BOLU ABANT IZZET BAYSAL UNIVERSITY
THE GRADUATE SCHOOL OF NATURAL AND APPLIED SCIENCES
DEPARTMENT OF BIOLOGY

THE EFFECTS OF THE PHOTOPERIOD, THE TIME OF THE DAY AND THE PINEAL GLAND ON DEPRESSION AND AGRESSION LIKE BEHAVIOURS IN WISTAR ALBINO RATS

MASTER OF SCIENCE

UĞUR GÜRBÜZ

BOLU, JANUARY 2020

APPROVAL OF THE THESIS

THE EFFECTS OF THE PHOTOPERIOD, THE TIME OF THE DAY AND THE PINEAL GLAND ON DEPRESSION AND AGRESSION LIKE BEHAVIOURS IN WISTAR ALBINO RATS submitted by **UĞUR GÜRBÜZ** and defended before the below named jury in partial fulfillment of the requirements for the degree of **Master of Science** in **Department of Biology, The Graduate School of Natural and Applied Sciences of Bolu Abant Izzet Baysal University in 14.01.2020** by

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To my family, Alper KARAKAŞ and Fatma PEHLİVAN KARAKAŞ

DECLARATION

I hereby declare that all information in this document has been obtained and presented in accordance with academic rules and ethical conduct. I also declare that, as required by these rules and conduct, I have fully cited and referenced all material and results that are not original to this work.

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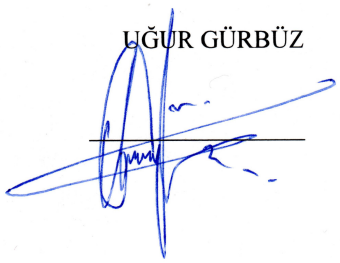

ABSTRACT

THE EFFECTS OF THE PHOTOPERIOD, THE TIME OF THE DAY AND THE PINEAL GLAND ON DEPRESSION AND AGRESSION LIKE BEHAVIOURS IN WISTAR ALBINO RATS
MSC THESIS
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BOLU ABANT IZZET BAYSAL UNIVERSITY GRADUATE SCHOOL OF NATURAL AND APPLIED SCIENCES
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BOLU, JANUARY 2020

Recently, with the development of technology, the differentiation of modern living conditions may cause the disturbance of the natural sleep rhythm and may trigger mental disorders such as anxiety, depression and aggression by changing the release rhythm of melatonin hormone. There are no studies in the literature that test the effects of different photoperiods, different measurement times of the day and the pineal gland together on these conditions. Our aim in this study, was to investigate the effects of the photoperiod, measurement time of the day and the pinealectomy on anxiety, depression and aggression-like behaviors in male Wistar albino rats by using open field, Porsolt forced swim test and resident-intruder test. The tested subjects were firstly divided into two groups as control and pinealectomy, then each group was divided into 3 subgroups as short (8L:16D), normal (12L:12D) and long (16L:8D) photoperiod, randomly. Experimental measurements were performed at the four different measurement time (6:00, 12:00, 18:00 and 24:00 h). In open field, pinealectomized subjects in the normal photoperiod at 24:00 h were the least anxious of all other conditions for most of the measurements. In Porsolt forced swim test, control subjects in the long photoperiod at 12:00 h were the least depressive of all other conditions for most of the measurements. In the resident-intruder test, the subjects in the short photoperiod were more aggressive than those in the normal and long photoperiod. Taken together these findings showed that the pinealectomized animals with low level of melatonin were less anxious, the subjects under the long photoperiod with low level of melatonin were less depressive compare to those in short photoperiod subjects with high level of melatonin. However, animals under the short photoperiod with high level of melatonin were more aggressive than those in long and normal photoperiod subjects. The outcomes of the study indicated that the effects of the pinealectomy, the measurement time of the day, and photoperiod were significant on the anxiety, depression and aggression-like behavior of the Wistar albino rats.

KEYWORDS: Photoperiod, Time of the day, Pinealectomy, Melatonin, Anxiety, Depression, Aggression

ÖZET

WISTAR ALBINO RATLARDA DEPRESYON VE SALDIRGANLIK BENZERİ DAVRANIŞLARI ÜZERİNE FOTOPERİYOT, GÜNÜN ZAMANI VE PİNEAL BEZİN ETKILERI
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Son zamanlarda, teknolojinin gelişmesiyle birlikte modern yaşam koşullarının farklılaşması, doğal uyku ritminin bozulmasına ve dolayısıyla melatonin hormonunun salınım ritminin değişmesine neden olarak, anksiyete, depresyon ve saldırganlık gibi zihinsel bozuklukları tetikleyebilmektedir. Literatürde, farklı fotoperiyotların, günün farklı ölçüm zamanlarının ve pineal bezinin bu durumlar üzerindeki etkilerini birlikte test eden herhangi bir çalışma bulunmamaktadır. Bu çalışmada amacımız, açık alan, Porsolt zorla yüzme testi ve resident-intruder testi kullanılarak, erkek Wistar albino sıçanlarında fotoperiyodun, günün ölçüm zamanının ve pinealektominin anksiyete, depresyon ve saldırganlık benzeri davranışlar üzerindeki etkilerini araştırmaktır. Test edilen denekler ilk olarak kontrol ve pinealektomi olmak üzere iki ana gruba ayrıldı, daha sonra her grup rastgele olarak, kısa (8L: 16D), normal (12L: 12D) ve uzun (16L: 8D) fotoperiyot olmak üzere 3 alt gruba ayrıldı. Deneysel ölçümler dört farklı ölçüm zamanında (6:00, 12:00, 18:00 ve 24:00 s) yapıldı. Açık alanda, pinealektomili denekler, normal fotoperiyotta ve saat 24:00'de alınan ölçümde, diğer tüm koşullara göre en az endişe duyuyorlardı. Porsolt zorla yüzme testinde, uzun fotoperiyotta, saat 12: 00'de ölçüm alınan kontrol denekler, diğer tüm koşullara göre en az depresifdi. Resident-intruder testinde, kısa fotoperiyottaki denekler, normal ve uzun fotoperiyottakilerden daha agresifdi. Tüm bulgular birlikte ele alındığında, düşük melatonin düzeyine sahip pinealektomili hayvanların daha az endişeli olduğu, düşük melatonin seviyesine sahip uzun fotoperiyottaki deneklerin, yüksek melatonin seviyesine sahip kısa fotoperiyottaki deneklerden daha az depresif olduğunu göstermektedir. Bununla birlikte, yüksek melatonin seviyesine sahip kısa fotoperiyottaki hayvanlar, uzun ve normal fotoperiyottaki deneklerden daha agresifdi. Çalışmanın sonuçları, pinealektomi, günün ölçüm zamanı ve fotoperiyodun Wistar albino sıçanların kaygı, depresyon ve saldırganlık benzeri davranışları üzerinde önemli etkileri olduğunu göstermektedir.

ANAHTAR KELİMELER: Fotoperiyot, Günün Zamanı, Pinealektomi, Melatonin, Ansiyete, Depresyon, Saldırganlık

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LIST OF ABBREVIATIONS AND SYMBOLS

AANAT **:** N acetyltransferase

AGP **:** Aggressive Posture

AOS **:** Attack to other subjects

BAIBU **:** Bolu Abant Izzet Baysal University

CMDP **:** Center Point Moving Duration In porsolt

DHEA : Dehydroepiandrosterone

FCOF **:** Frequency at the Center of the Open Field

FEOF **:** Frequency at the Edge of the Open Field

GEAG **:** General aggression

GMG **:** Grooming

HIOMT **:** hydroxyindole-O-methyltransferase

MDOF **:** Mobility Duration in the Open Field

MDP **:** Mobile Duration in the Porsolt (Force Swim Test)

MFOF **:** Mobility Frequency in the Open Field

MFP(FST) **:** Mobile Frequency in the Porsolt (Force Swim Test)

NAS **:** N-acetylserotonin

NAT **:** N-acetyltransferase

PHPLB **:** Push and Pull Behaviour

PINX **:** Pinealectomy

RFOF **:** Rotary Frequency in the Open Field

RIT : Resident/Intruder Test

TDTOF **:** Total Distance Travelled on the Open Field

TDTP **:** Total Distance Travalled in the Porsolt

TSCOF **:** Time Spent at the Center of the Open Field

TSEOF **:** Time Spent at the Edge of the Open Field

VELOF **:**Velocity in the Open Field

VELP(FST) **:** Velocity in Porsolt

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1. INTRODUCTION

1.1 Circadian Rhythm

Most organisms must make adaptation to their environment in the world. Seasonal rhythms in light time, ambient temperature, and humidity are regulated by the rotation of earth around itself and around the sun. In order to make adaptations to these rhythms, most organisms developed rhythms in almost every direction of their body, ranging from gene expression, physiology (e.g., heart rate, metabolism, reproduction, and hormone secretion), cognitive functions (e.g., learning and memory) and activity-rest patterns (Kronfeld-Schor and Einat, 2012).

Approximately 24-h dark-light cycle is regarded as circadian (from circadiem) rhythms. On the other hand, cycle that is shorter or longer that the 24-h cycles are defined as ultradian and infradian rhythmicity, respectively.

1.2 Photoperiod

As Suprachiasmatic nucleus (SCN) located in hypothalamus namely called a circadian oscillator, generate and controls some parameters of endogenous circadian rhythm such as release of some hormones (e.g. melatonin, serotonin etc.), locomotor activity, and body temperature (Le Sauter and Silver, 1994; Nelson et al., 2010). In mammals, this brain region is known as a center for photoperiodism, the ability of plants and animals to measure environmental day length (photoperiod), a process that underlies the so-called biological calendar (Nelson et al., 2010). Photoperiodism or the biological ability to measure day length, leads living organisms to understand the time of year and engage in seasonally appropriate adaptations. The individuals that respond to day length can exactly understand the time of year with just two bits of data: (1) the length of the daily photoperiod and (2) whether day lengths are increasing or decreasing (Walton et al., 2011). The ablation of SCN disorganizes such activity or photoperiodism (Le Sauter and Silver, 1994). Circadian rhythms are

synchonized by some factors such as light/dark circle, hormones, food availability, and temperature (Goldmann, 1999; Prosser and Bergeron, 2003; Rusak and Zucker, 1979). In mammals, photoperiodic information (day-length) can even be passed to developing fetuses in utero so that the summer or winter phenotype can begin to develop prior to birth (Weaner et al., 1987). In mammals, hamster and sheep are often used in such studies, because they also show robust photoperiodic responses. Despite this well-known evidence, little is known about how circadian rhythms are affected by which factors such as anxiety, depression or aggression-like behaviours that have adoptive value for mammals

1.3 Pineal Gland

Pineal gland is a small pine shape structure located in the epithalamus of the brain (Klein, 1993). Since ancient times, the pineal gland was called 'enigmatic organ' by Van Gehuchten (1937). According to the Indian culture, there was a belief that humans would be equipped with a 'third eye' or mystical organ (pineal gland) (Baker, 1985). One of the most prominent proponents of the role of the pineal gland was the French philosopher Rene Descartes (1596-1650), who suggested as the physical seat of the human soul (Lopez-Munoz et al., 2011). Subsequently, the important scientific advances that have come out the middle of the 20th century, culminating in the discovery of melatonin, the hormone secreted and released by the pineal gland. Pineal gland secretes melatonin hormone which is known as mediator for photoperiodic time (day-length) measurement in mammals (Hazlerigg, 2010; Makino et al., 2001). The secretion of the melatonin demonstrates a diurnal function; with the highest levels during the dark cycle of the day and this circadian rhythm is similar in all organisms whether they are nocturnal or diurnal. The pinealectomy or removal of the pineal gland inhibits responsiveness to photoperiod in every mammalian species (Hare, 1978). It also decreases the plasma levels of melatonin significantly (Hoffman and Reiter, 1965). Its primary function is to transduce light/dark period information to whole body physiology via releasing melatonin (Arendt, 2005). The pineal gland adrenergic innervation, which activates a cascade of circadian events that leads to the nightly formation of melatonin from serotonin

and the pineal, is also innervated by parasympathetic system (Larsen, 1999); the role and regulation of which are presently unclear.

1.4 Melatonin Hormone

Melatonin (5-methoxy-N-acetyltryptamine) was discovered in the late 1950s by Lerner and co-workers (Lerner et al., 1958; Lerner et al., 1959; Lerner et al., 1960). Melatonin is released from pineal gland into the blood especially at night (Reiter, 1981; Stetson and Watson-Whitmyre, 1984). Thoughout the daytime, concentrations of melatonin keep very low, as exposure to light strongly repress its secretion in an intensity-dependent manner (I.M. McIntyre et al., 1989). Therefore, melatonin is a signal of darkness that encodes time-of-day and length-of-day information to the brain including the Suprachiasmatic Nucleus, brain and peripheral organs (Pandi-Perumal et al., 2006b) As a result; melatonin is involved in the processing of photoperiodic information in various organisms. However, this way is not the only the way for melatonin production. Melatonin is also in part produced by gastrointestinal tract (Huether, 1993., Reiter, 1991) and retina (Zawilska and Nowak., 1992), thymus, bone marrow, respiratory epithelium, skin, lens (Lerner et al., 1958, Pandi-Perumal SR et al., 2006) in vertebrates.

The amphilicity of the melatonin is allowing the molecule to passes by diffusion from peripheral circulation to other fluids or cells (Poeggeler et al., 1994). 70% melatonin is bound to albumins in serum the remaining 30% diffuses to the surrounding tissues (Hardeland R et al., 2006). Tryptophan and serotonin are precursors of melatonin hormone. N-acetyltransferase (AANAT) and hydroxyindole-O-methyltransferase (HIOMT) enzymes play important role in its synthesis. The first–rate limiting enzyme is AANAT in melatonin synthesis, converts serotonin to N-acetylserotonin (NAS), HIOMT converts NAS to melatonin hormone (Figure 1). By the axons of retinal ganglion cells running in the optic nerves and forming the retino-hypothalamic tract, light-dark cycle acts on the neuronal activation of the anterior hypothalamus which also controls the regulation of the melatonin production.

In addition to regulating sleep, melatonin regulates many physiological functions such as neuroendocrine, circadian visual, reproductive, cerebrovascular, and neuroimmunological functions (Arendt, 2000; Borjigin et al., 1999; Brzezinski, 1997; Masana and Dubocovich, 2001; Vanecek, 1999; Wirz-Justice, 2001). Additionally, its neuroendocrine functions, many pharmacological effects of melatonin have been reported in rodents, such as sedative, analgesic anticonvulsant and anxiolytic activities (Albertson et al., 1981; Datta and King, 1977; Datta and King, 1979; Golombek et al., 1993; Golus and King, 1981; Sugden, 1983). Moreover, along with these functions, melatonin also regulates behavior processes. For instance, recent studies have shown that several rodent species are affected by changes in photoperiod, which, in turn, influence some behaviors such as anxiety and depression like behaviors. Exposure to short photoperiod generates anxiety-like and depressive-like responses in some rat species (Weil et al., 2007), Siberian hamsters (Prendergast and Nelson, 2005; Pyter and Nelson, 2006), nocturnal rodents (Benabid et al., 2008; Molina-Hernandez and Tellez-Alcantara, 2000; Prendergast and Kay, 2008) and diurnal rodents (e.g., Nile grass rats (Ashkenazy-Frolinger et al., 2010) and sand rats (Ashkenazy et al., 2009). These affective replies to short days are clearly linked directly to pineal melatonin secretion duration (Ashkenazy et al., 2009) but exact mechanism is still unknown. These behaviors are affected not only by acute short-day photoperiod administration but also by prenatal melatonin administrate in utero (Workman et al., 2008). There is evidence that high level of melatonin release induced by short-day photoperiod increases serotonin transport in the mid brain (Benca et al., 2009). This suggests that the theupatic use of serotonin-reuptake inhibitors play roles in combating anxiety and depression in humans (Benca et al., 2009).

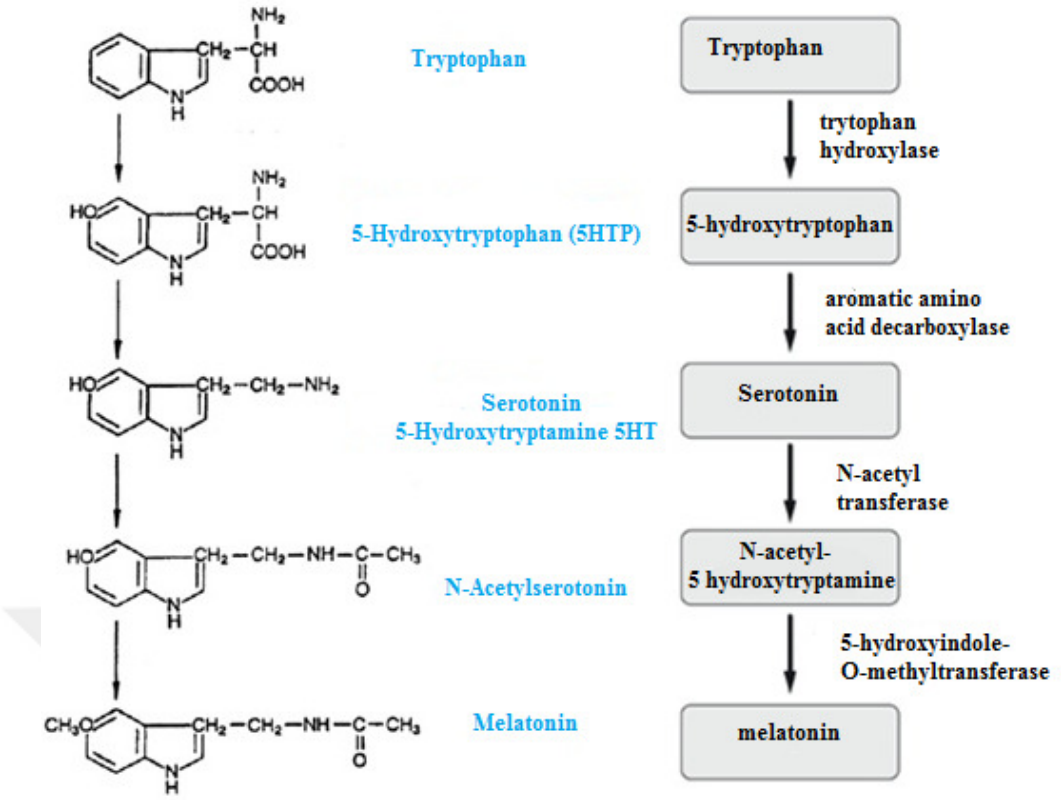

Figure 1. 1 Metabolic pathway of melatonin synthesis

Some specific melatonin receptors (M1/M2) and its binding sites have been found in the areas involved in the brain (Cardinalli et al., 1979; Weaver et al., 1989). Melatonin receptors (MT_1, MT_2, CA3, and CA1) are located in the hippocampus of several mammals. $MT_1$and MT_2 receptors exist in dentate gyrus, CA_3 and CA_1 areas and subiculum of the hippocampus (Musshoff et al., 2002). In recent times, the non-selective MT1/MT2 receptor antogonists agomelatine and Neu-P11 have been found to possess anxiolytic properties in preclinical models of anxiety-like behaviors (Millan et al., 2005; Rainer et al., 2011; Tian et al., 2010).

