythms of stress hormones both in stressful and normal life conditions. In other words, the SCN regulates the functioning of the HPA axis; as a result the SCN can be related to affective disorders, especially those based on stress and the HPA axis (Bernstein et al., 2005; Buijs, Wortel, van Heerikhuize, & Kalsbeek, 1997; Cascio, Shinsako, & Dallman, 1987; Sage, Maurel, & Bosler, 2001).

The SCN, Circadian Rhythms and Depression

Many neurological and affective disorders are related to abnormal circadian rhythms; in particular, abnormally phase advanced rhythms are found in affective disorders (Hallonquist, Goldberg, & Brandes, 1986; Turek et al., 2001; Wehr & Wirz-Justice, 1982). Depressed patients who display abnormal circadian rhythms are treated with sleep deprivation or with phase shifting light pulses that entrain the endogenous circadian clock and show improvements in their moods thus supporting the relationship between depression and the SCN (Solberg, Horton, & Turek, 1999). Sleep deprivation in hamsters can rapidly reset the circadian clock and alter gene expression within the circadian system (Antle & Mistlberger, 2000). It is well known that some depressed patients show an improvement in depressive mood after a night of total sleep deprivation (Kuhs & Tölle, 1991). In addition, phototherapy is generally effective in seasonal affective disorders and major depression. Moreover, there is a reciprocal association between the SCN, circadian rhythms and depression in that depressed patients show abnormal circadian rhythms (Yamada et al., 1995), while abnormal rhythms can lead to depression (Wehr et al., 2001).

Stress, Depression and Circadian Rhythms in Juvenile Rats

All of the studies mentioned above generally were conducted with adult animals or humans. Yet age-dependent differences in the sensitivity of depression in both humans and

animals are found (Bourin et al., 1998; Sapolsky, 2004; Slotkin, Seidler, & Ritchie, 2000; Yates et al., 1991). A noticeable increase in the prevalence of depression during adolescence is well-known (Kaltiala-Heino, Marttunen, Rantanen, & Rimpela, 2003; Laitinen-Krispijn, der Ende, & Verhulst, 1999). Radloff (1991) found that depression dramatically increases between the ages of 13 and 15 years and reaches a peak at the age of 17 years. After that point, a subsequent decline is seen in the depression levels of individuals. In a similar study conducted on rats, it was found that chronic social stress and restraint stress have greater effects on adolescents than adults, and that the food intake and subsequently body weights of adolescents were remarkably decreased. Additionally, the number of entries into and time spent in the open arms of a plus maze were noticeably suppressed in adolescents (Stone & Quartermain, 1998).

Overall, there are remarkable differences in the occurrence, treatment and neurobiological correlates of depression among children, adolescents and adults (Kaufman, 1999; Kaufman et al., 2001; Spear, 2000). Adolescence is a time of brain growth, remodeling of brain and reorganization of many neurotransmitter systems. Additionally adolescence is a period of extensive pruning of synapses as well as formation of additional connections (Spear, 2000). The most prominent changes in the adolescents' brain are the alternations in the prefrontal cortex, limbic brain areas and their dopamine input that is highly sensitive to stressors (Kaufman et al., 2001; Michael & Crowley, 2002; Spear, 2000). Early stress may have different effects on adolescents due to different patterns of brain organizations and less developed brain regions and systems in young animals. The differences between adults and adolescents in terms of depression can be explained in this way. Correspondingly, different antidepressants have different effects according to age of the subjects. It is shown that the serotonergic system is susceptible to age related changes, whereas dopaminergic and noradrenergic systems are less vulnerable (Bourin et al., 1998). In old age, a reduced effectiveness of serotonin based antidepressants was shown, whereas in juveniles, selective

serotonin reuptake inhibitors (SSRIs) have an essential role in the treatment of depression (Kaufman et al., 2001; Michael & Crowley, 2002; Petersen et al., 1993). These results are clarified by considering early maturation of serotonin activity and later and slower maturation of dopamine and norepinephrine activity (De Jong et al., 2006; Wongwitdecha, Kasemsook, & Plasen, 2006).

In early development, infants exhibit a reduced response to stress during the stress hyporesponsive period, which is characterized by a reduced capacity to secrete ACTH and corticosterone in response to stressful stimuli (Walker & Vrana, 1993). Moreover, although infants are capable of responding to certain stressful stimuli after the stress hyporesponsive period, the HPA axis does not develop evenly (Hodes & Shors, 2005). The circadian rhythms of corticosterone (cortisol) and negative feedback regulation are not developed fully until early adulthood (Levine, 2001). The basal (non-stress) glucocorticoid level is also higher in juveniles than in adults (Hodes & Shors, 2005; Kaufman, 1999; Kaufman et al., 2001; Levine, 2001; Petersen et al., 1993; Spear, 2000). In addition, when both juvenile and adult rats are subjected to stress, returning to basal levels of glucocorticoids is slower in juveniles than in adults (Hodes & Shors, 2005). All in all, juveniles show more vulnerability to stress, and the induction of depression is easier in juveniles. Therefore, differences in induction, progress and the treatment of depression among different age groups are to be expected. Accordingly, in the present study, it is hypothesized that depressed juvenile rats would exhibit aggravated behavioral despair in the form of longer duration of immobility in the second swim test compared with adult rats.

Adolescence is a time of great change; however, there is a paucity of research on circadian rhythms during adolescence. Input and output pathways of the SCN are established in late gestation in humans and during the early postnatal period in the rats (Kennaway, 2002). The SCN is nearly rhythmic around 9-12 weeks of age in humans, while it is rhythmic at birth in rodents (Kennaway, 2002). In adolescents, daily rhythms are almost regular. However, with

the onset of puberty, adolescents begin to experience a phase delay in the onset of their sleep time (Dagan, 2002; Dawson, 2005). The sleep patterns of individuals are regulated by the synthesis of melatonin by the pineal gland. The daily rhythms of this hormone are controlled by the SCN (Brzezinski et al., 2005). Toward the morning, the synthesis of melatonin declines while the synthesis of cortisol is increased preparing the individual for waking (Brzezinski et al., 2005; van Esseveldt et al., 2000). During adolescence, patterns of melatonin secretion show differences that lead to sleep phase delay, causing individuals to sleep less (Dawson, 2005). Dahl et al. (1992) showed that some depressed adolescents display impaired sleep onset mechanisms. Another study showed that depressed mood and sleep problems are more frequently encountered in adolescents when compared with their mothers (Rutter et al., 1976; cited in Spear, 2000).

Johnson and Breslau (2001) concluded that sleep problems in adolescents might be the most pronounced indicator and causal pathway of psychiatric problems and substance use. In adolescence, the circadian typology of individuals changes systematically (Natale et al., 2005); even diurnal variations in suicide by age are observed (Preti & Miotto, 2001). The different age distribution of mental disorders might be explained by considering diurnal variations in biological rhythms with age. Therefore, further consideration should be given to the biological rhythms and depression in adolescents.

Stress is one of the most pronounced precipitating factors for the development of depression and stress hormones are regulated by the SCN. Additionally, bright light has a preventive effect on depression in humans and on behavioral despair in animals and this effect can show differences based on age. Thus, the preventive effects of light should be studied by considering various ages. Bright light generally is used in the treatment of depression in humans and the prevention of behavioral despair in animals. To see which mechanisms are involved in the effectiveness of light, both the preventive and ameliorative effects of light should be studied in animal models of depression at various ages to reach a reliable

conclusion. The preventive and ameliorative effects of light exposure can be assessed by applying behavioral despair. If light exposure before FSTs reduces immobility levels in FST2, it can be said that light exposure has preventive effects on behavioral despair. On the other hand, if light exposure after the induction of depression lessens immobility levels in FST2, it has an ameliorative effect on behavioral despair. The present study therefore investigates the potentially ameliorative and preventive effects of a short light pulse exposure late at night (0400 h, ZT21) in both juvenile and adult rats. Animals were maintained on a 12L/12D cycle (lights on at 0700 h) except for the days of light treatment in an insulated chamber. It was expected that a 10-minute light pulse at ZT21 can both prevent and alleviate behavioral despair as measured by forced swimming tests. Additionally, the preventive and ameliorative effects of short light pulse were expected to be less effective in the prevention and treatment of behavioral despair in juvenile rats.

CHAPTER TWO

METHOD

Subjects

Approximately, 1.5 and 4.5 months old, experimentally naïve, male Wistar rats were obtained from the Psychobiology Laboratory of Boğaziçi University. Forty adult rats were assigned randomly to one of five groups (n = 8 each), and 24 juvenile rats were assigned randomly to one of three groups (n = 8 each). Subjects were maintained on a 12L/12D cycle (lights on at 0700 h) in a temperature controlled environment (22 ± 2 ^{o}C) except for the days of light (or control) treatments in an insulated chamber. Food and water were available ad libitum.

Apparatus

Light treatment was administered in a ventilated chamber with sound and light insulation. Light was provided by a 100W tungsten lamp, approximately 50 cm above the cage housing the subjects.

Design

Male Wistar rats were used in the present study. There were two age groups 1.5 month old juveniles and 4.5 month old adults. For adult rats, there were three experimental and two control groups. For juvenile rats, there was one experimental and two control groups.

In adult rats, the first experimental group (ZT21-B group) received light pulse before the first forced swimming test; the second one (B-ZT21 group) received light pulse after the first forced swimming test and the last one (ZT21-ZT21 group) received light pulse in both before and after the first forced swimming test. The chamber control group (C-B group) was placed in the experimental chamber on both days, but did not receive any light pulse. The animals of vivarium control group (C-V group) was taken from the vivarium and put into their home cages after forced swimming tests.