Melatonin is basicly known as a hormone synthesized in the tryptophan/serotonin pathway and secreted into blood circulation by the pineal gland, following a day-lenght, in coherence with its role as organizer of the circadian rhythm. The highest melatonin plasma level occurs generally 3 - 5 hours after the begining of darkness, and its concentration during the period of day light is low or not able to be detected (Corpas et al., 2018).

1.5 Anxiety

Anxiety is one of the psychological common disorders that show itself by unpleasant sentimental state, horror and distressing physical symptoms in answer to stressor conditions. It occurs with a life time prevalence of 16.6 % in general population (Somers et al., 2006) and with prevalence estimates ranging from 5 to 18 % in the pediatric populations (Ramsawh et al., 2010). Scientists show growing interest discovering novel pharmacotherapautic targets for the treatment of anxiety.

As mentioned previously, in addition to its neuroendocrine functions such as mood, circadian rhythms, sleep and reproduction (Reiter 1991; Ribelayga, and Simonneaux 2003), endogenous melatonin produces anxiolytic effects in both preclinical (Crupi et al., 2010; Golombek et al., 1993; Golus and King, 1981; Papp et al., 2006) and human studies (Caumo et al., 2009; Srinivasan et al., 2006). The non-selective MT1/MT2agonist agomelatine and Neu-P11 have been demonstrated to possess anxiolytic properties in preclinical models of anxiety. Photoperiodic conditions can also affect anxiety like reponses. For instance, hamsters exposed to short-days early in life have increased anxiety- and depressive-like responses as adult (Pyter and Nelson, 2006). This evidence also suggests that other related parts of phoperiods such as SCN and pineal gland play a regulatory role in anxiety like behaviors. However, which parts of these structures are mostly involved in anxiety are still unknown and needs to be illuminated with further studies. In addition, there is no strong evidence for the role of circadian systems on anxiety in the literature. The anxiolytic-like effects of melatonin have experimentaly been tested in the passive avoidance test, the open field and the elevated plus maze (Lister, 1990).

1.6 Depression

Depression is a status of low mood and reluctance to activity that can negatively affect a person's thoughts, sense, feelings and behavior of well-being with syptoms such as feelings of hopelessness, anhedonia and guilt, suicidal opinion, distortion of sleep and appetite as well as cognitive function (Cryan et al., 2002). It occurs with a prelevance of 17-20 % in general population (Kessler et al., 1994) and with episode at any age from childhood to elderly.

In the literature, there have been some explanations proposed for underlying pathophysiology of this disease. The first explanation deals with lack of monoamines and serotonin (5-hydroxytryptamine, 5-HT). Serotonin is converted to melatonin, a neurohormone crucial for regulation on circadian cycles. The second explanation is that dysregulation of circadian cycles brings about depression (Lanfumey et al., 2013). There is evidence in humans as well as in animal models that obviously show connection between depression and circadian rhythms. Circadian clock is frequently phase-delayed in major depressive disorder, with intensity of depression associating with extent of delay (Emens et al., 2009). Circadian abnormalities are important in depressive disorder. Third explanation focuses on interactions between depression and activity patterns across the day/night cycle. For instance, depressed patients reduced activity levels during wake time and to demonstrated higher motor activity during sleeping time (Volkers et al., 2003). Final explanation is related to chonic exposure to light at night though shift work. This is associated with higher prevalence of mood disorders (Dumont and Beaulieu, 2007). Elevated exposure to light at night correlates with increasing rates of depressive disorders (Emens et al., 2009).

Melatonin is shown to produce antidepressant-like effect in the rodent tail suspension test (Mantovani et al., 2003; Prakhie and Oxenkrug, 1998), and in the rat forced swimming test (Micale et al., 2006). Another study indicated that melatonin treatment prevents behavioral and physiological responses triggered by the chronic mild stress model of depression in mice (Detanico et al., 2009), hence reinforcing cue that melatonin possesses antidepressant features and is effective in rodent models of depression. Shaji and Kulkarni, (1998) indicated that melatonin was shown to decreased the spent time of immobility in the forced swim test.

1.7 Aggression

Aggression is complex social behaviors (biting, chasing, groming and etc.) that are displayed by all organisms. This behavior serves a wide range of adaptive functions (acquiring and maintaining food or mates) in both sexes and requires integration of both environmental (photoperiod, temperature, seasonal changes) and physiological factors (Cutton-Brock, 2009). Previous studies have shown that male

hamsters housed in short photoperiods showed more aggression than those in long one considering the significant decrease in circulating testosterone (Bronson and Heideman, 1994; Garrett and Campbell, 1980; Caldwell and Arbes, 2004; Jasnow et al., 2002). The mechanisms liable for the enhance in aggression in short photoperiod exposed hamsters are not known. A possible explanation for the higher levels of aggression observed in short photoperiod housed hamsters was due to the longer duration of melatonin secretion induced by short photoperiod. It is known that day length (photoperiod) is the primary cue used by mammals to coordinate seasonal changes in morphology, physiology and behavior (Hardeland 2009) Short photoperiod patterns of melatonin induce a suite of traits including gonadal regression and decreases in sex steroids. Furthermore, receptors of melatonin are deployed thoughout the brain and periphery, containing at the level of both the gonads and adrenal glands (Hardeland 2009).

Despite these new advancements, whether short photoperiod increases aggression or not in various species is still unknown. All previous studies lack an appropriate comparison condition; that is, normal photoperiod. Thus, a new research is needed and requires a condition where long, short, and normal photoperiods are compared in a single research paradigm.

1.8 Time of Measurement

The time of measurement seems to be important factor for experimental behavioral measures such as anxiety, depression, and aggression like-behaviors. For instance, Kaya et al (2011) in their research found that rats were more mobile (thus less anxious) at 24:00 hour (h) than 06:00 h early in the morning at the open field test. On the other hand, rats were more mobile (thus less anxious) at 24:00 hour than 18:00 hour early in the evening at the elevated plus maze test. Taken together, these findings suggest that time of the measurement and type of the test are very important factors that need to be taken in to consideration in all behavioral measures such as anxiety, depression, and aggression-like behaviors.

2. OBJECTIVES

Given the consideration discussed earlier, the aim of this research was to investigate how behavior, such as anxiety, depression and aggression, is affected by day length which is an environmental factor and the time of measurement, and pinealectomy. There are number of reasons for this study. First, pineal gland plays an active role in photoperiodic regulations. There have been limited numbers of studies in the literature, suggesting that depression, anxiety, and aggression are affected by some seasonal changes. Also a few studies examined the influence of pinealectomy (removing the pineal gland). Second, influence of the time of measurement on behavioral measures has been rarely studied in the literature. Third, even though day length (long photoperiod, short photoperiod, and normal photoperiod) is one popular topic for affecting behavior, its interaction effect with other variables such as pineal gland needs to be investigated since there is no research conducted on this matter. Fourth, in order to replicate the previous findings in the literature, the effect of melatonin induced by the day length on aggression requires a new research paradigm where normal, long, and, short photoperiod will be investigated together. Therefore, the present study investigated effects of these variables by analyzing data with 2 treatment (pinealectomy and control) X 3 photoperiod (short photoperiod, normal photoperiod and long photoperiod) X 4 time of measurement (06:00, 12:00, 18:00, and 24:00 hour).

3. MATERIALS AND METHODS

3.1 Animal Care

Adult male Wistar albino rats (200-250 g) that approximately 8 weeks old were obtained from our laboratory colony maintained at the Bolu Abant Izzet Baysal University (BAIBU) (see Figure 3.1). The all experimental procedures in this research were carried out in accordance with the Animal Scientific procedure and approved by the Institutional Animal Care and Use Committee at BAIBU. Animals (male Wistar albino rats) were housed in plastic cages (16x31x42 cm) with pine shavings used as bedding. Food pellets and tap water were accessible ad libitum and these regurarly changed every two days. All cages were exposed to 200 lux light that was provided by the cool-white fluorescent tubes controlled by automatic programmable timers. Ambient temperatures in the animal facilities were held constant at 22 ± 2 °C in air-ventilated rooms.

Figure 3. 1 Wistar albino rats in cages

In the present study, a total of 60 male adult Wistar albino rats were selected and randomly divided into the six experimental groups [control normal photoperiod (n=10), pinealectomy normal photoperiod (n=10), control short photoperiod (n=10), pinealectomy short photoperiod (n=10), control long photoperiod (n=10), pinealectomy long photoperiod (n=10)]. Experimental design was shown at Figure 3.2. The behaviors of all these animals were observed in different measurement times at 06:00, 12:00, 18:00 and 24:00 h. Anxiety-like behaviors, depression-like behaviors, and aggression-like behaviors of male Wistar rats were measured by a means of open field, force swimming, and resident-intruder tests.

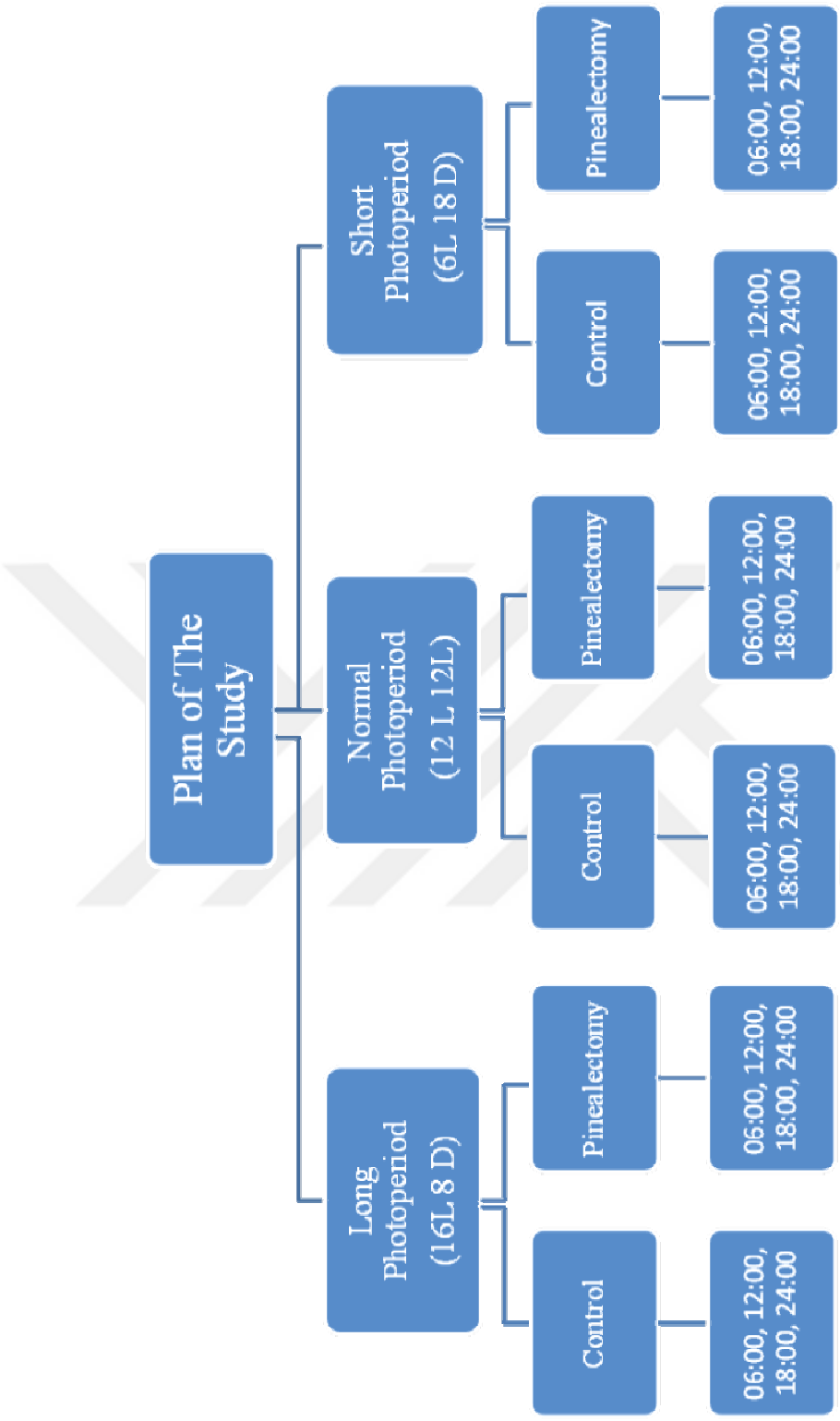

Figure 3. 2 Experimental design

3.2 Pinealectomy

The pinealectomy process was made according to the method described by Karakas and Coskun (2012). Briefly, male Wistar albino rats were anesthetized subcutaneously with Ketamine (20 mg/kg), and intraperitoneally with pentobarbitol (32.5 mg/kg) before surgery. The anesthetized animals were placed on stereotaxic instrument and then the pineal gland was removed by setting the appropriate coordinate according to the described rat pinealectomy method (Karakas and Coskun, 2012).

3.3 Open Field

Open-field test consists of 80 cm × 80 cm arena with 40 cm high walls. The open field has been the most generelly used test in animal psychology for anxiety-like behaviors. In this test, an animal (usually a rodent) is introduced into a plain and illuminated arena and its behavior is commonly regarded as a fundamental index of general behavior. In this experiment a video camera (Gkb CC-28905S, Commat LTD.ŞTİ. Ankara/Turkey) was mounted above the arena, recording behavior into the Ethovision videotracking system (Noldus Ethovision, Version 6, Netherland; Commat LTD.ŞTİ. Ankara/Turkey) that ensured a variety of anxiety-like behaviors such as total distance travelled, time spent at the edge of open field, time spend at the center of open field, entry frequency at the edge of open field, entry frequency at the center of open field, mobility duration on open field, mobility frequency on open field, and velocity on the open field (see Figure 3.3).

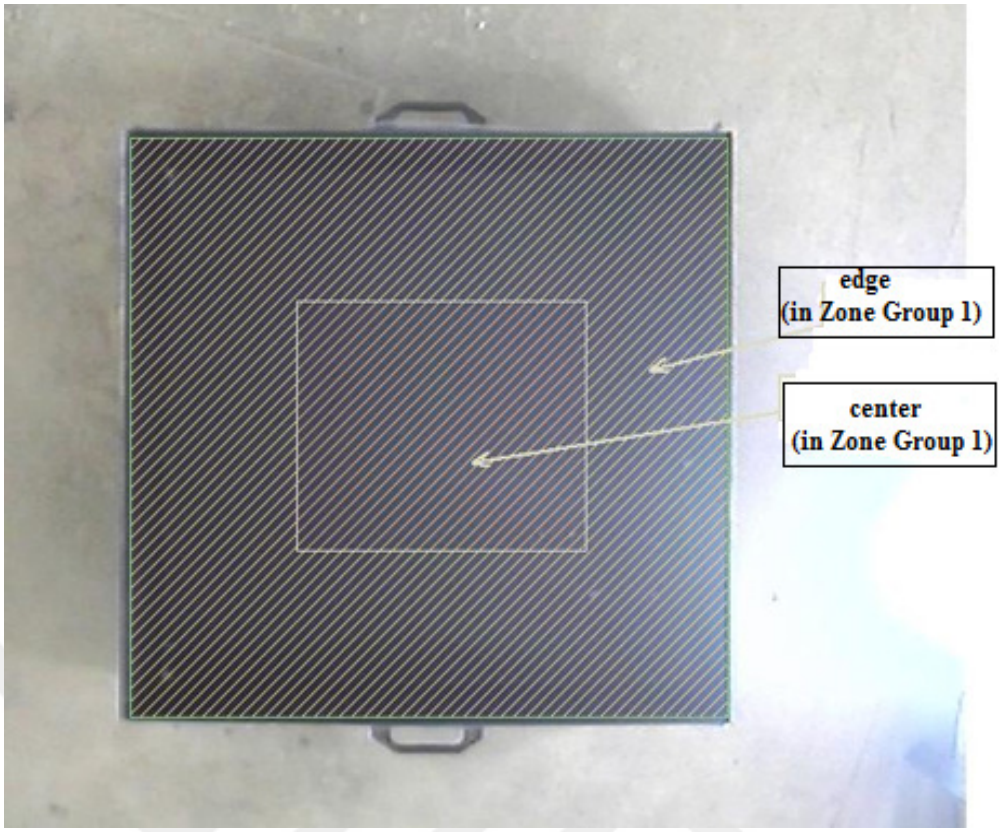

Figure 3. 3 Open field apparatus and Ethovision video tracking system

3.4 Forced Swim Test (Porsolt)

To examine depressive- like behaviours, the subjects were put in porsolt, including an opaque cylinder tank (24 cm diameter, 53 cm height) (see Figure 3.4.) which was filled with 17 cm deep water kept at 28 °C. Swimming behaviors of the rats were recorded on video for 5 minutes. In this experiment a video camera (Gkb CC-28905S, Commat LTD.ŞTİ. Ankara/Turkey) was mounted above the arena, recording behavior into the Ethovision videotracking system (Noldus Ethovision, Version 6, Netherland; Commat LTD.ŞTİ. Ankara/Turkey) that had a variety of behavioral measures such as the mobility time, mobility frequency, total distance travelled, immobility time, rotation, and velocity.

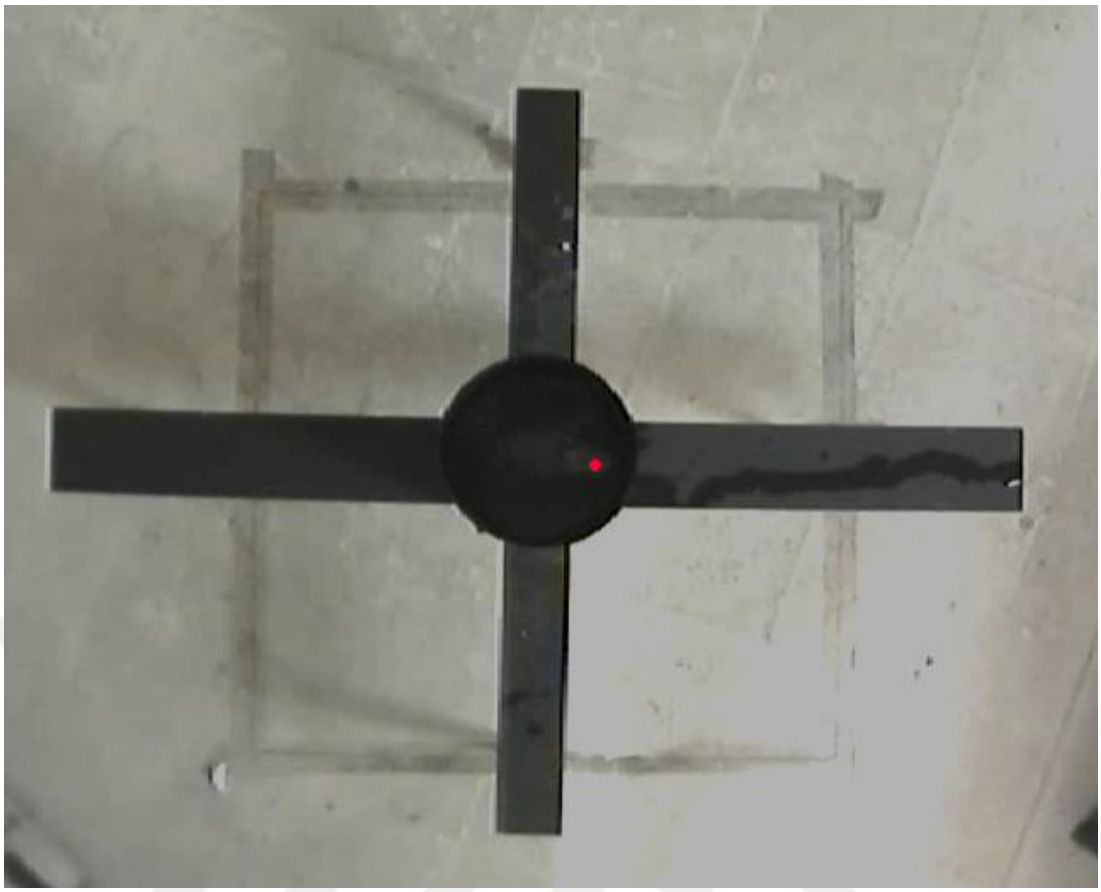

Figure 3. 4 Force swimming test

3.5 Animals for Aggression

The experiments were performed using male Wistar albino rats taken from the colony maintained by the Central Animal Facility of the Medical School of Bolu Abant Izzet Baysal University. Animals that were at the age of 8 old weeks were group-housed (5 animals/cage) in a temperature controlled (24□±□1□°C) room, under normal photoperiod (12L:12D) (lights on at 6:00□am) with food and water ad libitum, for approximately 2□weeks. After this processes, resident rats were single-housed for 15□days. First, intruders were kept group-housed (5 animals/cage) until the resident-intruder test day. Then, intruders were kept single housed for 15 days. A total of 60 male Wistar albino rats were used in this experiment.

3.6 Resident/Intruder Test (RIT)

The RIT measures aggressive behaviour based on the criteria of previous theories (Floody and Pfaff 1977; Malkesman et al. 2006). Briefly, an intruder animal of same age and weight was placed into the home cage of the experimental rat (resident). The aggressive behavior was determined when a resident male rat exposed to an intruder. All paired rat of resident/intruder were from the same experimental group. The resident was scored for grooming, aggressive posture, push and pull behavior, attack to the other subject, and general aggression (Harrison et al., 2000). These aggressive behaviors were numbered from 0 (not at all) to 4 (the most). The measures were taken in the home cage of the experimental rat. The intruders were placed directly into the home cage of the experimental animal. Each test was performed for 20 min duration and was videotaped and coded by the two observers who were unaware of the rats' experimental treatments. Each intruder was used only once.

3.7 Statistical Analyses

Data of all measurements (the open field, Porsolt, and resident/intruder test) were analyzed using SPSS Statistical Software version 22.0 (SPSS Inc., Chicago). Data were analyzed by 3 (short, normal and long photoperiod) × 2 (control and pinealectomy) × 4 (time of the day: 6:00, 12:00, 18:00, and 24:00 h) analysis of variance (ANOVA) analysis with the last factor as a within subject or repeated design. After ANOVA, significant comparisons were also analyzed by the post-test, namely the Duncan test, a strong test for comparison of groups that has equal variance and sample size. Values were considered statistically significant at $p \leq 0.05$. Data were illustrated as mean ± SD after back transforming from ANOVA results. Aggression experiments were analyzed using an ANOVA with 3 (photoperiod: short, normal and long photoperiod) and 2 (treatment: control and pinx).

4. RESULTS

4.1 Results of Anxiety Study (Open Field Measurements)

4.1.1 Total Distance Travelled on the Open Field (TDTOF)

The main effect of the treatment (control and pinx) was significant on the total distance travelled, F(1,69) = 16.57, p = 0001, n^2 = .19. The subjects in the pinx condition (M = 2051.25 cm) travelled more distance than those in the control condition (M = 1610.70 cm) (Figure 4.1).

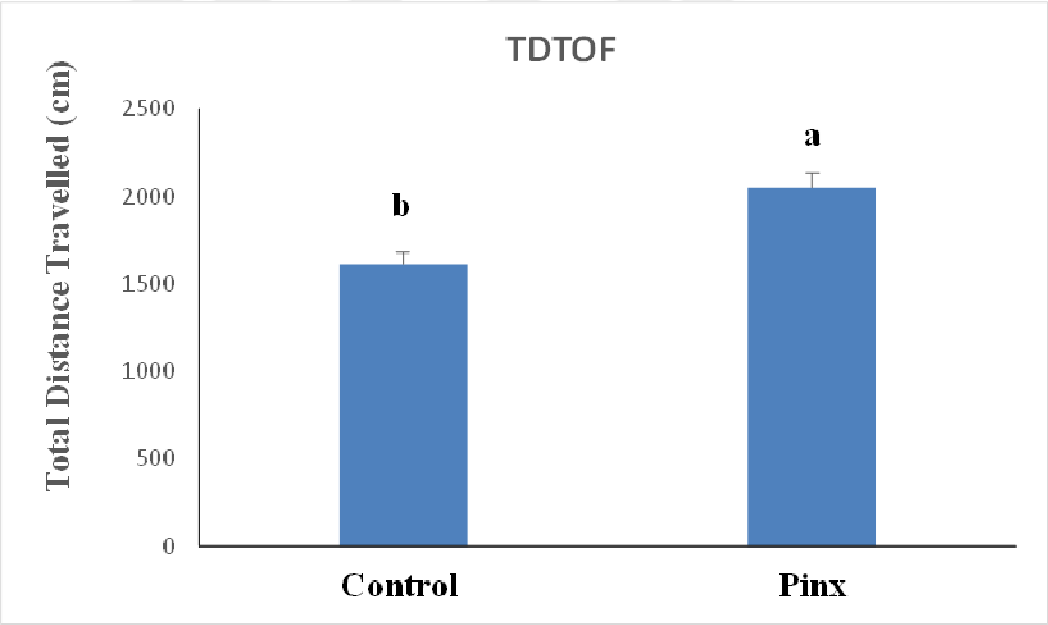

Figure 4. 1 Mean ± Standart Deviation (S.D.) of the total amount of distance travelled on the open field in control and pinealectomy group. Mean-values with the different letters above vertical columns are significantly different (p<0.05)

The effect of photoperiod was also significant F(2,69)=41.13, p=.0001, n^2=.54. The Duncan test showed that the subjects in the normal photoperiod (M=2387.67 cm) travelled more distance than those in the short (M=1449.38 cm)

and long (M=1516.33 cm) photoperiod, but with no significant difference between the last two conditions (e.g., short and long photoperiod) (Figure 4.2).

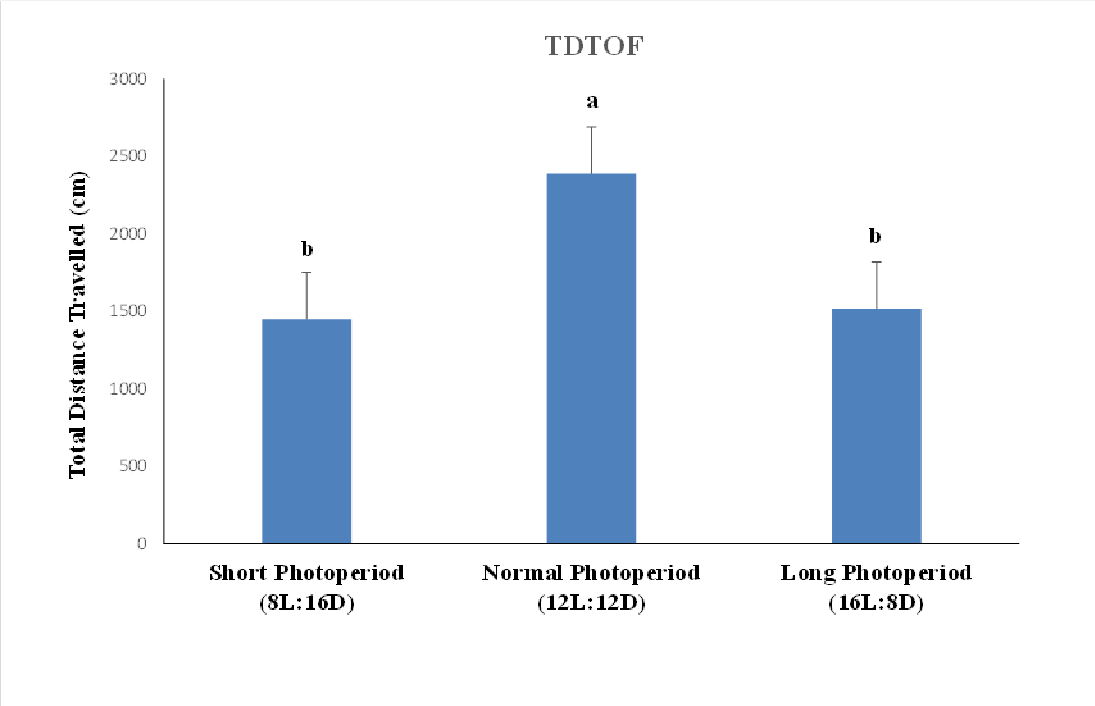

Figure 4. 2 Mean ± S.D. of the total amount of distance travelled in the different photoperiod conditions (short photoperiod-8L:16D, normal photoperiod-12L:12D, and long photoperiod 16L:8D)

However there was no significant interaction effect between treatment and photoperiod $F(2,69) = 1.55$, $p = .22$, $\eta^2 = .04$. Measurement times had significant effect on the total distance travelled, Wilks' Lambda = .50, $F(3,67) = 22.17$, $p = .001$, $\eta^2 = .50$. The subjects that were tested at 24:00 h (M = 2336.57 cm) travelled more distance than those at 18:00 h (M = 2083.04 cm), 12:00 h (M = 1448.48 cm) and 06:00 h (M = 1455.82 cm) (Figure 4.3). However, there was no significant difference between those at 6:00 and 12:00 h. The interaction effect between measurement time and treatment was significant, Wilks' Lambda = .83, $F(3, 67) = 4.67$, $p = .005$, $\eta^2 = .17$. There was no significant difference between pinx (pinealectomy) and control condition at the 12:00 h; however, the subjects in the pinx travelled more distance than those in control at 6:00 h 18:00 h and 24:00 h (Figure 4.4).

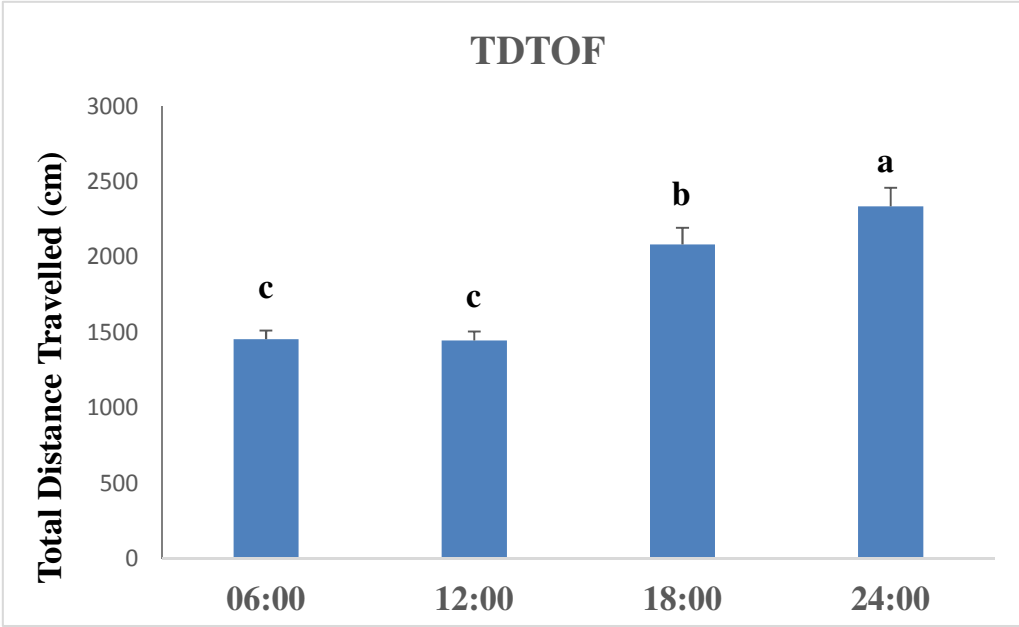

Figure 4. 3 Mean ± S.D. of the total amount of distance travelled in four different measurement time of the day (06:00, 12:00, 18:00, and 24:00 h)

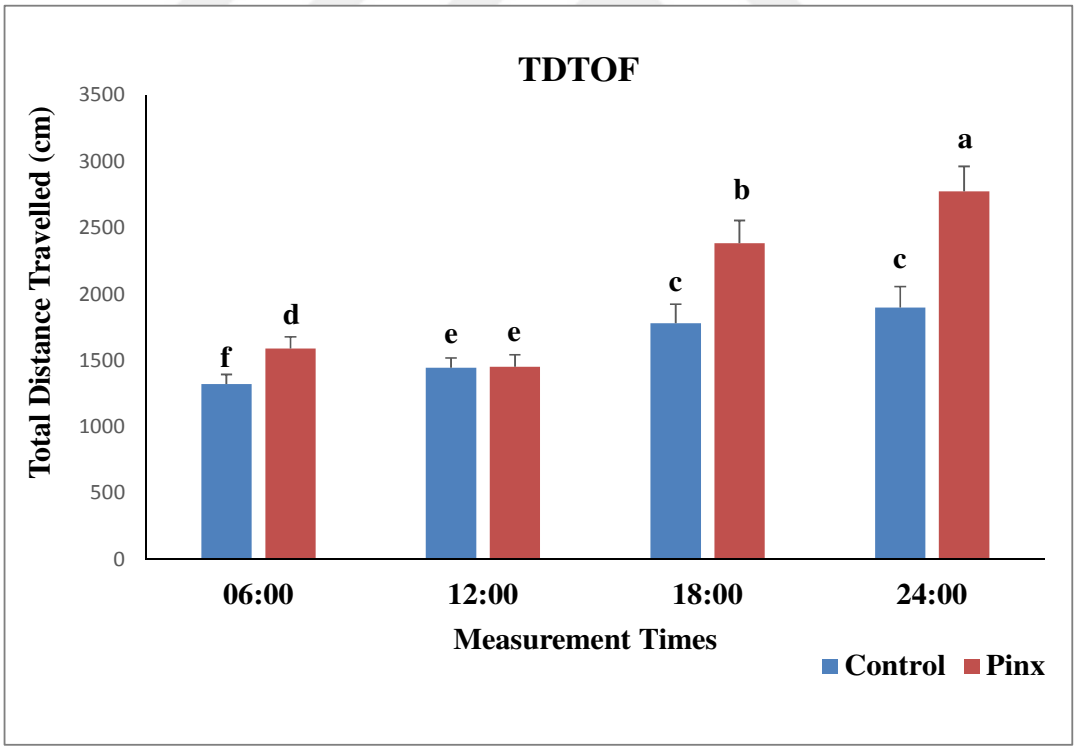

Figure 4. 4 Mean ± S.D. of the total amount of distance travelled of control and pinealectomy subjects at four different measurement time of the day (06:00, 12:00, 18:00, and 24:00 h)

The interaction effect between measurement time and photoperiod was significant, Wilks' Lambda = .68, F(6,134) = 4.72, p = .001 , η^2 = .18. There was no significant effect between short and long photoperiod at 6:00 h, 12:00 h and 18:00 h, except to 24:00 h measurement time. The subjects in the normal photoperiod travelled more distance than those in the short and long photoperiod across all measurement times. In other words, normal photoperiod was significantly more active than short and long photoperiod at all tested measurement times (06:00, 12:00, 18:00, and 24:00 h) of the day (Figure 4.5).