For juvenile Wistar rats, there were three groups, one experimental and two control groups. The procedure for the two control groups was the same as that for the adult rats (C-B and C-V groups). The procedure for the experimental group was the same as that for the first experimental group of adult rats (ZT21-B group) that received a light pulse before the first forced swimming test. Among the experimental groups of adult rats, although not significant, only ZT21-B group showed a distinct pattern; therefore, for the juvenile rats, only ZT21-B group was used as experimental group.

Experimental Procedure

For light treatment, the subjects were taken from their home cages ten 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 individually and two cages, thus two animals, were run on any 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, 100W light pulse exposure at ZT21 (0400 h-3 hours before lights on) for three experimental groups. The first control group (the chamber control group) was also kept in the chamber, but did not receive light pulses. The second control group (the vivarium control group) was taken directly from the vivarium for tests. For all four groups, temporally housed in the chamber, light was turned on at 0700 h (ZT0) in the insulated chamber to start the light phase of the L/D cycle. The animals were kept in the insulated chamber until 1500 h (ZT8) when the first of the forced swimming test was conducted as specified below. The animals in all groups, except for the vivarium control group (C-V group), were returned to the insulated chamber after the first test and kept there until the second forced swimming test conducted 24 h later as specified below.

Following is a graphical representation of the experimental procedure:

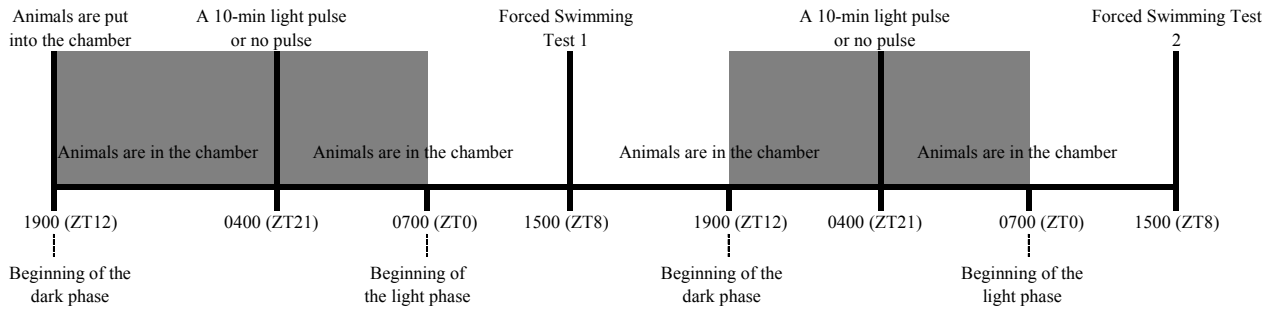

Fig. 1. The experimental procedure.

Forced Swimming Test

The 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 filled each time with fresh water (25º) to a height of 20 cm. In the first swim test, the animals were individually forced to swim for 15 minutes followed 24 h later by a five minutes second test. After each test, the animal was towel dried and placed under a lamp for 30-minutes for drying. The swim tests were recorded on videotape. The total duration of immobility is defined as staying motionless or floating by keeping the head above the water surface without leaning against the wall of the cylinder.

Comparison of the duration of immobility in the first and second swim tests revealed behavioral despair, depression of the animals. In addition to immobility, swimming duration, number of head shakes, jumps and dives were coded. Jumps were counted when the animal lifted itself up and attempted an escape from the cylinder with at least the upper half of its body out of water. Dives were recorded when the rat's body was totally immersed in water. Last, the frequency of total struggling was obtained by summing up the numbers of head shakes, diving and jumping. Due to the insufficient number of diving and jumping, they both were considered under the title of total struggling in all statistical analyses. Head shakes also were considered separately in statistical analyses since recent studies show that the serotonergic system that is important in depression is involved in swimming and head shaking behaviors of rats in FSTs (Lino-de-Oliveira, De Lima, & Carobrez, 2005; Page et al., 1999) and also the serotonergic regulation and treatment of depression with SSRIs show differences in adults and juveniles (De Jong et al., 2006; Wongwitdecha et al., 2006).

CHAPTER THREE

RESULTS

Adult Rats

The mean durations of immobility and swimming (mean ± SEM) of five groups of adult rats in two forced swim tests (FST1 and FST2) are shown in Fig. 2.

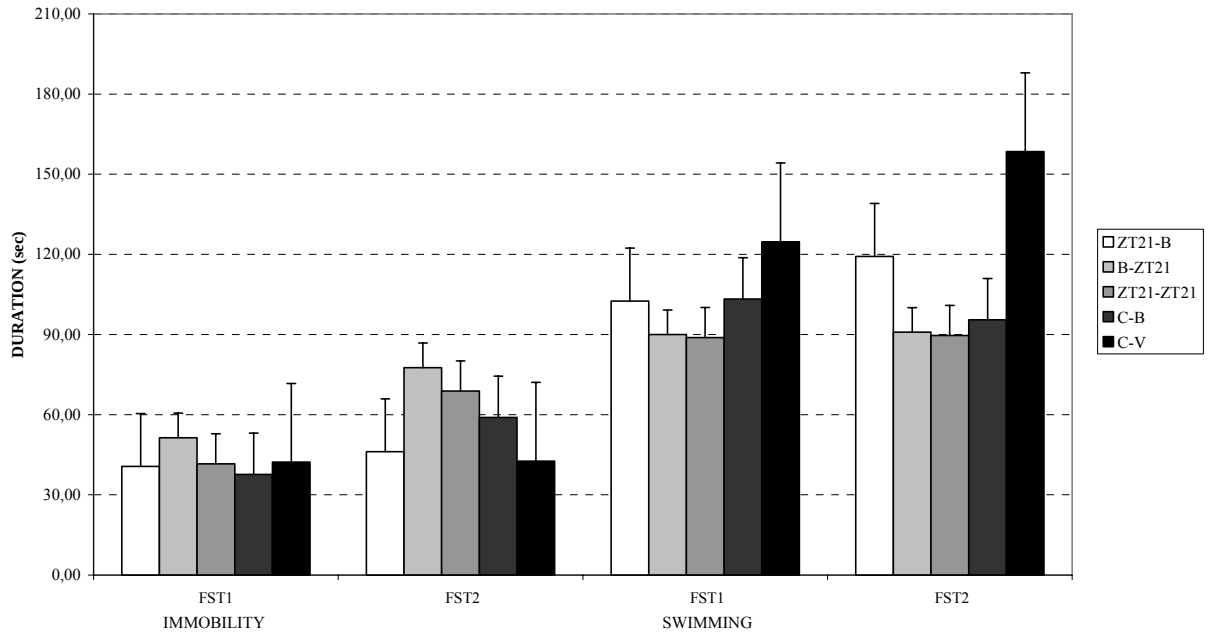

Fig. 2. Durations of immobility and swimming (mean ± SEM) in two forced swim tests (FST1 and FST2) conducted 24 h apart for the groups of adult rats. For three experimental groups, animals were exposed to a 10-minute, 100W light pulse at ZT21 (0400 h-3 hours before lights on) either before the FST1 (ZT21-B), or FST2 (B-ZT21) or before both (ZT21-ZT21). The first control group (C-B) was also kept in the light exposure chamber, but did not receive light pulses. The second control group (C-V) was taken directly from the vivarium for tests.

An analysis of variance (ANOVA) with repeated measures indicated that the second swim test was significantly different from the first with respect to durations of immobility [F (1, 35) = 4.81, $p < 0.05$] (Fig. 2) and head shakes [F (1, 35) = 6.14, $p < 0.01$] (Fig. 3). However, there was no significant factor X group interaction in any of the behaviors

measured. Additionally, an analysis of variance (ANOVA) with repeated measures comparing durations of swimming showed no significant results that adult rats did not decrease their duration of swimming in FST2 compared to FST1.