Lastly, the interaction effect among the measurement time, treatment and photoperiod was significant, Wilks' Lambda = .75, F (6,134) = 3.42, p = .004, η^2 = .13. The pinx subjects in the normal photoperiod had the highest performance of all other conditions (Figure 4.6).

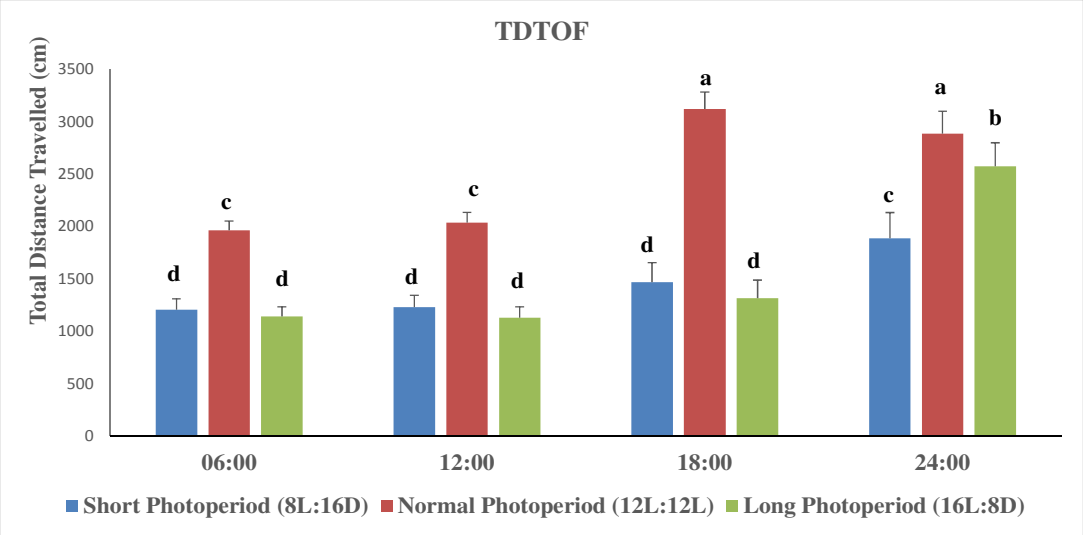

Figure 4. 5 Mean ± S.D. of the total amount of distance travelled in four different measurement time (06:00, 12:00, 18:00, and 24:00 h) of the day with different photoperiod conditions

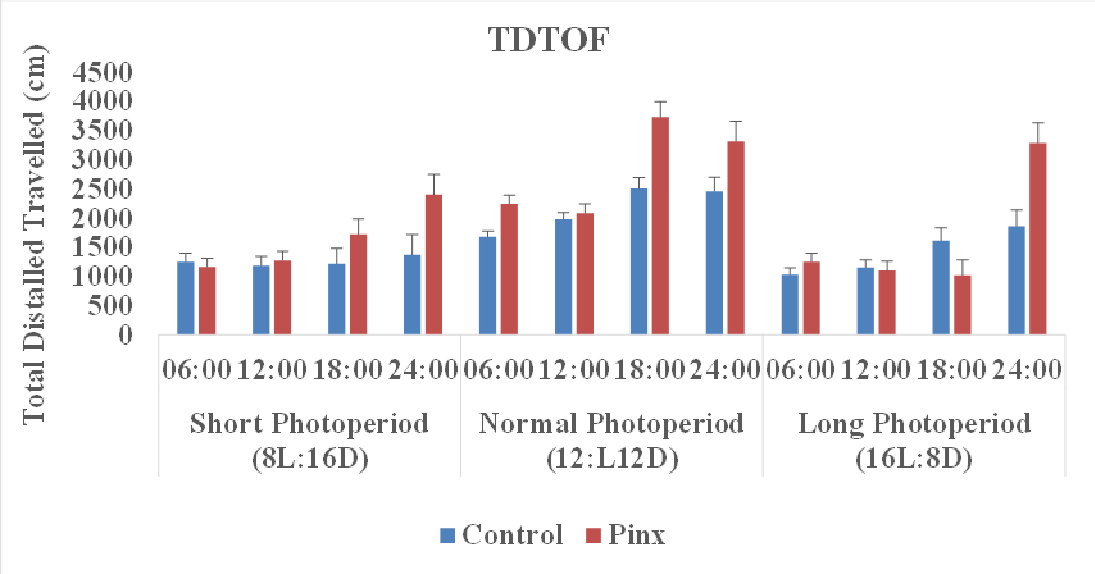

Figure 4. 6 Mean ± S.D. of the total amount of distance travelled of treatments subjects (control and pinx) in four different measurement time of the day with different photoperiod conditions

4.1.2 Time Spent at the Edge of the Open Field (TSEOF)

The effect of treatment was significant, $F(1,69) = 7.32$, $p = .009$, $\eta^2 = .10$. The subjects of pinx (pinealectomy) group (M = 4.96 min) had more duration at the edge than those in the control group (M = 4.92 min) (Figure 4.7). However, the effect of photoperiod was not significant, $F(2,69) = 1.09$, $p = .34$ (Figure 4.8) However, the interaction effect between measurement time and photoperiod was significant, Wilks' Lambda = .52, $F(6,134) = 8.59$, $p = .001$, $\eta^2 = .28$ (Figure 4.9). Also, the interaction effect among the measurement times, treatments, and photoperiods was not significant, Wilks' Lambda = .83, $F(6,134) = 2.09$, $p = .06$, $\eta^2 = .09$ (Figure 4.10).

The subjects in the normal and long photoperiod spent more time at the edge of the open field than those in the short photoperiod at 6:00 h. The subjects in the short and normal photoperiod spent more time than those in the long photoperiod at 12:00h. However, the subjects in the short photoperiod spend more time than those in the normal and long photoperiod at 18:00 h and 24:00 h (Figure 4.10).

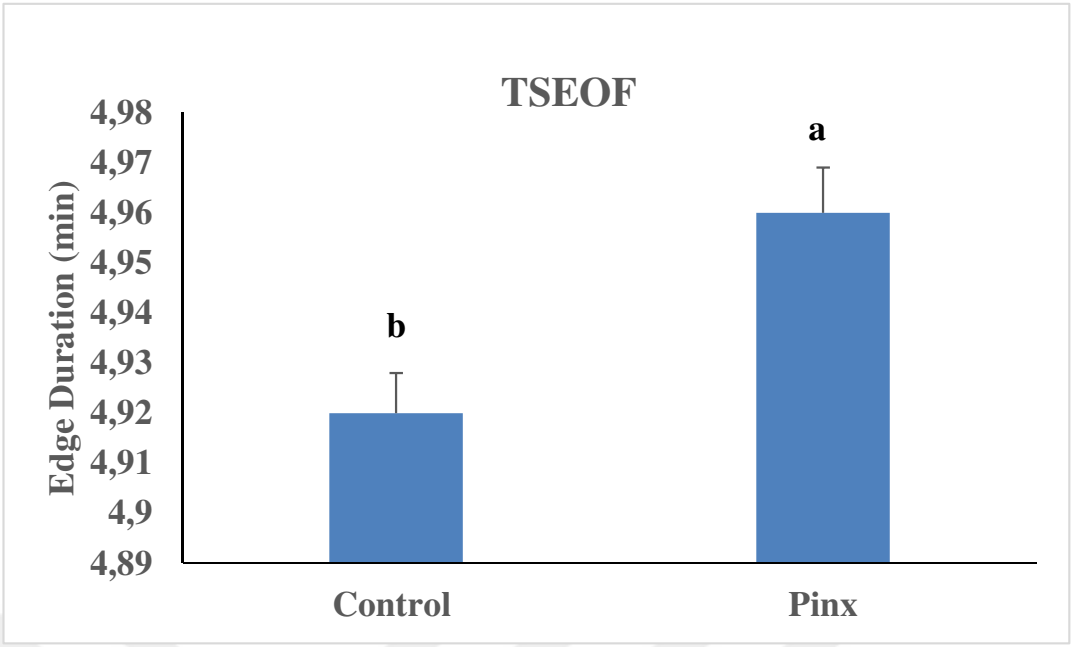

Figure 4. 7 Mean ± S.D. of the time spent at the edge of the open field at treatments (control and pinx)

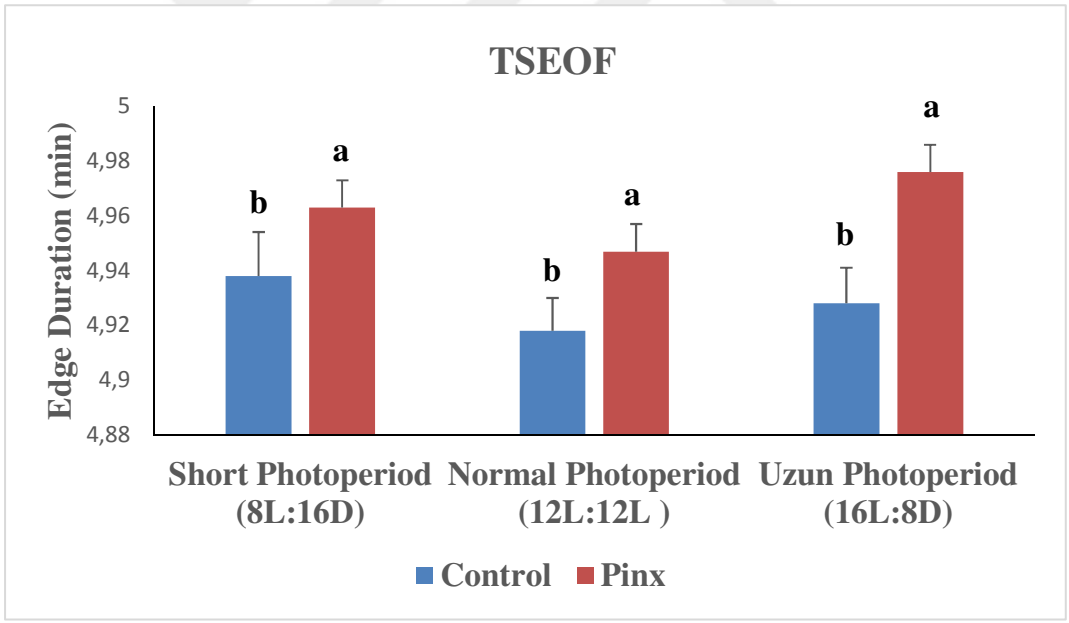

Figure 4. 8 Mean ± S.D. of the time spent at the edge of the open field at treatments (control and pinx) and different photoperiod conditions

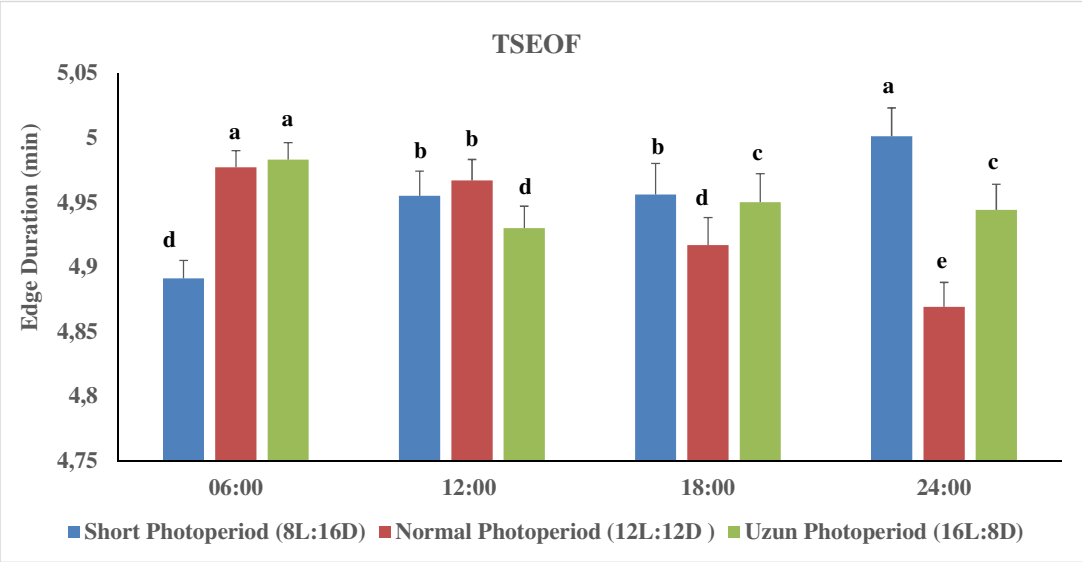

Figure 4. 9 Mean ± S.D. of the time spent at the edge of the open field for measurement times and different photoperiod conditions

The treatments (control and pinx), photoperiods (short photoperiod-8L:16D, normal photoperiod-12L:12D, and long photoperiod-16L:8D), and the four different measurement times (06:00, 12:00, 18:00, and 24:00 h) mean values are given at figure 4.10, collectively.

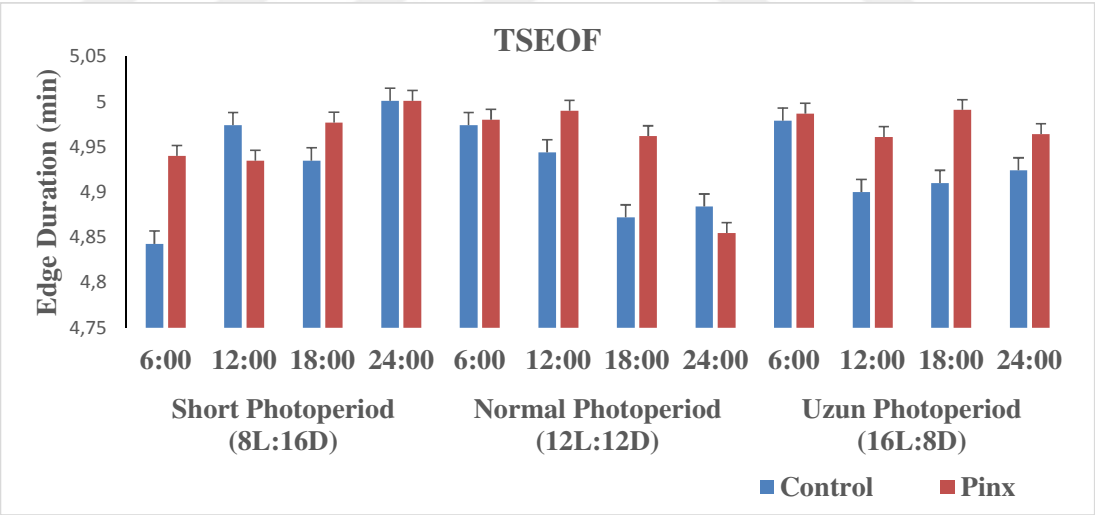

Figure 4. 10 Mean ± S.D. of the time spent at the edge of the open field for treatment (control and pinx), different photoperiod conditions, and measurement times

4.1.3 Frequency at the Edge of the Open Field (FEOF)

The effect of the photoperiod was signification the frequency at the edge of the open field, F(2,69) = 12.27, p = .0001, n^2 =.26. This means that the subjects in the normal photoperiod (M = 4.51) had more frequency than those in the long photoperiod (M = 2.65) and those in the short photoperiod (M = 1.85) by the Duncan test (Figure 4.11).

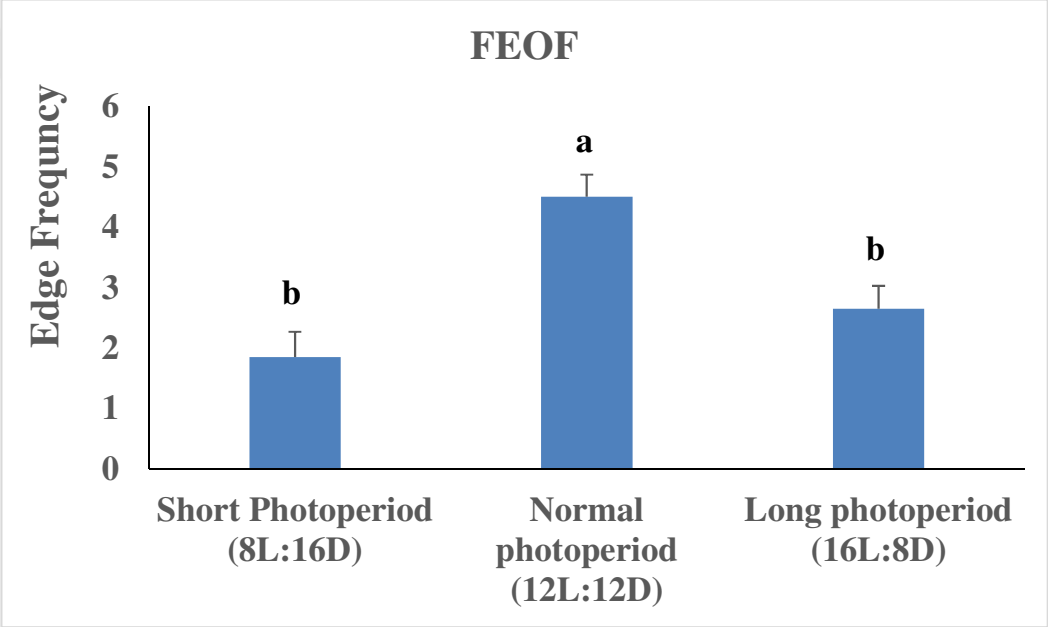

Figure 4. 11 Mean ± S.D. of the entry frequency of the edge in the different photoperiod conditions

4.1.4 Time Spent at the Center of the Open Field (TSCOF)

The effect of treatment was significant on the time spent at the center of the open field, F(1,69) = 12.86, p = .0001, n^2 =.16. The subjects in the control condition (M = .081 min) stayed longer time in the center than those in the pinx condition (M = 0.39 min) (Figure 4.12). The interaction effect between photoperiod and treatment was not significant, F(2,69) =.06, p = .94, n^2 = .002. However, the interaction effect between measurement time and photoperiod was significant, Wilks' Lambda = .51,

F(6,134) = 8.93, p = .0001, η^2 = .29. The subjects in the short photoperiod stayed longer time at the center then those in the normal and long photoperiod in the morning (at 06:00 h); however, there was no difference among conditions at 12:00 h. On the other hand, the subjects in the normal photoperiod stayed longer at center then those in the short and long photoperiod at 18:00 h and 24:00 h (Figure 4.13). In addition, the interaction effect between the measurement time and treatment was not significant, Wilks' Lambda = .94, F (3,67) = 1.41, p = .25, η^2 = .06.

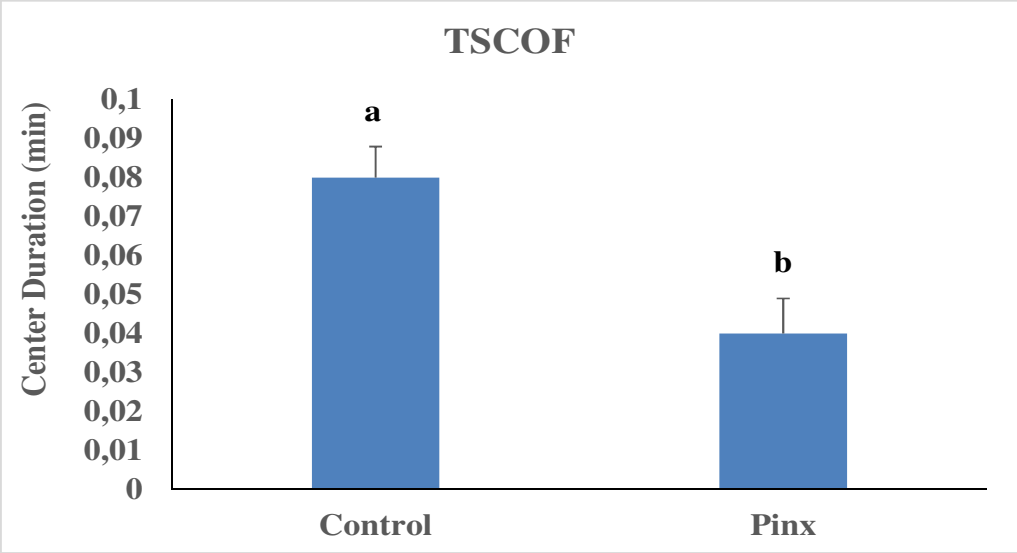

Figure 4. 12 Mean ± S.D. of the time spent at the center of the open field in treatments (control and pinx)

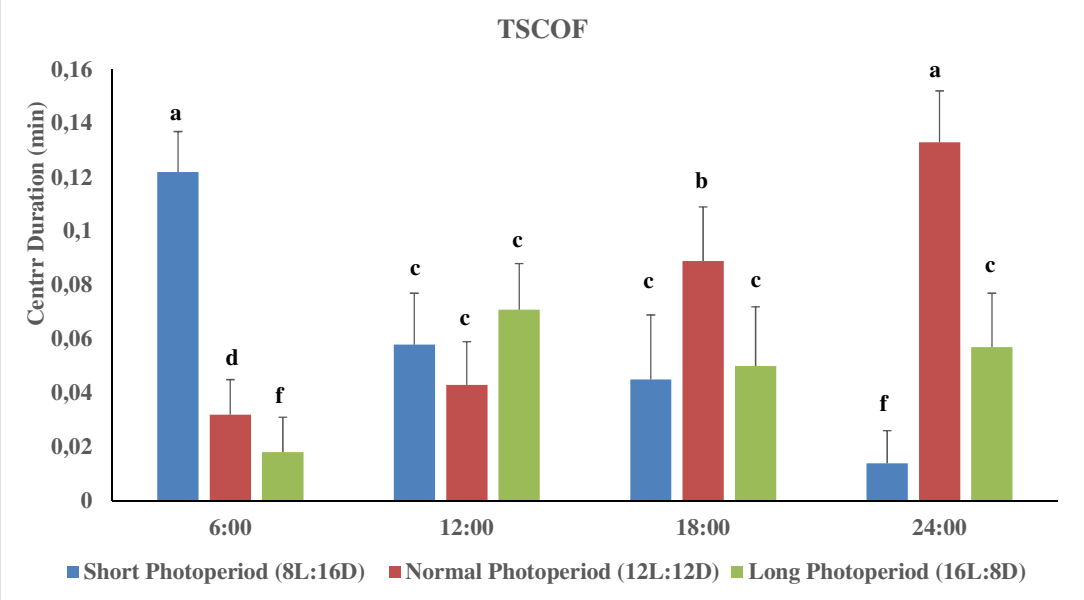

Figure 4. 13 Mean ± S.D. of the time spent at the center of the open field in different photoperiod conditions

The interaction effect among measurement time, treatment and photoperiod was not significant, Wilks' Lambda = .83, F (6,134) = 2.19, p = .05, n^2 = .09 (data not shown).

4.1.5 Frequency at the Center of the Open Field (FCOF)

The effect of photoperiod was significant on the frequency at the center of the open field, F(2,69) = 10.55, p = .0001, n^2 = .23. This means that the subjects in the normal photoperiod (M = 3.51) had more frequency than those in the long photoperiod (M = 1.96) and those in the short photoperiod (M = 1.03) (Figure 4.14) by the Duncan test.

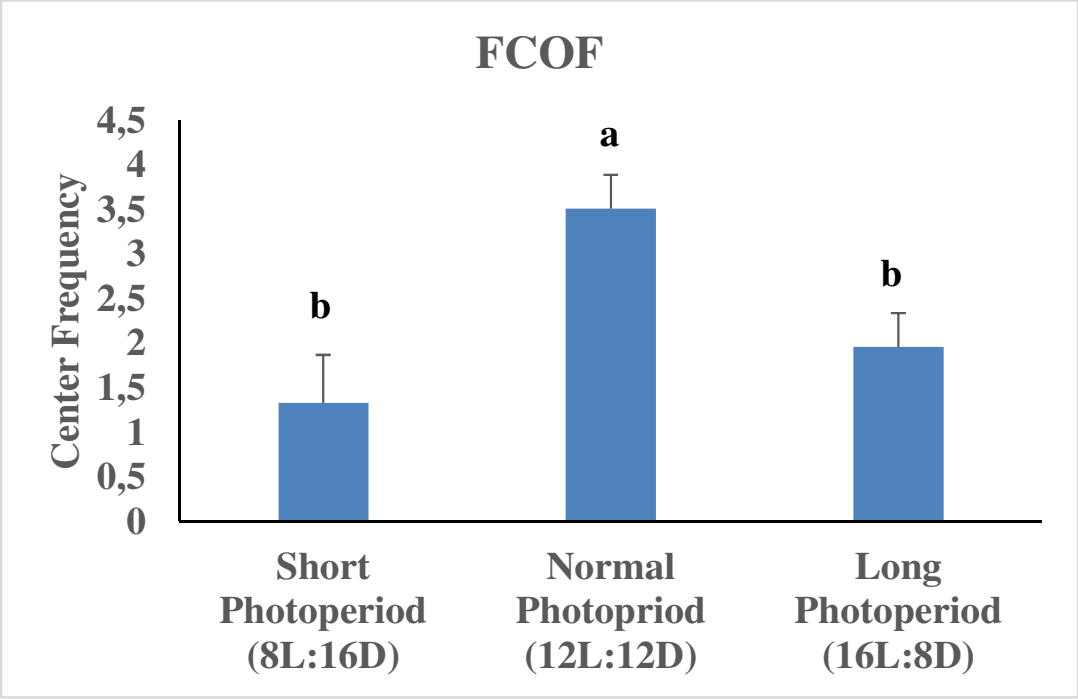

Figure 4. 14 Mean ± S.D. of the entry fequency of the center in the different photoperiod conditions

4.1.6 Mobility Duration in the Open Field (MDOF)

The effect of treatment was significant on mobile duration (or mobility), F (1,69) = 64.13, p = .0001, n^2=.48. The subjects in the pinx group (M = .244 min)

were more mobile than those in the control group (M = .066 min) (Figure 4.15). The effect of photoperiod was significant, F (2,69) = 16.99, p = .0001, n^2 = .34. The subjects in the normal photoperiod (M = .243 min) were more mobile than those in the short photoperiod (M = .121 min) and those in the long photoperiod (M = .102 min) (Figure 4.16). The interaction effect between treatment and photoperiod was significant, F (2,69) = 9.76, p = .0001, n^2 = .22. The difference in mobility between pinx and control conditions was greater in normal photoperiod than those between those conditions in short and long photoperiod (Figure 4.17).

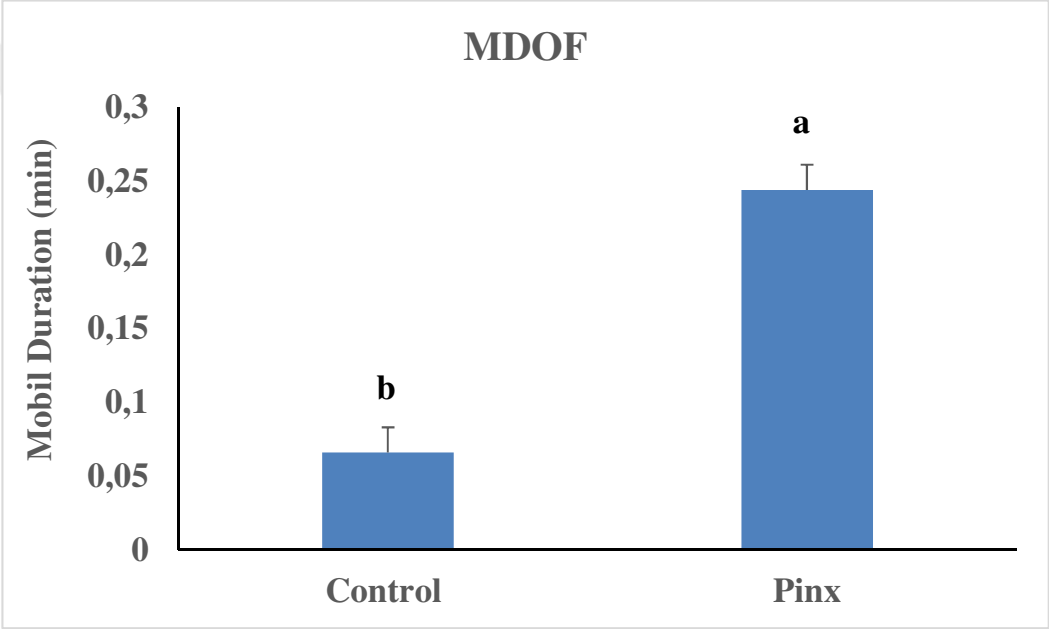

Figure 4. 15 Mean ± S.D. the mobility duration on the open field for treatment conditions (control and pinx)

The effect of measurement times was significant, F (3,67) = 79.30, p = .0001, n^2 = .78. The subjects were more mobile (M = .33 min) at 24:00 h than those at 18:00 h (M = .17 min) and those at 12:00 h (M = .10 min) and those at 6:00 h (M = .02 min) with all differences being significant ($p<0.05$) (Figure 4.18).

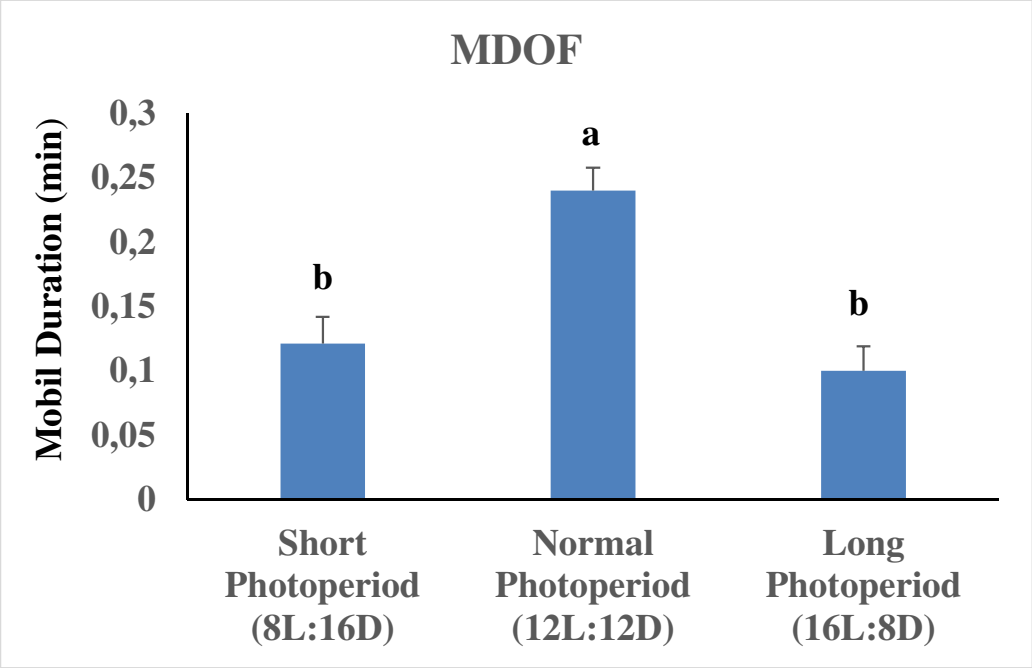

Figure 4. 16 Mean ± S.D. the mobility duration on the open field for different photoperiod conditions

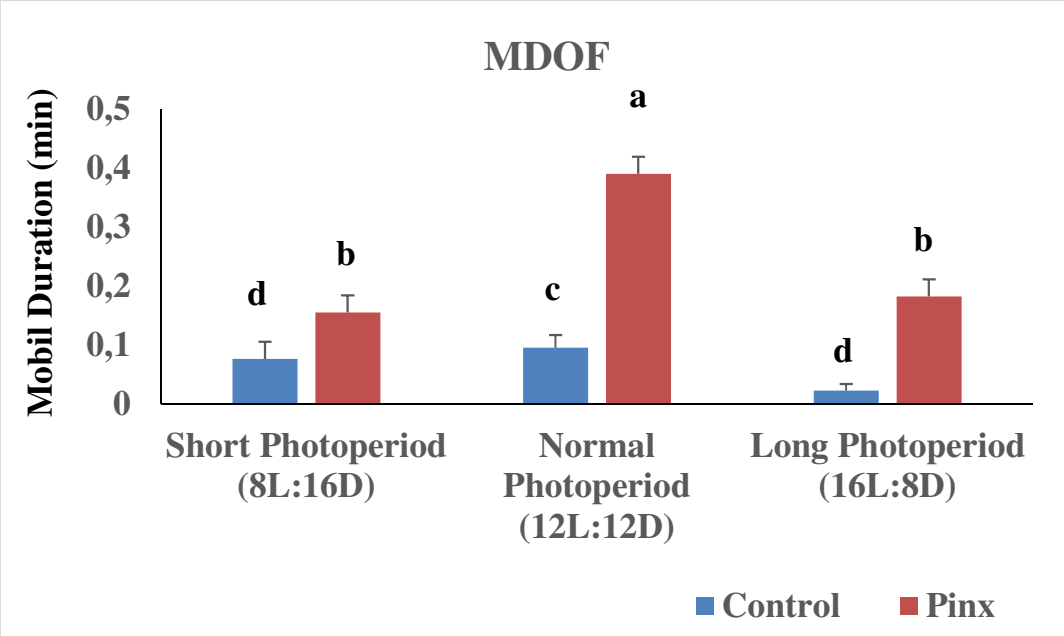

Figure 4. 17 Mean ± S.D the mobility duration on the open field for treatment conditions (control and pinx) and different photoperiod conditions

The interaction effect between measurement time and treatment was significant, Wilks' Lambda = .50, F (3,67) = 22.16, p = .0001, n^2 = .50. The

difference between control and pinx were greater at 24:00 h and 18:00 h than 12:00 h and 06:00 h (Figure 4.19).