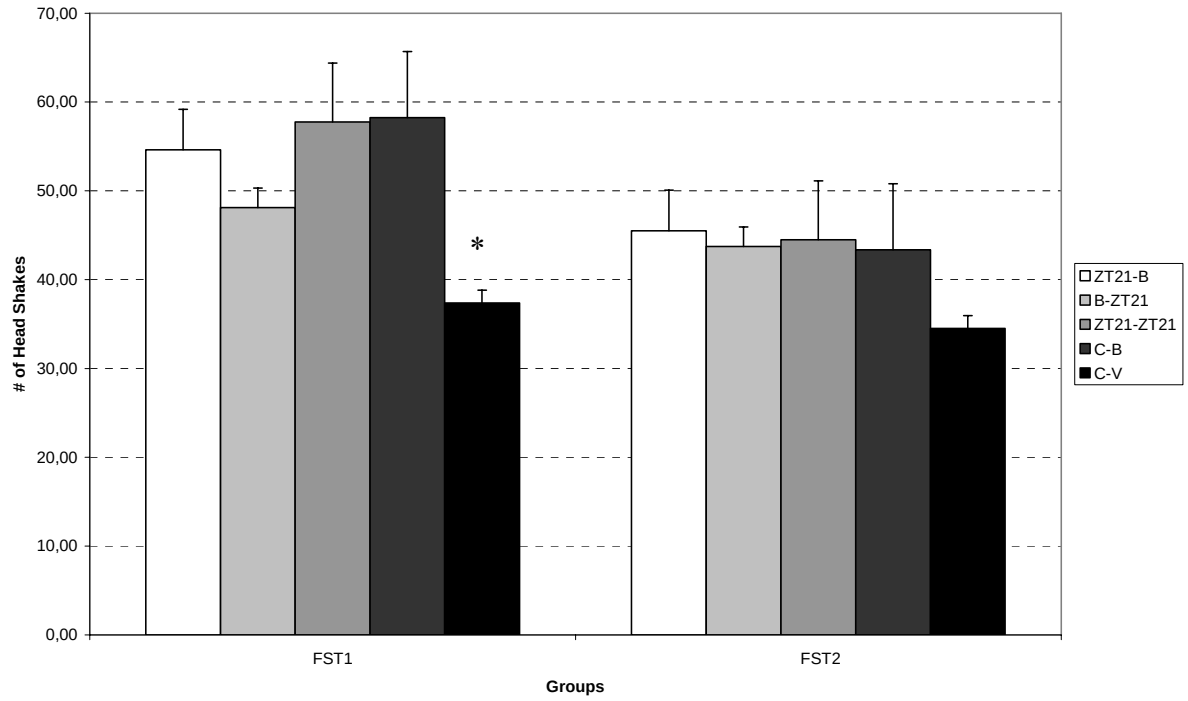

Fig. 3. Number of the head shakes in two forced swim tests (FST1 and FST2) conducted 24 h apart for the groups of adult rats. For three experimental groups, animals were exposed to a 10-minute, 100W light pulse at ZT21 (0400 h-3 hours before lights on) either before the FST1 (ZT21-B), or FST2 (B-ZT21) or before both (ZT21-ZT21). The first control group (C-B) was also kept in the light exposure chamber, but did not receive light pulses. The second control group (C-V) was taken directly from the vivarium for tests. Asterisk indicates significant difference ($p < 0.05$) between C-V group and others groups (ZT21-B, B-ZT21, ZT21-ZT21, C-B) for the indicated swim test.

Table 1 shows the frequency of diving, jumping, head shaking and total struggling of the five groups.

Table 1
Frequencies (mean ± SEM) of diving, jumping, head shakes and total struggling in two forced swim tests (FSTs) conducted 24 h apart for two control (n=8, for each group) and three experimental (n=8, for each group) groups of adult rats

	Diving		Jumping		Head Shakes		Total Struggling	
	FST1	FST2	FST1	FST2	FST1	FST2	FST1	FST2
B-ZT21	1.75 ± 1.91	1.88 ± 3.23	0.00 ± 0.00	0.13 ± 0.35	48.13 ± 16.48	43.75 ± 10.48	49.88 ± 15.79	45.75 ± 11.50
ZT21-B	2.38 ± 3.42	0.63 ± 1.41	0.00 ± 0.00	0.00 ± 0.00	54.63 ± 15.48	45.50 ± 18.71	57.00 ± 13.48	46.13 ± 19.06
ZT21-ZT21	2.63 ± 2.88	0.63 ± 1.41	0.00 ± 0.00	0.00 ± 0.00	57.75 ± 12.50	44.50 ± 20.18	60.68 ± 13.67	45.13 ± 20.63
C-B	3.25 ± 4.06	1.75 ± 3.45	0.13 ± 0.35	0.63 ± 1.41	58.25 ± 10.83	43.38 ± 21.10	61.63 ± 8.78	45.75 ± 21.27
C-V	1.25 ± 2.38	0.50 ± 1.07	0.88 ± 1.13 *	0.13 ± 0.35	37.38 ± 14.71 *	34.50 ± 15.68	39.50 ± 14.51 *	35.13 ± 15.69

* Indicates significant difference ($p < 0.05$) between C-V group and the other groups in the indicated swim test.

In the first swim test, there was a significant difference in the number of head shakes [F (4, 39) = 3.07, $p < 0.05$] (Fig. 3) and total struggling [F (4, 39) = 3.67, $p < 0.05$] (Fig. 4). The post-hoc test (LSD) showed that animals in the second control group (C-V) displayed fewer head shakes and fewer total struggling than the other groups ($p < 0.05$). In the second swim test, there was no significant difference among the groups in these measures.

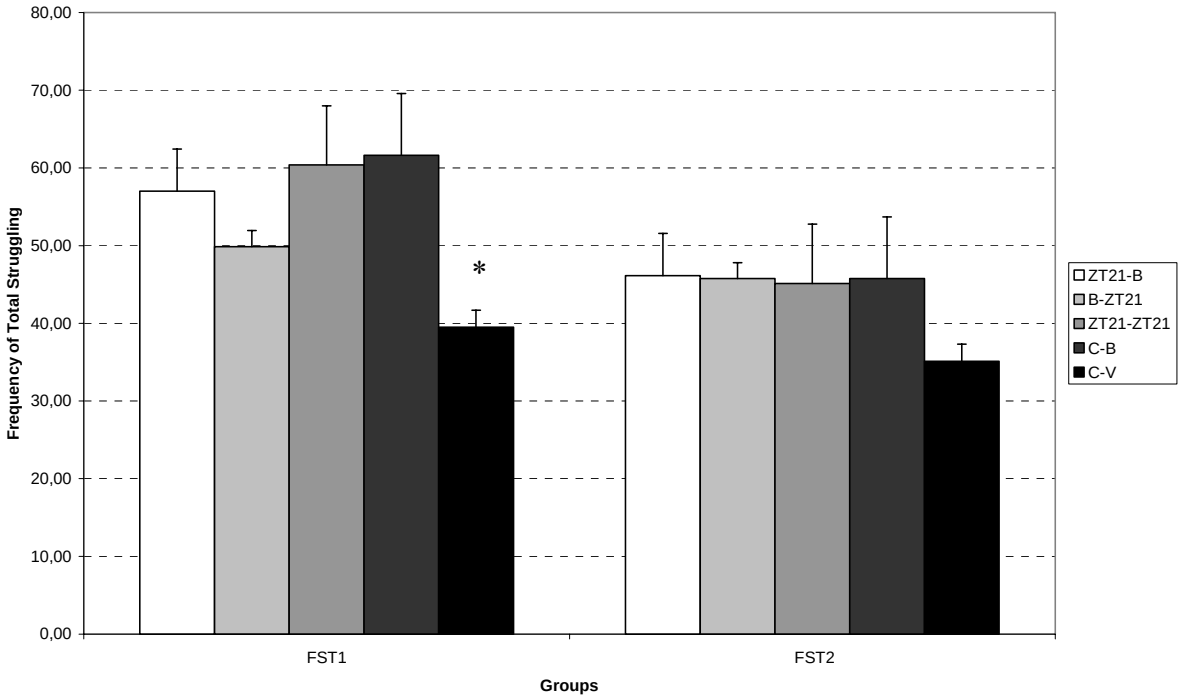

Fig. 4. Number of the total struggling in two forced swim tests (FST1 and FST2) conducted 24 h apart for the groups of adult rats. For three experimental groups, animals were exposed to a 10-minute, 100W light pulse at ZT21 (0400 h-3 hours before lights on) either before the FST1 (ZT21-B), or FST2 (B-ZT21) or before both (ZT21-ZT21). The first control group (C-B) was also kept in the light exposure chamber, but did not receive light pulses. The second control group (C-V) was taken directly from the vivarium for tests. Asterisk indicates significant difference ($p < 0.05$) between C-V group and other groups (ZT21-B, B-ZT21, ZT21-ZT21, C-B) for the indicated swim test.

Juvenile Rats

The mean durations of immobility and swimming (mean $\pm$ SEM) of three groups of juvenile rats in two forced swim tests (FST1 and FST2) are shown in Fig. 5.

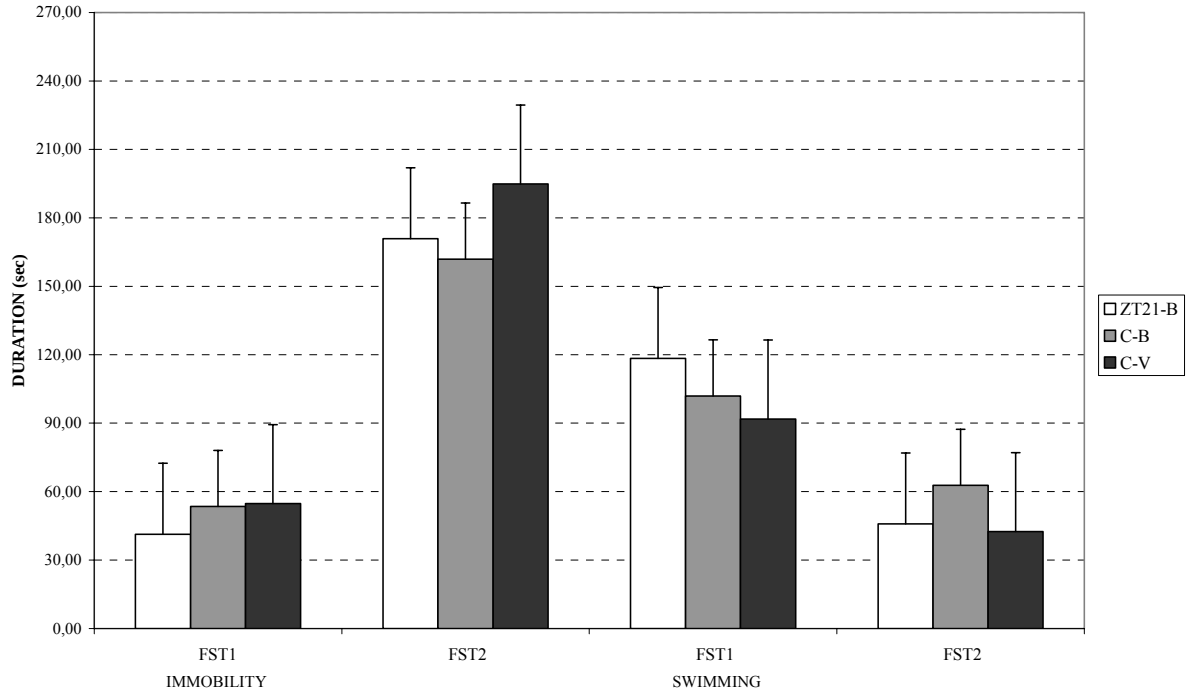

Fig. 5. Durations of immobility and swimming (mean ± SEM) in two forced swim tests (FST1 and FST2) conducted 24 h apart for the groups of juvenile rats. For one experimental group, animals were exposed to a 10-minute, 100W light pulse at ZT21 (0400 h-3 hours before lights on) before the FST1 (ZT21-B). The first control group (C-B) was also kept in the light exposure chamber, but did not receive light pulses. The second control group (C-V) was taken directly from the vivarium for tests.