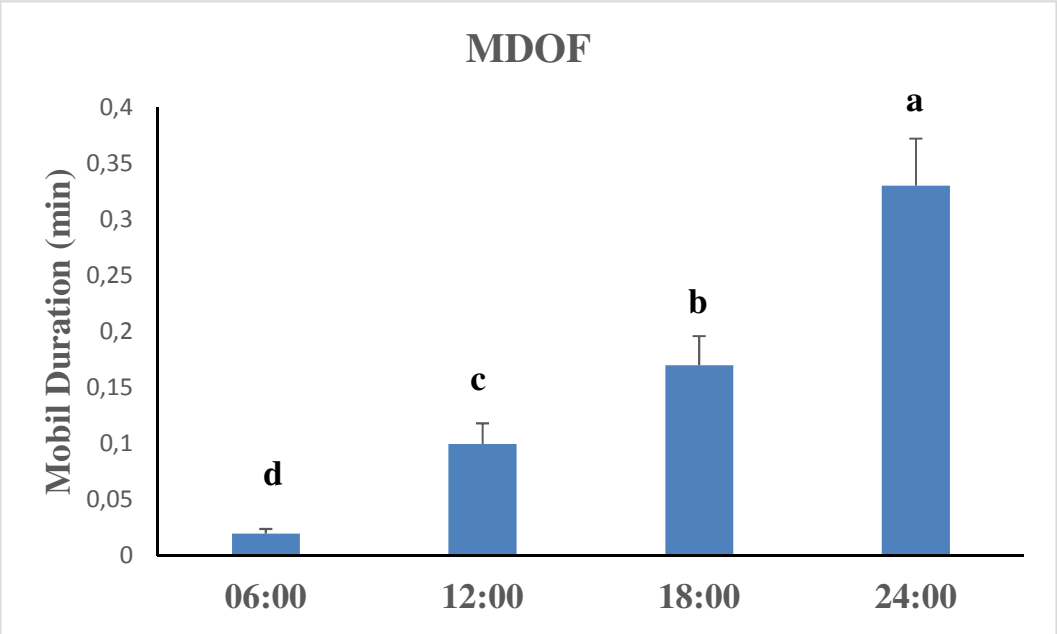

Figure 4. 18 Mean ± S.D. of the mobility duration on the open field for measurement times (06:00, 12:00, 18:00, and 24:00 h)

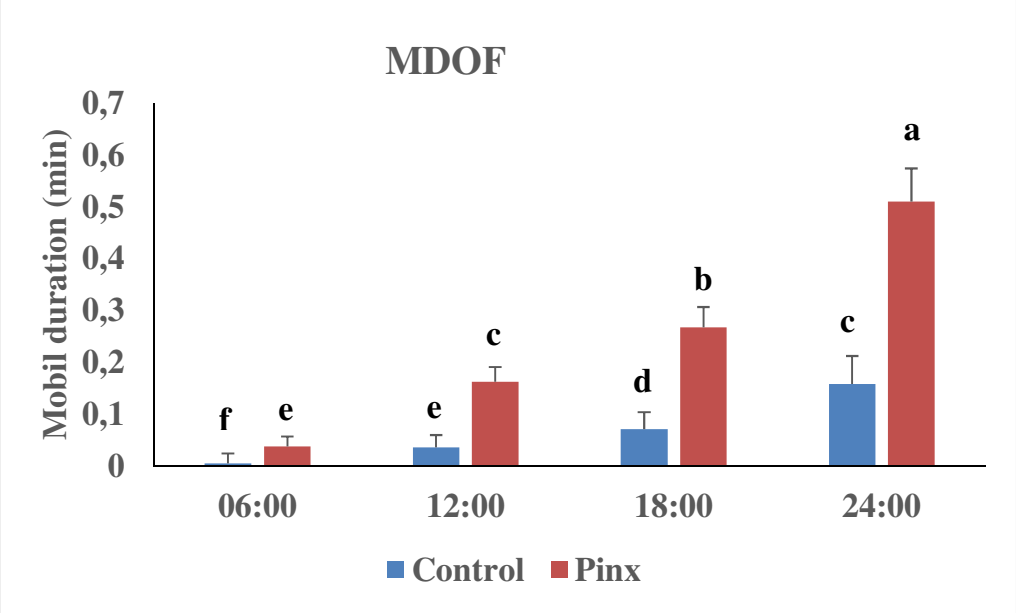

Figure 4. 19 Mean ± S.D. of the mobility duration on the open field for treatment conditions (control and pinx) and measurement times (06:00, 12:00, 18:00, and 24:00 h)

The interaction effect between measurement times and photoperiod was significant, Wilks' Lambda = .40, F (6,134) = 13.13, p = .0001, η^2 = .37. There was no significant difference across short, long and normal photoperiod in the morning (at 18:00 h). However, the subjects in normal photoperiod were more mobile than those in short photoperiod and those in the long photoperiod at 12:00 h. However, the subjects in the long and normal photoperiod with being not significant from each other, were more mobile than those in the short photoperiod at 24: 00 h (Figure 4.20).

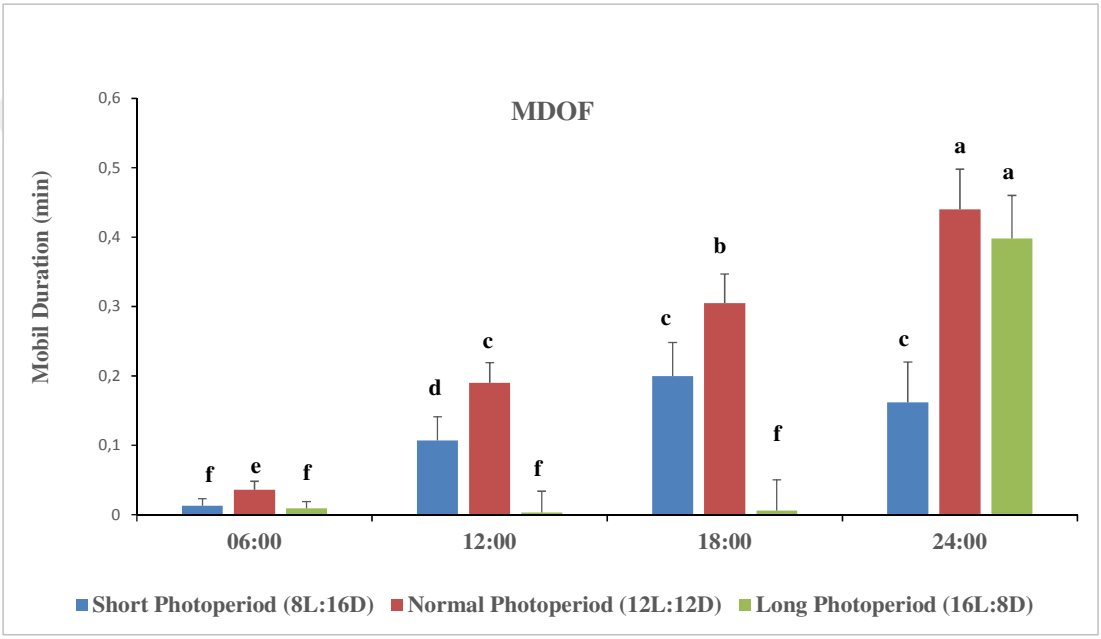

Figure 4. 20 Mean ± S.D. of the mobility duration on the open field in different photoperiod conditions, and measurement times

The interaction effect among measurement time, treatment and photoperiod was significant F (6,134) = 11.10, p = .0001, η^2 = .33. The subjects in the pinx condition were the most mobile of control conditions in short, normal and long photoperiod at 24:00 h. The subjects in the control condition were mobile than those pinx condition at 12:00 h and 18 h for short period, and at 06:00 h, 12:00 h and 18:00 h for normal and long photoperiods (Figure 4.21).

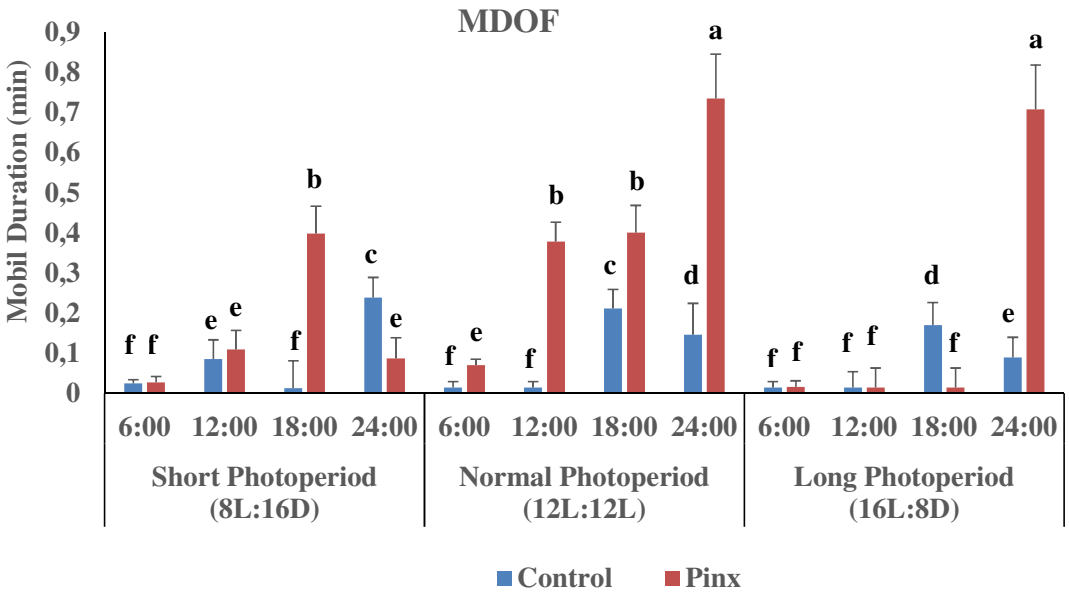

Figure 4. 21 Mean ± S.D. of the mobility duration on the open field in different photoperiod conditions, and measurement times (06:00, 12:00, 18:00, and 24:00 h) and, treatment conditions (control and pinx)

4.1.7 Mobility Frequency in the Open Field (MFOF)

The effect of treatment was significant on mobile frequency (or mobility), $F(1,69) = 90.44$, $p = .0001$, $\eta^2 = .57$. The subjects in the pinx group ($M = .139.29$) were more mobile than those in the control group ($M = 33.84$) (Figure 4.22). The effect of photoperiod was significant, $F(2,69) = 26.12$, $p = .0001$, $\eta^2 = .43$. The subjects in the normal photoperiod ($M = 140.79$) were more mobile than those in the short photoperiod ($M = 63.99$) and those in the long photoperiod ($M = 54.91$) (Figure 4.23). The main effect of the measurement times was significant on the mobile frequency in open field, Wilks' Lambda = .23, $F(3, 67) = 75.42$, $p = .0001$, $\eta^2 = .77$. The mobile frequency mean values of the subjects was the highest at night (24:00 h). The subjects were faster at 24:00 h ($M = 167.62$) than those at evening (18:00) ($M = 102.17$), those at 12:00 ($M = 59.50$) and at 06:00 ($M = 16.96$). This indicated that the subjects at night and in the evening had less anxiety than those at noon and in the morning (Figure 4.24).

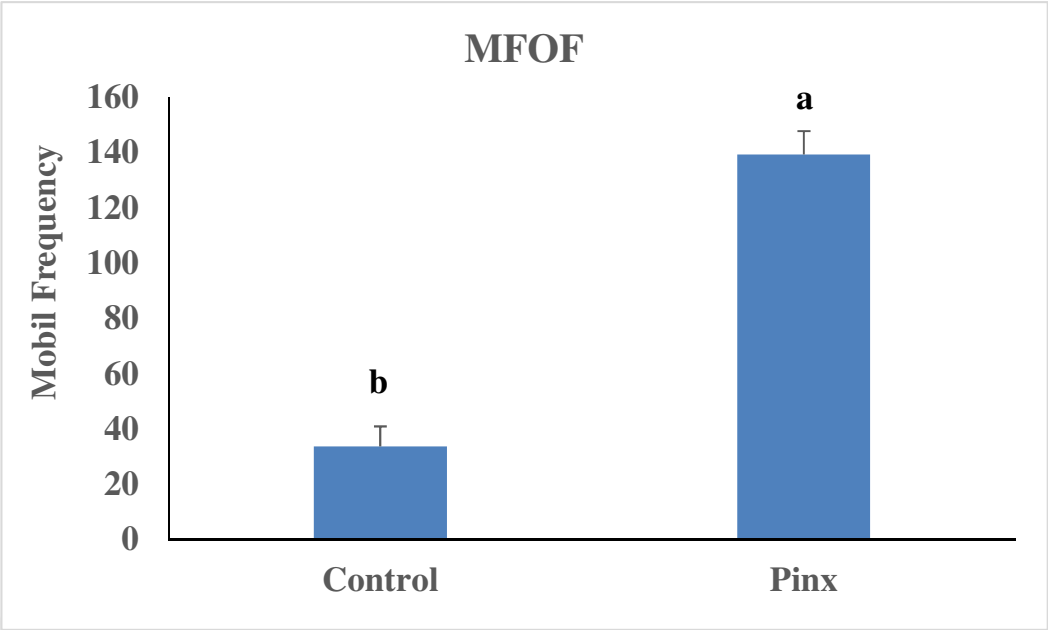

Figure 4. 22 Mean ± S.D. of the mobility frequency on the open field for treatment conditions (control and pinx)

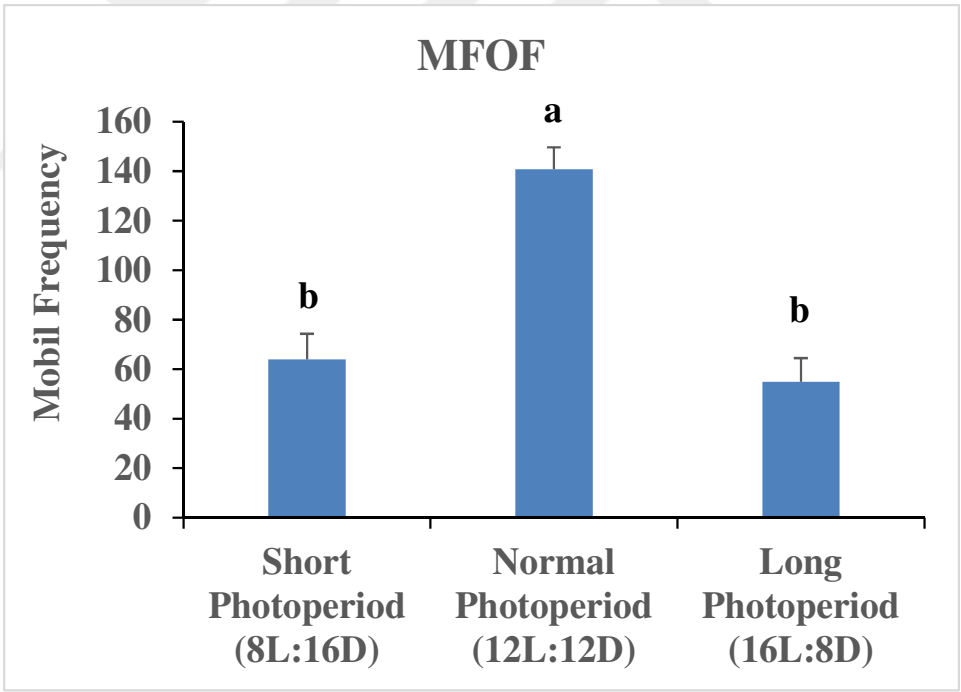

Figure 4. 23 Mean ± S.D. of the mobility frequency on the open field for different photoperiod conditions

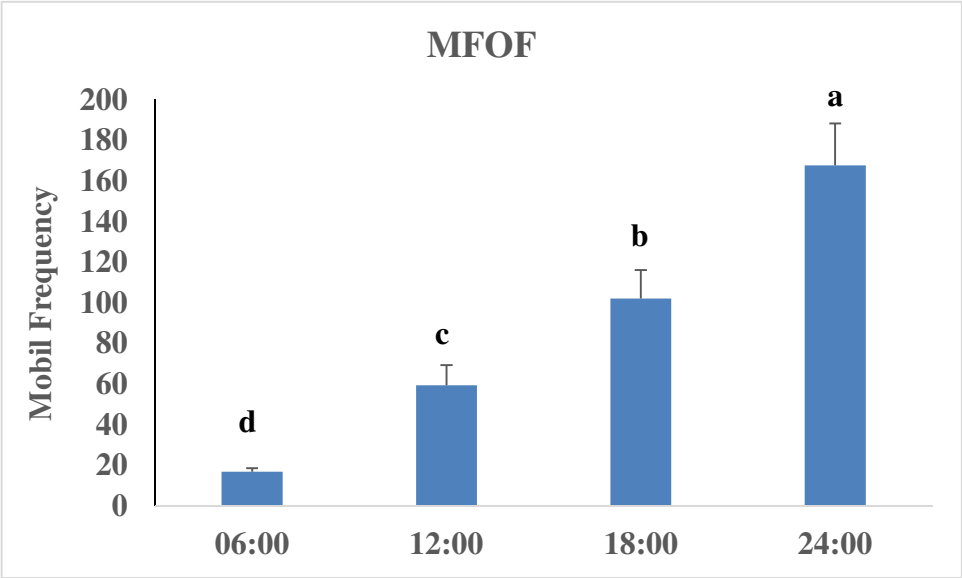

Figure 4. 24 Mean ± S.D. of the mobility frequency on the open field for measurement times (06:00, 12:00, 18:00, and 24:00 h)

The interaction effect between treatment and photoperiod was significant, F (2,69) = 13.57, p = .0001, n^2 = .28. The difference in mobility between pinx and control conditions was greater in normal photoperiod than those between those conditions in short and long photoperiod (Figure 4.25)

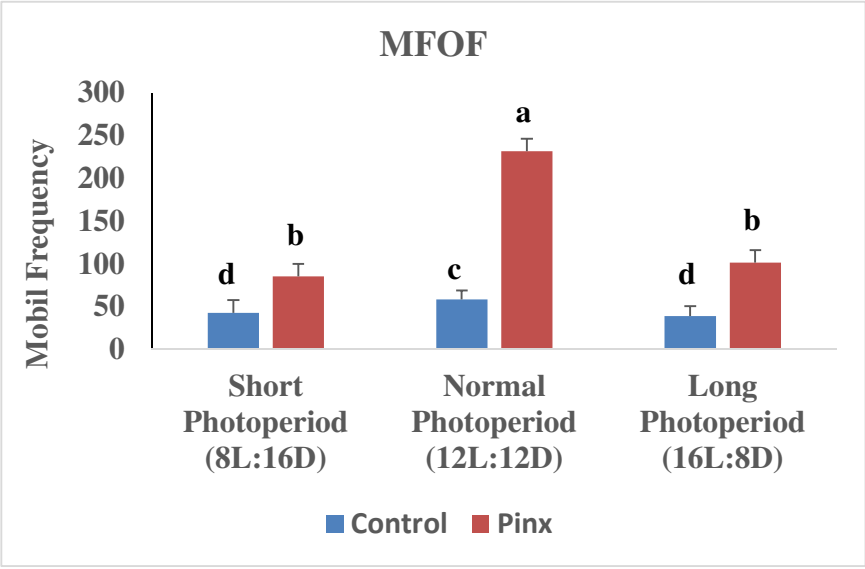

Figure 4. 25 Mean ± S.D. of the mobility frequency on the open field for treatment conditions and different fotoperiod conditions

The interaction effect between measurement time and treatment was significant, Wilks' Lambda = .51, F(3,67) = 21.52, p = .0001, η^2 = .49. The pinx subjects were higher than control subjects all measurement times (As can be seen on Figure 4.26).