An analysis of variance (ANOVA) with repeated measure indicated that the second swim test was significantly different from the first with respect to duration of immobility [F (1, 21) = 54.71, $p < 0.001$] (Fig. 5), swimming [F (1, 21) = 27.82, $p < 0.001$] (Fig. 5) and head shakes [F (1, 21) = 38.31, $p < 0.001$] (Fig 6). There was a significant group difference only in the number of head shakes; the second control group (C-V) displayed more head shakes than other groups ($p < 0.05$) in the first swim test.

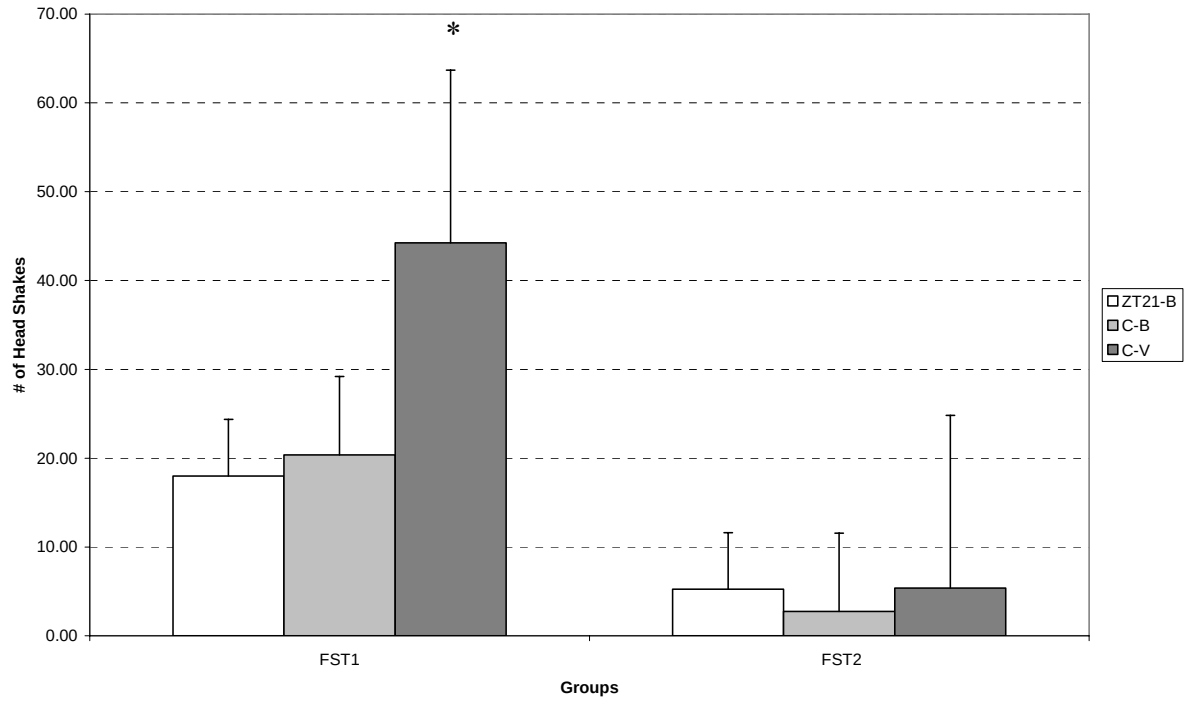

Fig. 6. Number of the head shakes in two forced swim tests (FST1 and FST2) conducted 24 h apart for the groups of juvenile rats. For one experimental group, animals were exposed to a 10-minute, 100W light pulse at ZT21 (0400 h-3 hours before lights on) before the FST1 (ZT21-B). The first control group (C-B) was also kept in the light exposure chamber, but did not receive light pulses. The second control group (C-V) was taken directly from the vivarium for tests. Asterisk indicates significant difference ($p < 0.01$) between C-V group and others groups (B-ZT21 and C-B) for the indicated swim test.

Table 2 shows the frequency of diving, jumping, head shaking and total struggling scores of the three groups.

Table 2

Frequencies (mean ± SEM) of diving, jumping, head shakes and total struggling in two forced swim tests (FSTs) conducted 24 h apart for two control (n=8, for each group) and one experimental (n=8) groups of juvenile rats

	Diving		Jumping		Head Shakes		Total Struggling	
	FST1	FST2	FST1	FST2	FST1	FST2	FST1	FST2
ZT21-B	2.75 ± 4.13	2.13 ± 3.14	0.00 ± 0.00	0.00 ± 0.00	18.00 ± 16.00	5.25 ± 9.45	20.75 ± 17.76	7.38 ± 12.45
C-B	2.38 ± 3.16	0.38 ± 1.06	0.00 ± 0.00	0.00 ± 0.00	20.38 ± 15.91	2.75 ± 3.88	22.75 ± 18.11	3.13 ± 4.36
C-V	3.00 ± 3.38	0.38 ± 1.06	0.00 ± 0.00	0.00 ± 0.00	44.25 ± 16.83 *	5.38 ± 5.83	47.25 ± 17.56 *	5.75 ± 6.52

* Indicates significant difference ($p < 0.05$) between C-V group and the other groups in the indicated swim test.

In the first swim test, there was a significant difference in the number of head shakes [F (2, 23) = 6.38, $p < 0.01$] (Fig. 6) and total struggling for treatment [F (2, 23) = 5.49, $p < 0.05$] (Fig. 7). The post-hoc test (LSD) showed that animals in the second control group (C-V) displayed more head shakes and total struggling than the other groups ($p < 0.05$). In the second swim test, there was no significant difference among the groups in the number of head shakes and total struggling.

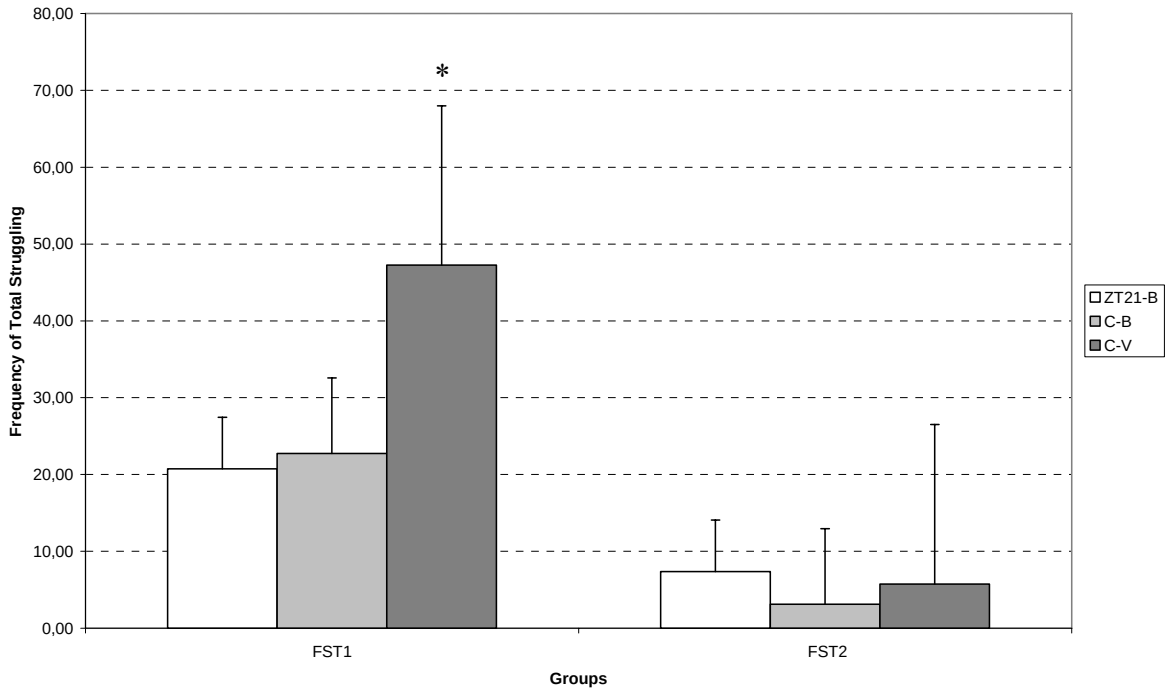

Fig. 7. Number of the total struggling in two forced swim tests (FST1 and FST2) conducted 24 h apart for the groups of adult rats. For one experimental group, animals were exposed to a 10-minute, 100W light pulse at ZT21 (0400 h-3 hours before lights on) before the FST1 (ZT21-B). The first control group (C-B) was also kept in the light exposure chamber, but did not receive light pulses. The second control group (C-V) was taken directly from the vivarium for tests. Asterisk indicates significant difference ($p < 0.05$) between C-V group and others groups (B-ZT21 and C-B) for the indicated swim test.

Adult Rats vs. Juvenile Rats

The mean durations of immobility and swimming (mean $\pm$ SEM) of five groups of adult and three groups of juvenile rats in two forced swim tests (FST1 and FST2) are shown in Fig. 2 and Fig. 5, respectively. The mean durations of immobility (mean $\pm$ SEM) of three groups of juvenile and adult rats in the second forced swim test (FST2) are shown in Fig. 8. A 2X2 ANOVA comparing juvenile and adult rats (age X group) for immobility durations of the ZT21-B, C-B and C-V groups indicated a significant effect for age [F (1, 42) = 38.21, $p <$ 0.001], but no significant interaction revealed that juvenile rats displayed longer duration of immobility in the second swim test.

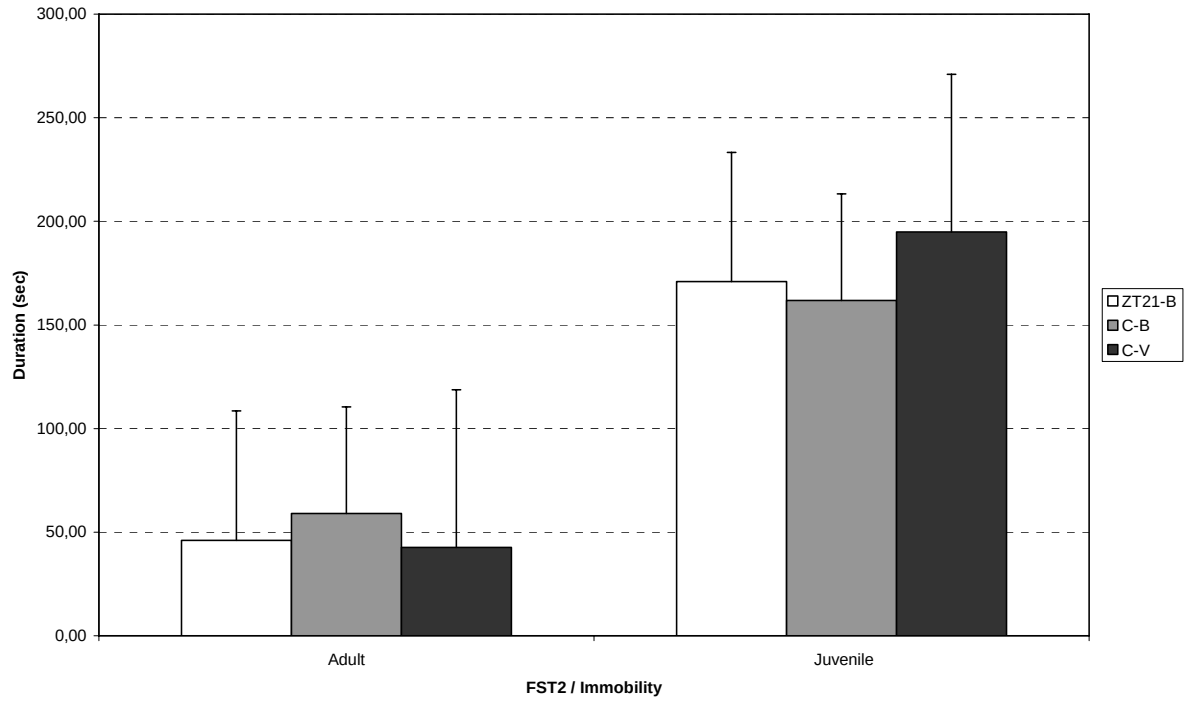

Fig. 8. Durations of immobility (mean ± SEM) in the second forced swim test (FST2) for the groups of adult and juvenile rats. For one experimental group, animals were exposed to a 10-minute, 100W light pulse at ZT21 (0400 h-3 hours before lights on) before the FST1 (ZT21-B). The first control group (C-B) was also kept in the light exposure chamber, but did not receive light pulses. The second control group (C-V) was taken directly from the vivarium for tests.

The mean durations of swimming (mean ± SEM) of three groups of juvenile and adult rats in the second forced swim test (FST2) are shown in Fig. 9. Additionally, A 2X2 ANOVA comparing juvenile and adult rats (age X groups) for swimming durations of the ZT21-B, C-B and C-V groups indicated a significant effect for age [F (1, 42) = 19.02, $p < 0.000$], but no significant interaction revealed that juvenile rats displayed shorter durations of swimming in the second swim test.

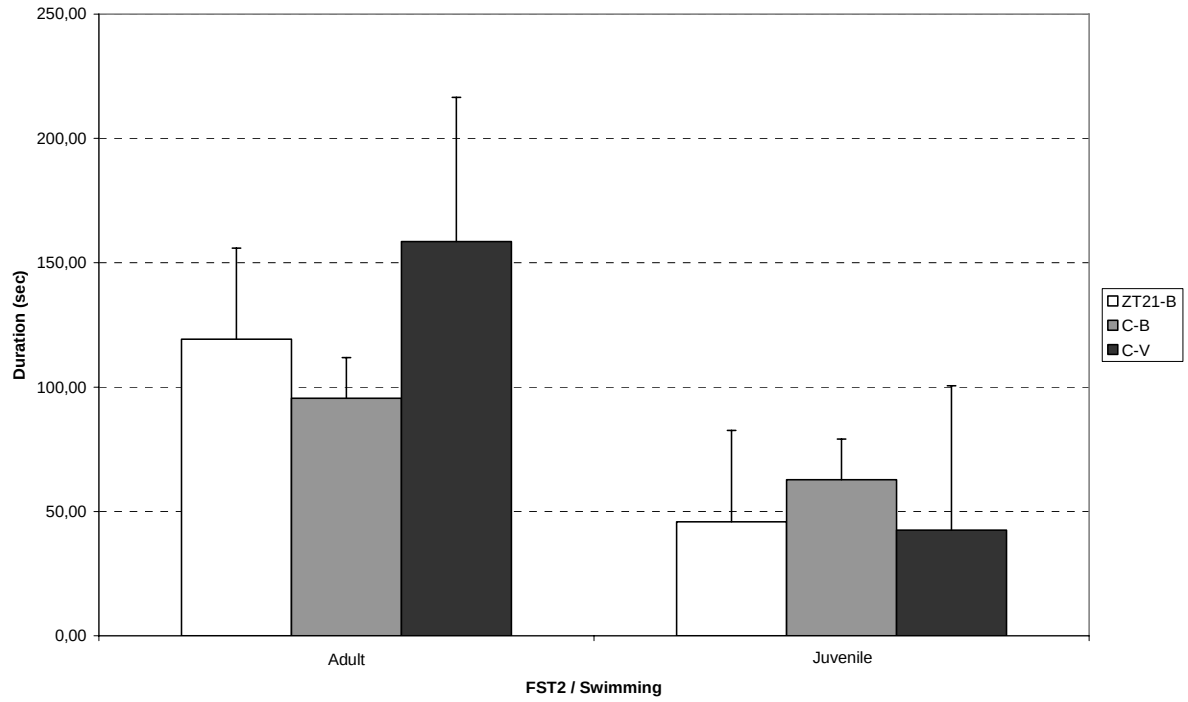

Fig. 9. Durations of swimming (mean ± SEM) in the second forced swim test (FST2) for the groups of adult and juvenile rats. For one experimental group, animals were exposed to a 10-minute, 100W light pulse at ZT21 (0400 h-3 hours before lights on) before the FST1 (ZT21-B). The first control group (C-B) was also kept in the light exposure chamber, but did not receive light pulses. The second control group (C-V) was taken directly from the vivarium for tests.

Additionally, juvenile rats displayed less head shakes and total struggling than adult rats in both FST1 [F (1, 42) = 26.79, $p < 0.001$] (Fig. 10), [F (1, 42) = 25.56, $p < 0.001$] (Fig. 11) and FST2 [F (1, 42) = 82.07, $p < 0.001$] (Fig. 10), [F (1, 42) = 77.02, $p < 0.001$] (Fig. 11), but there was no significant age X group interaction.