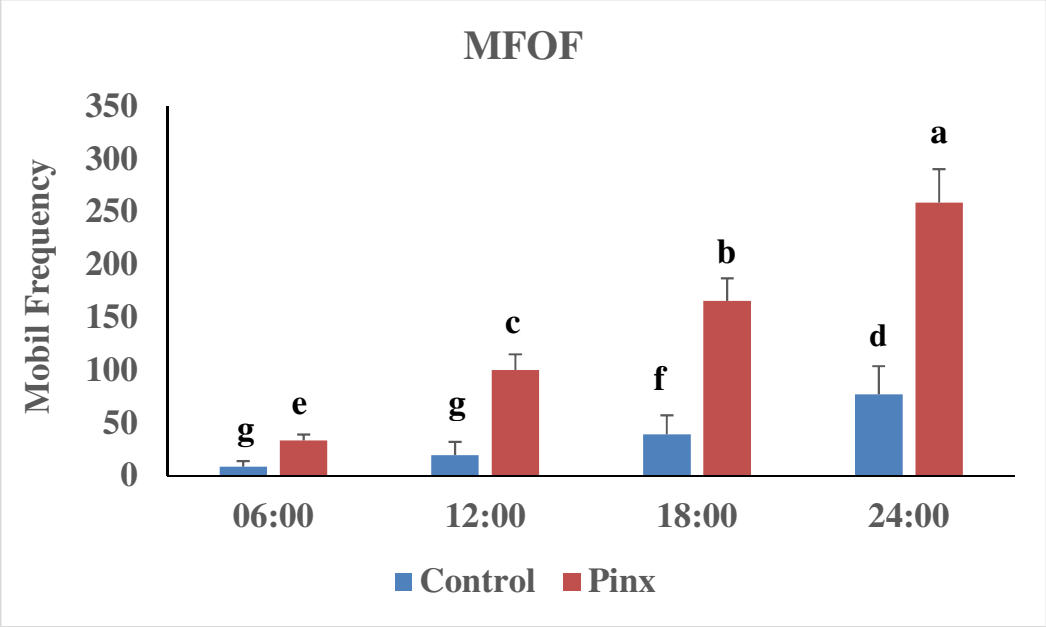

Figure 4. 26 Mean ± S.D. of the mobility frequency on the open field in different photoperiod conditions, and measurement times (06:00, 12:00, 18:00, and 24:00 h)

4.1.8 Velocity in the Open Field (VELOF)

The main effect of the measurement time was significant on the velocity in open field, Wilks' Lambda = .50, F(3, 67) = 22.67, p = .0001, η^2 = .50. The velocity mean values of the subjects were the highest at night (24:00 h). The subjects were faster at 24:00 h (M = 471.71) than those at evening (18:00) (M = 418.03), those at 06:00 (M = 291.85) and at 12:00 (M = 289.38). This indicated that the subjects in the evening and at night had less anxiety than those at noon and in the morning (Figure 4.27).

The effect of treatment was significant, F(1,69) = 16.24, p = .0001 , η^2 = .19. The subjects of pinx condition (M = 413.19) were faster than the subjects of control conditions (M = 322.29) (Figure 4.28).

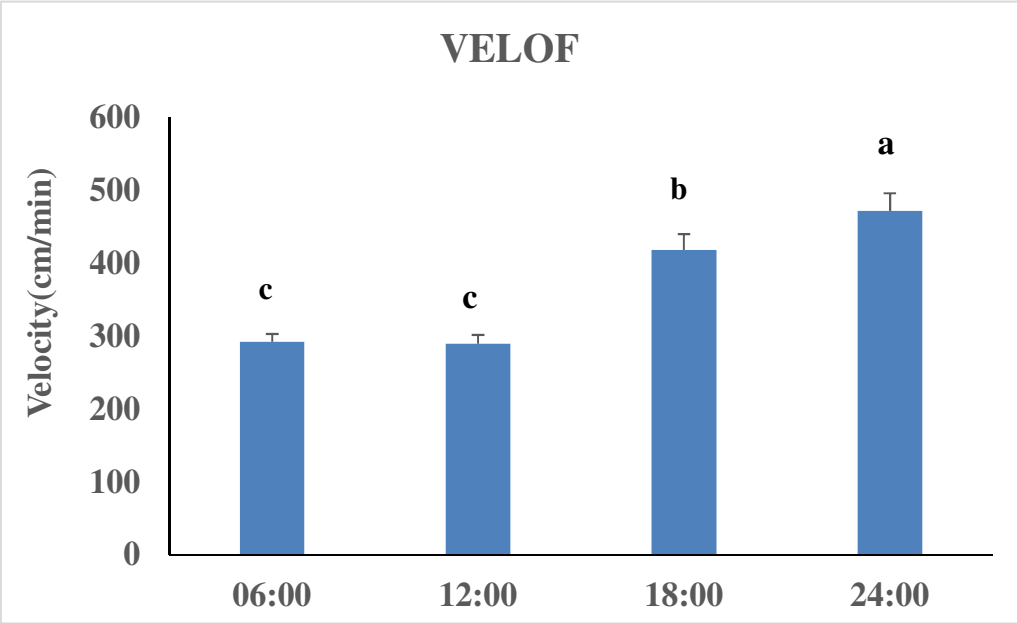

Figure 4. 27 Mean ± S.D. of the velocity of the subjects in the open field for measurements times (06:00, 12:00, 18:00, and 24:00 h) of the day

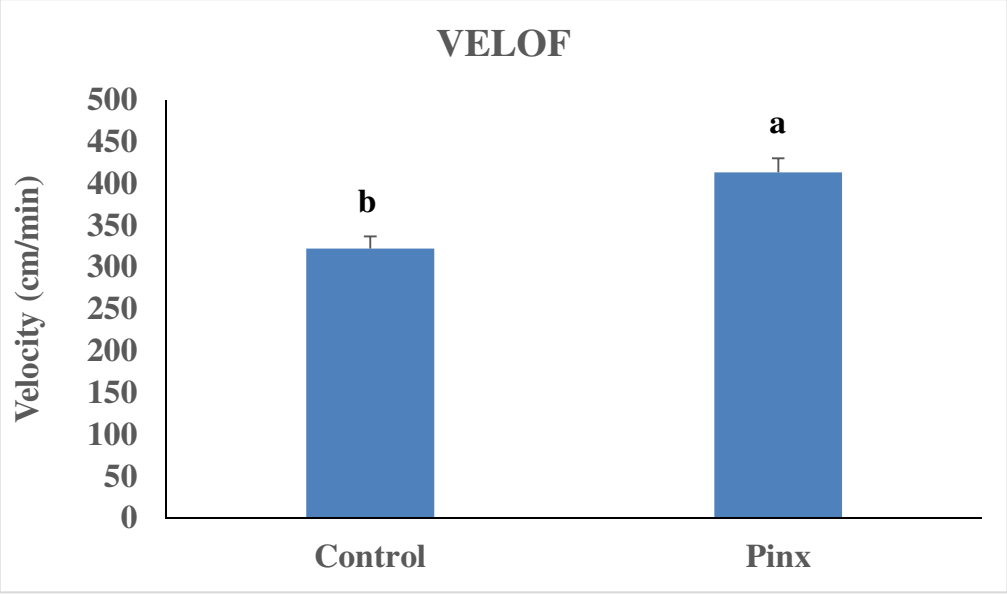

Figure 4. 28 Mean ± S.D. of the velocity of the subjects in the open field for treatments (control and pinx)

The main effect of the photoperiod was significant on the velocity, F(2, 69)=37.17, *p*=.0001, n^2=.52. The velocity of the subjects in normal photoperiod (M=500.76) was higher than long (M=309.43) and short (M=293.45) photoperiods (Table 1 and Figure 4.29).

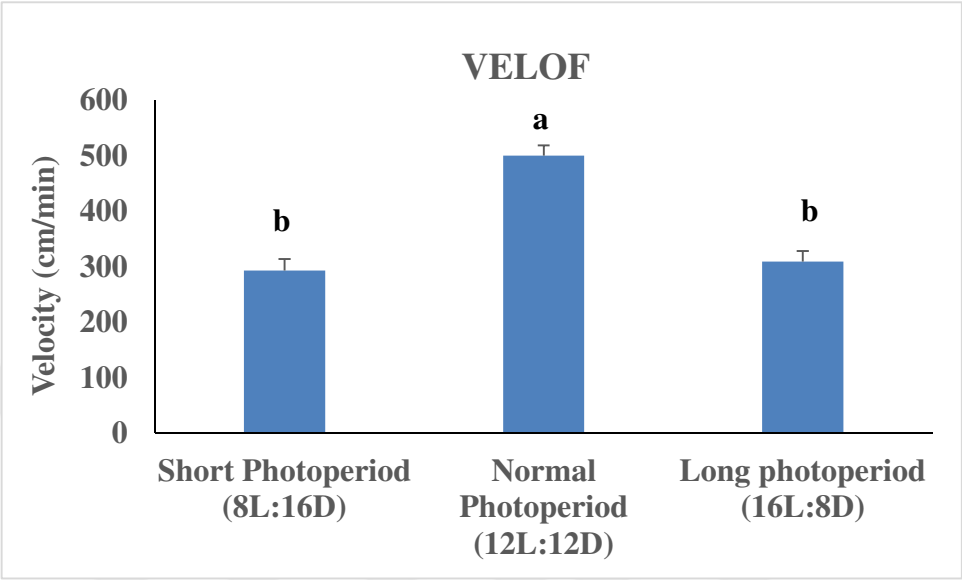

Figure 4. 29 Mean ± S.D. of the velocity of the subjects in the open field for different photoperiod conditions

Table 4. 1 Mean, standart error of velocity values (cm/min) of the subjects in the different photoperiod conditions.

Photoperiods	**Mean**	**Std. Error**	**95% Confidence Interval**	
	Lower Bound	Upper Bound	Lower Bound	Upper Bound
Short (8L:16D)	293,045	21,052	251,048	335,043
Normal (12L:12D)	500,756	18,232	464,385	537,127
Long (8L:16D)	309,434	19,218	271,096	347,773

The interaction effect between measurement time and treatment was significant, Wilks' Lambda=.84, F(3,67)=4.42, p=.007, n^2=.17. The pinx subjects were faster than control subjects all measurement times (06:00, 12:00, 18:00, and 24:00 h) (As can be seen on Figure 4.30).

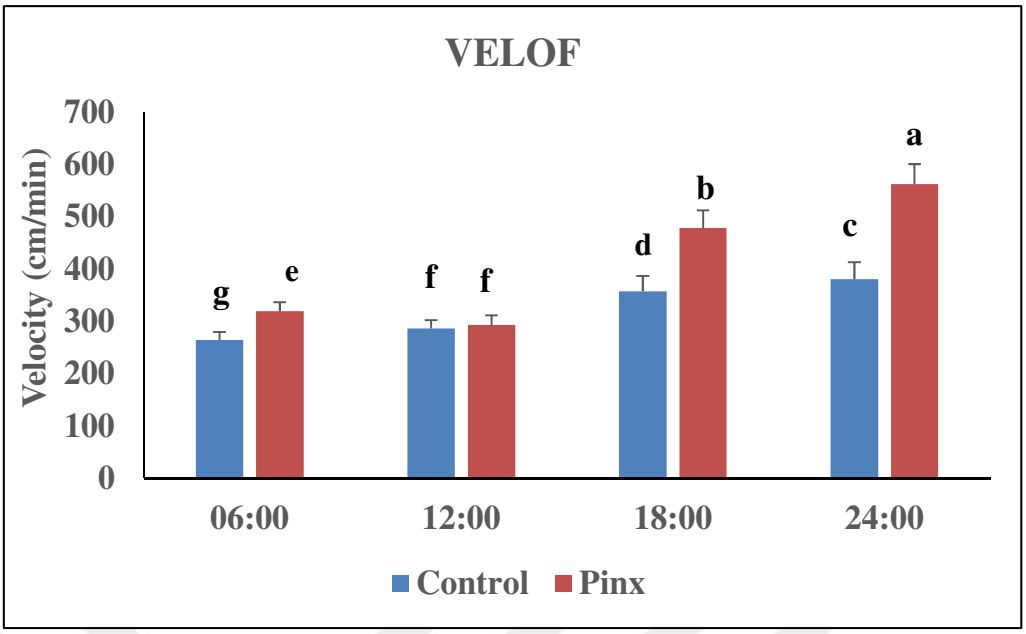

Figure 4. 30 Mean ± S.D. of the velocity of the subjects in the open field for measurements times (06:00, 12:00, 18:00, and 24:00 h) of the day and treatment conditions (control and pinx)

The interaction effect between measurement time and photoperiod was significant, Wilks' Lambda = .68, F (6,136) = 4.67, p = .001, η^2 = .17. Normal photoperiod was significantly more active than short and long photoperiod at all tested measurements times (06:00 h, 12:00 h, 18:00 h, 24:00 h) (Figure 4.31).

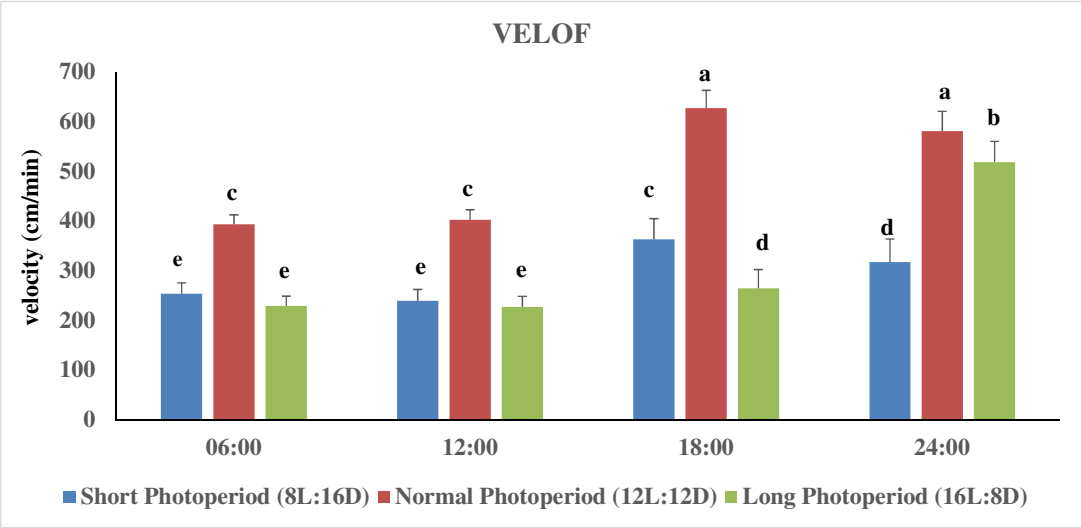

Figure 4. 31 Mean ± S.D. of the velocity of the subjects in the open field for measurements times (06:00, 12:00, 18:00, and 24:00 h) of the day and different photoperiod conditions

The interaction effect among measurement time, treatment and photoperiod was significant, Wilks' Lambda = .76, F (6,134) = 3.29, p = .006, n^2 = .13. The pinx subjects were more mobile at 6:00, 12:00, 18:00 and 24:00 h in normal photoperiod than of all other measurement times and photoperiods (Figure 4.32).