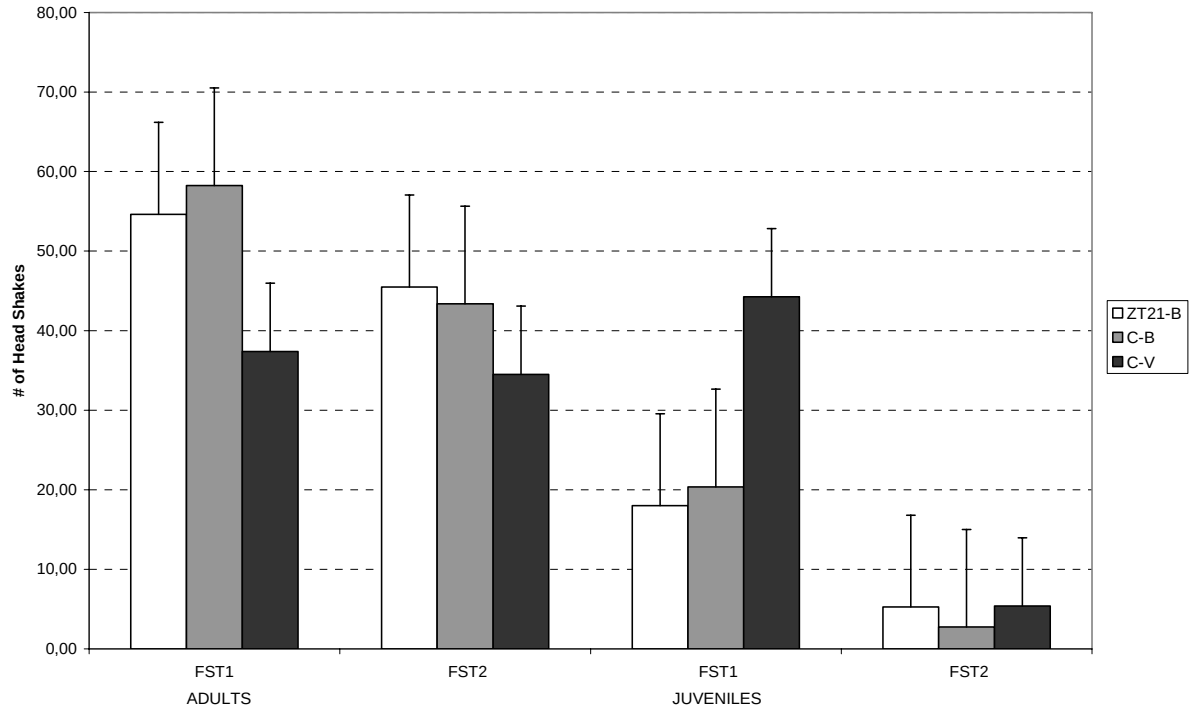

Fig. 10. Number of the head shakes in two forced swim tests (FST1 and FST2) conducted 24 h apart for the groups of adult and juvenile rats. For one experimental group, animals were exposed to a 10-minute, 100W light pulse at ZT21 (0400 h-3 hours before lights on) before the FST1 (ZT21-B). The first control group (C-B) was also kept in the light exposure chamber, but did not receive light pulses. The second control group (C-V) was taken directly from the vivarium for tests.

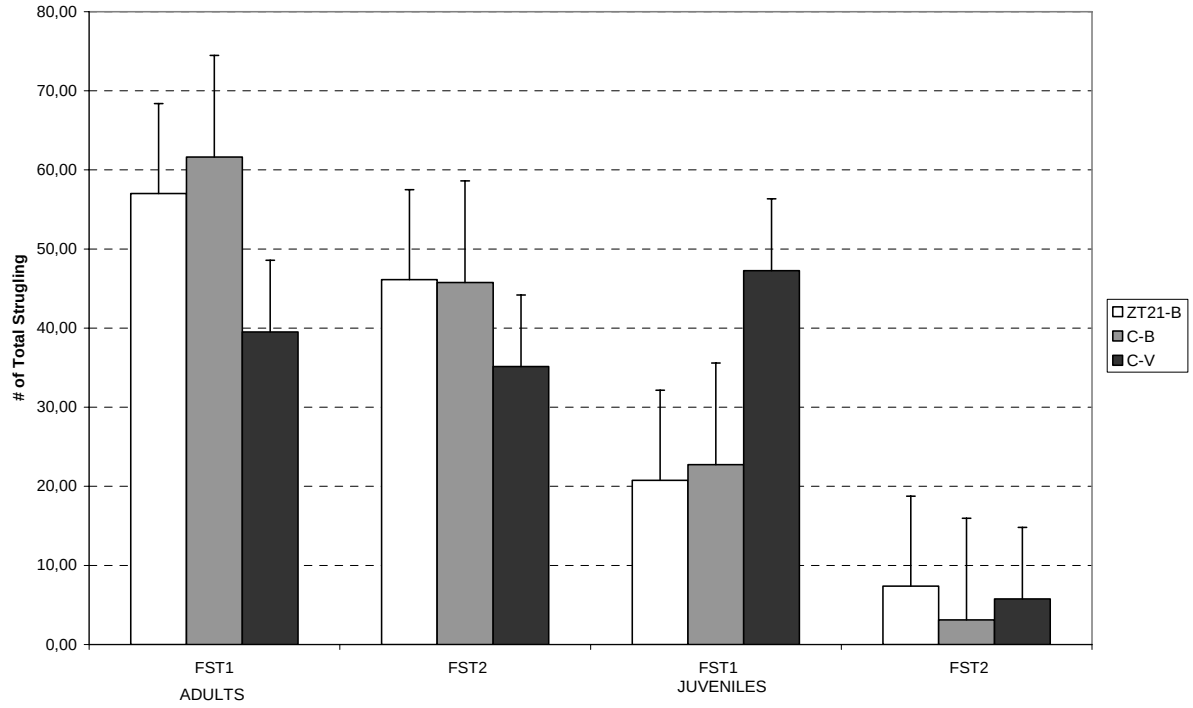

Fig. 11. Number of the total struggling in two forced swim tests (FST1 and FST2) conducted 24 h apart for the groups of adult and juvenile rats. For one experimental group, animals were exposed to a 10-minute, 100W light pulse at ZT21 (0400 h-3 hours before lights on) before the FST1 (ZT21-B). The first control group (C-B) was also kept in the light exposure chamber, but did not receive light pulses. The second control group (C-V) was taken directly from the vivarium for tests.

CHAPTER FOUR

DISCUSSION

The first aim of the present study was to assess the preventive and ameliorative effects of a short light pulse delivered late at night (0400 h, ZT21) on behavioral despair in juvenile and adult Wistar rats. Our findings indicated that although light pulse exposure leads to a shorter duration of immobility and longer duration of swimming when employed before first forced swimming test (ZT21-B group), it yields no significant results. Statistical analysis shows that a short light pulse exposure late at night has neither preventive nor ameliorative effect on behavioral despair measured by forced swimming test. The second aim of the present study was to examine the differences between juvenile and adult rats in terms of behavioral despair. The results showed that juvenile rats showed longer duration of immobility, shorter duration of swimming, fewer head shakes and instances of total struggling when compared with adults. There was, however, no difference between the two age groups in terms of experimental and control groups. These findings suggest that juvenile rats are more susceptible to behavioral despair than adult rats.

Lack of a significant effect of short light pulse exposure on behavioral despair might be a result of the shorter duration of light exposure as opposed to previous studies (Molina-Hernandez & Tellez-Alcantara, 2000; Prendergast & Nelson, 2005; Schulz et al., submitted; Yilmaz et al., 2004). It was shown that short light flashes can induce behavioral and cellular changes in the endogenous clock that are similar to those caused by longer light exposures (Arvanitogiannis & Amir, 1999). The unexpected results of the present study might be a consequence of the functioning of different systems as a result of the shorter light pulse exposure. Buijs et al. (1999) showed that the SCN has multi-synaptic pathways with the adrenal cortex. In the first pathway, the SCN transmits signal to the surrounding area of the PVN that produces corticosterone releasing hormone (CRH), which in turn activates the

anterior pituitary where it causes adrenocorticotrophic hormone (ACTH) secretion. Subsequently, ACTH stimulates the adrenal cortex to synthesize glucocorticoids. Thus, activation of the HPA axis and glucocorticoid synthesis can be influenced by the SCN and behavioral and physiological adaptation to the stressful environment can be maintained. In the second pathway, the SCN transmits signals to the surrounding area of the PVN that projects to the intermediolateral column (IML) of the spinal cord that influences the adrenal cortex directly to synthesize glucocorticoids (Buijs et al., 1999). This second pathway is thought to be responsible for the light induced inhibition of melatonin and corticosterone (Buijs et al., 1997; Buijs et al., 1999). The short light pulse exposure may cause a different interaction between these pathways or a completely different pathway might control the effects of short light pulses different than the long ones. In addition, it is known that the second pathway is activated by novelty stress (Buijs et al., 1997).

Explicitly, the novelty stress might be so effective that the short light pulse can not modify stress responses. In fact, putting animals into an isolated novel environment is distressing and replacing animals to a new environment influences the SCN (Buijs et al., 1997). Indeed, even though the C-V group was not included in the analyses, results were still nonsignificant. Accordingly, a combination of a novel environment and the short duration of light exposure used in the present experiment might be aversive to animals thereby lessening the potential ameliorative and preventive effects of light pulse treatment reported in previous studies. In addition, the tendency to freeze in an inescapable and novel environment, in this case the experimental chamber may have consequently been generalized to another similar inescapable and novel situation, forced swimming test (Bulduk & Canbeyli, 2004). Amir and Stewart (1999) found that the light induced phase changes of the SCN are affected by emotional states and aversive environments. Moreover, in previous studies in our laboratory, animals had been placed in the experimental chamber only for one day, namely on the day of light treatment (Schulz et al., submitted; Yilmaz et al., 2004). Staying in the experimental

chamber for two days of testing might have resulted in discrepant results reported in the present study. Indeed, it is seen that both the C-V (vivarium control) and the ZT21-B group display similar patterns in immobility and swimming in the forced swimming test. the immobility durations of these two groups increase minimally and also swimming durations increase in the FST2 compared to FST1. Although, statistical analyses show no groups differences, C-V and ZT21-B groups are seen to be more resistant to behavioral despair than the other groups. While the C-V group experiences only swim stress, other groups experience both swim and novelty stress, so that their stress level should be higher. As a result, the same tendency in the data for the C-V and the ZT21-B group can be considered as supporting the positive effect of light that fails to counteract the double stress on behavioral despair.

In later studies, the effects of longer durations of light pulse exposure delivered late at night should be tested on behavioral despair to obtain more conclusive results. As well, the depth of the water in FSTs was higher in the present study due to weighty adult rats. Abel (1993) showed that the depth and temperature of the water in FSTs are important covariates that affect the findings of forced swim tests. Testing both juvenile and adult rats in 20 cm. rather than 15 cm. water might have resulted in contradictory findings reported in the present study. Finally, circannual changes are revealed in the duration of immobility of rats in the forced swimming test that animals are most immobile during the winter months (Abel, 1995; Aksoy et al., 2004). The experimental procedure of the present study took place mostly throughout winter months, from February to April. This seasonal effect on behavioral despair might have attenuated the ameliorative and preventive effects of light pulse exposure late at night in the present study.

Adult versus Juvenile Rats

The differences between juvenile and adult rats in terms of behavioral despair indicate that juvenile rats are more susceptible to behavioral despair than adult rats. Juvenile rats

displayed longer durations of immobility and shorter durations of swimming in FST2 compared to adult rats that are the main indicators of behavioral despair. Additionally, the swimming durations of juveniles decreased sharply in FST2, while the swimming durations of adults lessened minimally which is also an indicator of the juveniles' susceptibility to behavioral despair.

Juveniles of many species show transformations in their social behaviors, with increasing social interaction and social play, especially with peers (Spear, 2000). It was revealed that even a 3.5 hour isolation from peers caused substantial changes in the basal states of stress hormones and endogenous opioid system (Niesink & Van Ree, 1989). Greater effects of social isolation during adolescence were reported in terms of object exploration in an open field and water intake that isolated juvenile rats shows higher scores (McGivern, Henschel, Hutcheson, & Pangburn, 1996). As a result, putting these juvenile rats into the experimental chamber and isolating them for two days might be an aversive manipulation (more than for adult rats) which affects the behavioral adjustment in forced swimming tests. Dal-Zotto et al. (2003) suggested that changes in the HPA axis as a result of previous stress exposure may sensitize the responses of animals to further stress exposures. Furthermore, basal (non-stress) glucocorticoid levels are higher in juveniles than in adults (Hodes & Shors, 2005; Kaufman, 1999; Kaufman et al., 2001; Levine, 2001; Petersen et al., 1993; Spear, 2000) and returning basal level of glucocorticoids in response to a stressor is slower in juvenile than in adult rats (Hodes & Shors, 2005). Additionally, recent studies show that testosterone is a protective factor against depression that rapidly reduces anxiety and despair in male rats (Aikey, Nyby, Anmuth, & James, 2002; Stone & Quartermain, 1998). Consequently, lack of sexual maturity in juvenile rats may be a factor in determining weakness to stress effects in the male animals (Stone & Quartermain, 1998).

Head Shakes and Total Struggling

There was no difference in the immobility and swimming durations of the experimental and control groups in both juveniles and adults. Nevertheless, there was a difference in head shakes and total struggling between the groups. In both age groups, the vivarium control group (C-V group) displayed different patterns in their total struggling and head shakes when compared with the other groups. In adult rats, the subjects of the C-V group displayed significantly fewer head shakes and total struggling than the other groups. On the other hand, in juvenile rats, the subjects of the C-V group displayed more head shakes and total struggling than the other groups. Higher basal glucocorticoids level (Kaufman et al., 2001; Levine, 2001), longer duration for returning to basal level of glucocorticoids in response to a stressor (Hodes & Shors, 2005), higher levels of anxiety (Doremus, Brunell, Varlinskaya, & Spear, 2003), lower levels of habituation (Laviola, Adriani, Terranova, & Gerra, 1999) and elevated effects of social isolation (Niesink & Van Ree, 1989) in juvenile rats might all be a cause for these findings. Juveniles show more vulnerability to stress, and induction of depression is easier in juveniles. Therefore, fewer head shakes and total struggling of juvenile rats kept in the experimental chamber for two days is not surprising when compared with the C-V group. On the other hand, adult rats that had been put in the experimental chamber displayed more head shakes and total struggling.

Lower basal glucocorticoid levels and rapid returning to basal level of glucocorticoids in response to a stressor in adult rats might be a cause for this difference. Moreover, although juvenile rats exhibit elevated levels of exploration in novel situations, they display greater startle responses and hyper-reactions to manipulations (Spear, 2000). Furthermore, juvenile rats show high levels of anxiety (Doremus et al., 2003) and lower levels of habituation (Laviola et al., 1999) to novel stimuli as compared to adults. More to the point, do-Rego et al. (2006) found that anxious mice displayed higher duration of immobility than non-anxious

ones. They compared elevated-plus maze, black and white compartments test and hole-board test in measuring anxiety levels of male Swiss albinos. It was found that in the forced swim test, animals in the higher anxiety group displayed higher immobility durations. Overall, lower anxiety levels and high adaptability to novel environment in adult rats might have lead to lower levels of behavioral despair, higher levels of head shakes and total struggling compared to juveniles. There are notable differences among different age groups of depressed patients in the neurobiological correlate and treatment response that necessitate careful consideration (Kaufman et al., 2001).

CHAPTER FİVE

CONCLUSION

The present findings indicate that juvenile and adult rats differ from each other in terms of the responses to the same stressors. However, short light pulse exposure late at night has no ameliorative and preventive effects on behavioral despair measured by forced swimming test in either of the groups. Further work would be necessary to investigate the neurobehavioral effects of light pulses of different length late at night. Additionally, the depth of the water would be changed more systematically to see more conclusive result. Moreover, more research is needed at the molecular level to elucidate the control of the HPA axis responses by light pulses delivered late at night.

References

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.

Aikey, J. L., Nyby, J. G., Anmuth, D. M., & James, P. J. (2002). Testosterone rapidly reduces anxiety in male house mice (Mus musculus). *Hormones and Behavioral, 42*, 448-460.

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

American Psychiatric Association Diagnostic and Statistical Manual of Mental Disorders (1994), (4th ed.). American Psychiatric Press.

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

Antle, M. C., & Mistlberger, R. E. (2000). Circadian clock resetting by sleep deprivation without exercise in the Syrian Hamster. *The Journal of Neuroscience, 20*, 9326-9332.

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

Bernstein, H. G., Heinemann, A., Krell, D., Dobrowolny, H., Bielau, H., Keilhoff, G., & Bogerts, B. (2005). Hypothalamic nitric oxide syntheses in affective disorders: Focus on the suprachiasmatic nucleus. *Cellular and Molecular Biology, 51*, 279-284.

Blackburn-Munro, G., & Blackburn-Munro, R. E. (2001). Chronic pain, chronic stress and depression: Coincidence or consequence? *Journal of Neuroendocrinology, 13*, 1009-1023.

Bourin, M., Colombel, M. C., Redrobe, J. P., Nizard, J., Hascoet, M., & Baker, G. B. (1998). Evaluation of efficacies of different classes of antidepressants in the forced swimming test in mice at different ages. *Progress in Neuro-Pharmacology and Biological Psychiatry 22*, 343-351.

Brzezinski, A., Vangel, M. G., Wurtman, R. J., Norrie, G., Zhdanova, I., Ben-Shushan, A., & Ford, I. (2005). Effects of exogenous melatonin on sleep: a meta-analysis. *Sleep Medicine Reviews, 9*, 41-50.

Buijs, R. M., Wortel, J., van Heerikhuize, J. J., Feenstra, M. G. P., Horst, G. J. T., Romijn, H. J., & Kalsbeek, A. (1999). Anatomical and functional demonstration of a multisynaptic suprachiasmatic nucleus adrenal (cortex) pathway. *European Journal of Neuroscience, 11*, 1535-1544.

Buijs, R. M., Wortel, J., van Heerikhuize, J. J., & Kalsbeek, A. (1997). Novel environment induced inhibition of corticosterone secretion: physiological evidence for a suprachiasmatic nucleus mediated neuronal hypothalamo-adrenal cortex pathway. *Brain Research, 758*, 229-236.

Bulduk, S., & Canbeyli, C. (2004). Effect of inescapable tones on behavioral despair in Wistar rats. *Progress in Neuro-Psychopharmacology and Biological Psychiatry, 24*, 471-475.

Cameron, J. L. (2004). Interrelationship between hormones, behavior, and affect during adolescence. *Annals of the New York Academy of Sciences, 1021*, 134-142.

Cascio, C. S., Shinsako, J., & Dallman, M. F. (1987). The suprachiasmatic nuclei stimulate evening ACTH secretion in the rat. *Brain Research, 423*, 173-178.

Connor, T. J., Kelliher, P., Shen, Y., Harkin, A., Kelly, J. P., & Leonard, B. E. (2000). Effect of subchoronic antidepresant treatments on behavioral, neurochemical, and endocrine changes in the forced-swim test. *Pharmacology Biochemistry and Behavior, 65*, 591-597.

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.

Dagan, Y. (2002). Circadian rhythms sleep disorders in psychiatry. *The Israel Journal of Psychiatry and Related Sciences, 39*, 19-27.

Dahl, R. E., Ryan, N. D., Williamson, D. E., Ambrosini, P. J., Rabinovich, H., Novacenko, H., Nelson, B., & Puig-Antich, J. (1992). Regulation of sleep and growth hormone in adolescent depression. *Journal of the American Academy of Child and Adolescent Psychiatry, 31*, 615-621.

Dal-Zotto, S., Marti, O., & Armario, A. (2003). Glucocorticoids are involved in the long-term effects of a single immobilization stress on the hypothalamic-pituitary-adrenal axis. *Psychoneuroendocrinology, 28*, 992-1009.

Dawson, P. (2005). Sleep and adolescents. *Principal Leadership, 5*, 11-15.

De-Jong, T. R., Snaphaan, L. J. A. E., Pattij, T., Veening, J. G., Waldinger, M. D., Cools, A. R., &Olivier, B. (2006). Effects of chronic treatment with fluvoxamine and paroxetine

during adolescence on serotonin-related behavior in adult male rats. European *Neuropsychopharmacology, 16*, 39-48.

Do-Rego, J. C., Viana, A. F., Maitre, E. L., Deniel, A., Rates, S. M. K., Leroux-Nicollet, I., & Costentin, J. (2006). Comparison between anxiety tests for selection of anxious and non anxious mice. *Behavioral Brain Research, 169*, 282-288.

Doremus, T. L., Brunell, S. C., Varlinskaya, E. I., & Spear, L. P. (2003). Anxiogenic effects during withdrawal from acute ethanol in adolescent and adult rats. Pharmacology, *Biochemistry and Behavior, 75*, 411-418.

Doremus, T. L., Varlinskaya, E. I., & Spear, L. P. (2004). Age-related differences in elevated plus maze behavior between adolescent and adult rats. *Annals of the New York Academy of Sciences, 1021*, 427-430.

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.

Hallonquist, J. D., Goldberg, M., A., & Brandes, J. S. (1986). Affective disorders and circadian rhythms. *Canadian Journal of Psychiatry, 31*, 259-272.

Hodes, G. E., & Shors, T. J. (2005). Distinctive stress effects on learning during puberty. *Hormones and Behavior, 48,* 163-171.

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

Johnson, E. O., & Breslau, N. (2001). Sleep problems and substance use in adolescence. *Drug and Alcohol Dependence, 64*, 1-7.

Kaltiala-Heino, R., Marttunen, M., Rantanen, P., & Rimpela, M. (2003). Early puberty is associated with mental health problems in middle adolescence. *Social Science and Medicine, 57*, 1055-1064.

Kaufman, J. (1999). Neurobiological correlates of child abuse. *Biological Psychiatry, 45*, 1235-1236.

Kaufman, J., Martin, A., King, R. A., & Charney, D. (2001). Are child-, adolescent-, and adult-onset depression one and the same disorder? *Biological Psychiatry, 49*, 980-1001.

Kennaway, D. J. (2002). Programming of the fetal suprachiasmatic nucleus and subsequent adult rhythmicity. *Trends in Endocrinology and Metabolism, 13*, 398-402.

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.

Kuhs, H., & Tölle, R. (1991). Sleep deprivation therapy. *Biological Psychiatry, 29*, 1129-1148.

Kupfer, D. J., & Woodward, H. R. (2004). Adolescent development and the regulation of behavior and emotion. *Annals of the New York Academy of Sciences, 1021*, 320-322.

Laitinen-Krispijn, S., der Ende, J., & Verhulst, F. C. (1999). The role of pubertal progress in the development of depression in early adolescence. *Journal of Affective Disorders, 54*, 211-215.

Laviola, G., Adriani, W., Terranova, M. L., & Gerra, G. (1999). Psychobiological risk factors for vulnerability to psychostimulants in human adolescents and animal models. *Neuroscience and Biobehavioral Reviews, 23*, 993-1010.

Leonard, B. E. (2001). Stress, norepinehprine and depression. *Journal of the Psychiatry and Neuroscience, 26*, 11-16.

Levine, S. (2001). Primary social relationships influence the development of the hypothalamic-pituitary-adrenal axis in the rat. *Physiology and Behavior, 73*, 255-260.

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.

McGivern R. F, Henschel D., Hutcheson M., & Pangburn T. (1996). Sex difference in daily water consumption of rats: effect of housing and hormones. P*hysiology and Behavior, 59*,653-658.

Michael, K. D., & Crowley, S. L. (2002). How effective are treatments for child and adolescent depression? A meta-analytic review. *Clinical Psychology Review, 22*, 247-269.

Molina-Hernandez, M., & Tellez-Alcantara, P. (2000). Long-photoperiod regimen may produce antidepressant actions in the male rat. *Progress in Neuro-Pharmacology and Biological Psychiatry 24*, 105-116.

Natale, V., Sansavini, A., Trombini, E., Esposito, M. J., Alessandroni, R., & Faldella, G. (2005). Relationship between preterm birth and circadian typology in adolescence. *Neuroscience Letters, 382*, 139-142.

Nestler, E. J., Barrot, M., DiLeone, R. J., Eisch, A. J., Gold, S. J., & Monteggia, L. M. (2002). Neurobiology of depression. *Neuron, 34*, 13-25.

Niesink, R. J. M., & Van Ree, J. M. (1989). Involvement of opioid and dopaminergic systems in isolation-induced pinning and social grooming of young rats. *Neuropharmacology, 28*, 411-418.

Page, M. E., Detke, M. J., Dalvi, A., Kirby, L. G., & Lucki, I. (1999). Seratonergic mediation of the effects of fluoxetine, but not desipramine, in the rat forced swimming test. *Psychopharmacology, 147*, 162-167.

Petersen, A. C., Compas, B. E., Brooks,Gunn, J., Stemmler, M., Ey, S., & Grant, K. E. (1993). Depression in adolescence. *American Psychologist, 48*, 155-168.

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.

Prendergast, B. J., & Nelson, R. J. (2005). Affective responses to changes in day length in Siberian hamsters. *Psychoneuroendocrinology, 30*, 438-452.

Preti, A., & Miotti, P. (2001). Diurnal variations in suicide by age and gender in Italy. *Journal of Affective Disorders, 65*, 253-261.

Radloff, L. S. (1991). The use of the Center for Epidemiological Studies Depression Scale in adolescents and young adults. *Journal of Youth and Adolescents, 20*, 149-166.

Rosenberg, R. S., Zee, P. C., & Turek, F. W. (1991). Phase response curves to light in young and old hamsters. *American Journal of Physiology, 261*, 491-495.

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.

Sage, D., Maurel D., & Bosler, O. (2001). Involvement of the suprachiasmatic nucleus in diurnal ACTH and corticosterone responsiveness to stress. *American Journal of Physiology, Endocrinology and Mutability, 280*, E260-E269.

Saitoh, Y., Nihonmatsu, I., & Kawamura, H. (1987). Transplantation of the suprachiasmatic nucleus in the rat. *Acta Neurochirurgica, Supplementum, 41*, 41-45.

Saper, C. B., Lu, J., Chou, T. C., & Gooley, J. (2005). The hypothalamic integrator for circadian rhythms. *Trends in Neurosciences, 28*, 152-157.

Sapolsky, R. M. (2004). *Why Zebras Don't Get Ulcers: An Updated Guide to Stress, Stress Related Diseases, and Coping* (pp. 238-251, 270-308). New York: W. H. Freeman.

Schulz, D., Aksoy, A., & Canbeyli, R. (submitted). Behavioral despair is differentially affected by the length and timing of photic stimulation in the dark phase of an L/D cycle.

Sher, L. (2003). Aetiology and pathogenesis of mood disorders. *QJM, 96*, 309-313.

Slotkin, T. A., Seidler, F. J., & Ritchie, J. C. (2000). Regional differences in brain monoamine oxidase subtypes in an animal model of geriatric depression: effects of olfactory bulbectomy in young versus aged rats. *Brain Research, 882*, 149–154.

Solberg, L. C., Horton, T. H., & Turek F. W. (1999). Circadian rhythms and depression: Effects of exercise in an animal model. *American Journal of Physiology, 276*, 152-161.

Spear, L. P. (2000). The adolescent brain and age-related behavioral manifestations. *Neuroscience and Biobehavioral Review, 24*, 417-463.

Stone, E. A., & Quartermain, D. (1998). Greater behavioral effects ıof stress in immature as compared to mature male mice. *Physiology and Behavior, 63*, 143-145.

Tataroglu, O., Aksoy, A., Yilmaz, A., & Canbeyli, R. (2004). Effects of lesioning the suprachiasmatic nuclei on behavioral despair in rats. *Brain Research, 1001*, 118-124.

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 den Berg, C. L., Pijlman, F. T., Koning H. A., Diergaarde, L., Van Ree, J. M., & Spruijt, B. M. (1999). Isolation changes the incentive value of sucrose and social behavior in juvenile and adult rats. *Brain Research Reviews, 106*, 133-142.

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.

Walker, S. J., & Vrana, K. F. (1993). Pituitary corticotrophins function during the stress hyporesponsive period in neonatal rats. *Neuroendocrinology, 57*, 1003-1010.

Wehr, T. A., Duncan, W. C:, Sher, L., AeschBach, D., Schwartz, P. J., Turner, E. H., Postolache, T. T., & Rosenthal, N. E. (2001). A circadian signal of change of season in patients with seasonal affective disorder. *Archives of General Psychiatry, 58*, 1108-1114.

Wehr, T. A, & Wirz-Justice, A. (1982). Circadian rhythm mechanisms in affective illness and in antidepressant drug action. *Pharmacopsychiatry, 1*, 31-39.

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

Wongwitdecha, N., Kasemsook, C., & Plasen, S. (2006). Social isolation alters the effect of desipramine in the rat forced swimming test. *Behavioral Brain Research, 167*, 232-236.

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.

Yates, G., Panksepp, J., Ikemoto, S., Nelson, E., & Conner, R. (1991). Social isolation effects on the "behavior despair" forced swimming test: Effect of age and duration of testing. *Physiology and Behavior, 49*, 347-353.

Yilmaz, 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.