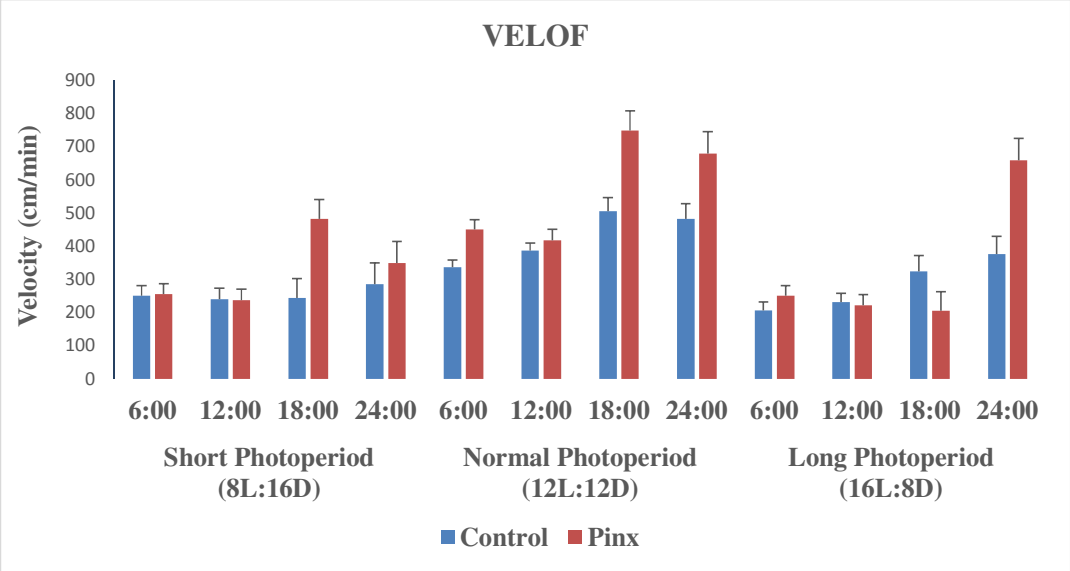

Figure 4. 32 Mean ± S.D. of the velocity of the subjects in the open field for treatment conditions (control and pinx), measurements times (06:00, 12:00, 18:00, and 24:00 h) of the day and different photoperiod conditions

4.1.9 Rotary Frequency in the Open Field (RFOF)

The effect of treatment was significant on the rotary frequency, F(1,69) = 27.77, p = .0001 , n^2 = .29. The subjects in the pinx group (M = 6.87) had more rotary frequency than those in the control group (M = 2.91) (Figure 4.33).

The effect of photoperiod was significant on rotary frequency, F (2,69) = 6.68, p = .002, n^2 =.16. The subjects in the normal photoperiod (M = 6.61) were more mobile than those in the long photoperiod (M = 4.8) and those in the short photoperiod (M = 3.25) (Figure 4.34).

The main effect of the measurement times was significant on the rotary frequency in open field, Wilks' Lambda = .76, F(3, 67) = 7.07, p = .0001, n^2 = .24.

The mean values of rotary frequency for the subjects were the highest at night (24:00 h). The subjects had higher rotary frequency at 24:00 h (M = 8.08) than those at evening (18:00 h) (M = 5.16), those at 06:00 (M = 3.38) and at 12:00 (M = 2.93). This indicated that the subjects in the evening and at night had less anxiety than those at noon and in the morning (Figure 4.35).

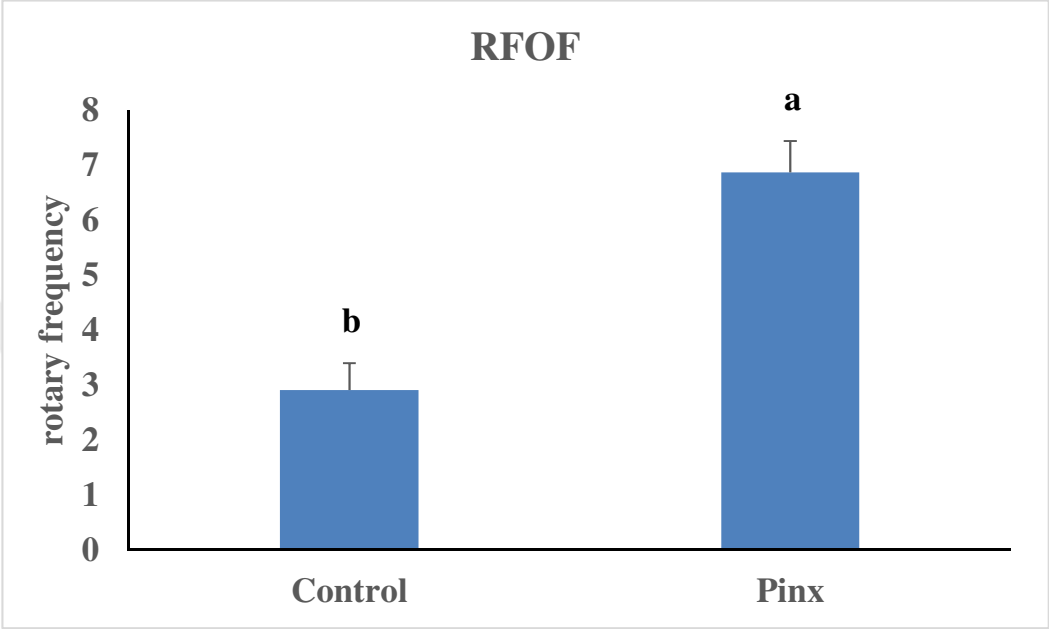

Figure 4. 33 Mean ± S.D. of the rotary frequency of the subjects in the open field for treatments (control, pinx)

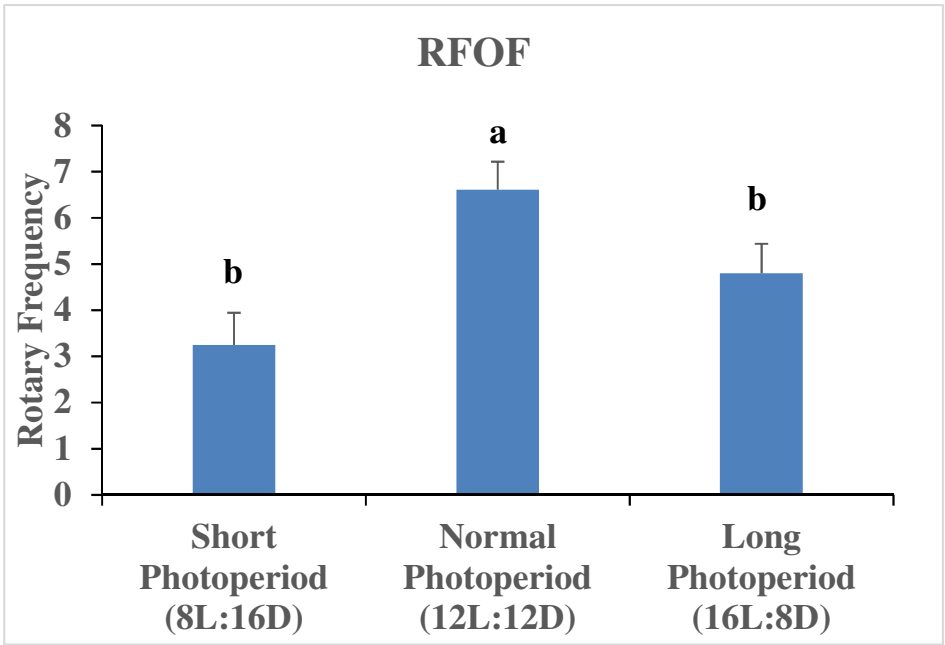

Figure 4. 34 Mean ± S.D. of the rotary frequency of the subjects in the open field for different photoperiod conditions

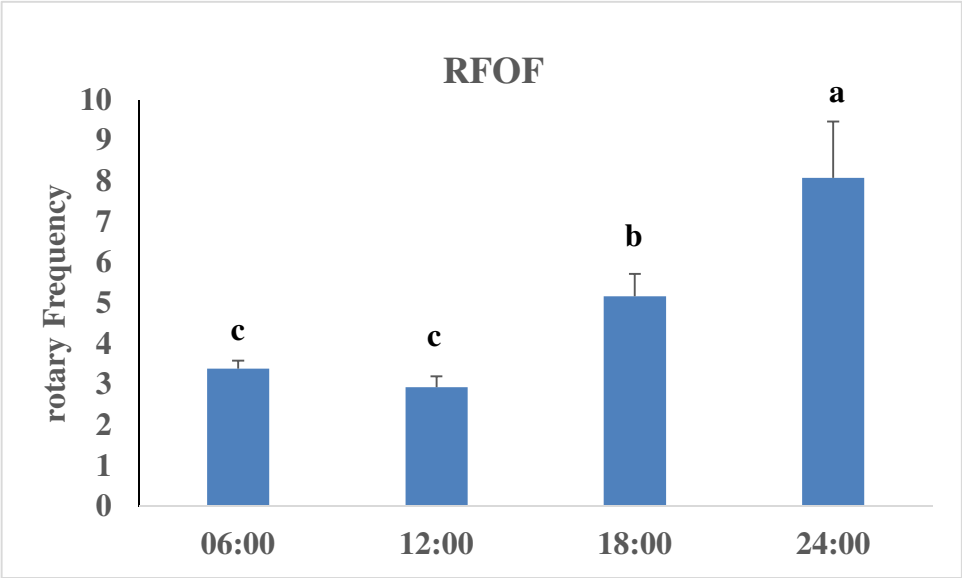

Figure 4. 35 Mean ± S.D. of the rotary frequency of the subjects in the open field for measurements times (06:00, 12:00, 18:00, and 24:00 h) of the day

The interaction effect between measurement time and treatment was significant, Wilks' Lambda = .81, F(3,67) = 5.22, p = .003, η^2 = .19. The pinx subjects had higher rotary frequency than control subjects all measurement times (06:00, 12:00, 18:00, and 24:00 h) (As can be seen on Figure 4.36).

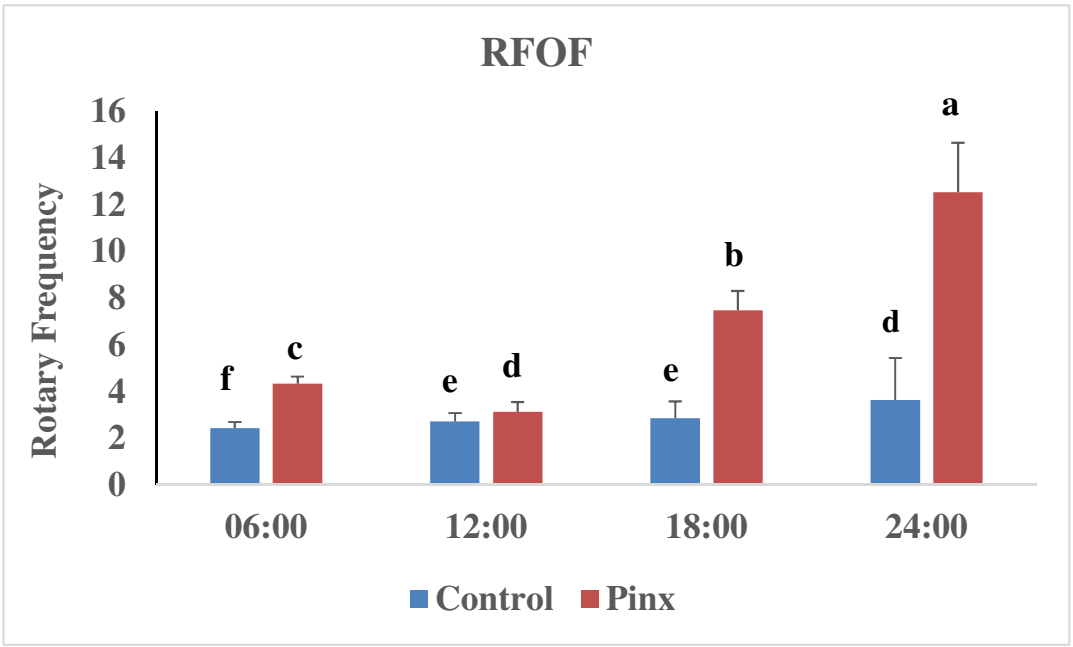

Figure 4. 36 Mean ± S.D. of the rotary frequency of the subjects in the open field for measurements times (06:00, 12:00, 18:00, and 24:00 h) of the day and treatment conditions (control and pinx)

4.2 Results of Depression Study (Forced Swim Test Porsolt) Measurements

4.2.1 Total Distance Travelled in the Porsolt (TDTP)

The main effect of the measurement time was significant on the total distance travelled, Wilks' Lambda = .70, F(3,67) = 9.38, p = .0001, n^2 = .30. The subjects in the evening (18:00 h) (M = 1743.51) travelled more distance than those at noon (12:00 h) (M = 1700.28), at night (24:00) (M = 1546.21) and in the morning (6:00 h) (M = 1544.7). This means that Wistar albino rats were less depressive in the evening (18:00 h) and at noon than at night and in the morning measurement times (Figure 4.37). The main effect of the treatments (control and pinx) was significant on the total distance travelled, F(1,69) = 6.70, p = .012, n^2 = .09. The subjects in the pinx condition (M = 1549.22 cm) travelled less distance than those in the control condition (M = 1718.14 cm). This means that pinx subjects were more depressive than subjects of controls condition (Figure 4.38).

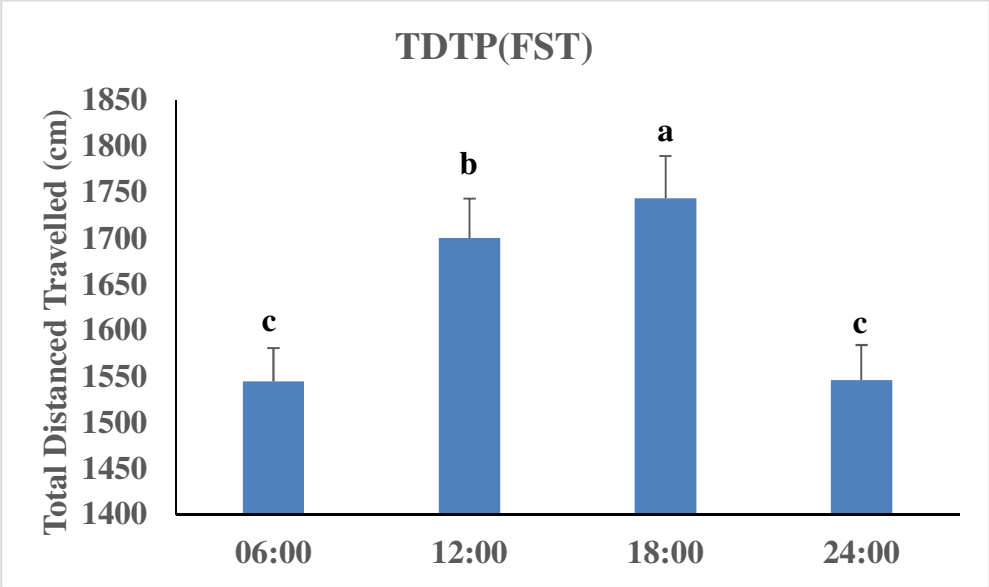

Figure 4. 37 Mean ± S.D. of the total distance travelled on the Porsolt in different measurement times (06:00, 12:00, 18:00, and 24:00 h) of the day

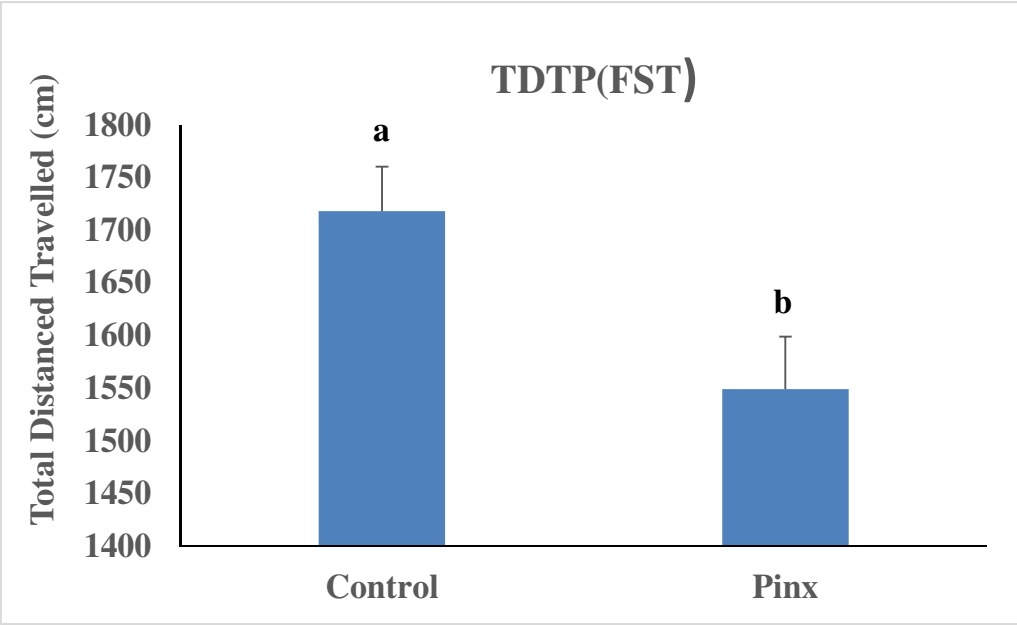

Figure 4. 38 Mean ± S.D. of the total distance travelled on the Porsolt of treatment conditions (control and pinx)

The effect of photoperiod was not significant F(2,69) = 1.37, p =.26, n^2 = .04 (Figure 4.39). In addition to this, there was no significant interaction effect between treatment and photoperiod F(2,69) = .39, p = .68, n^2 = .01 (Figure 4.40).

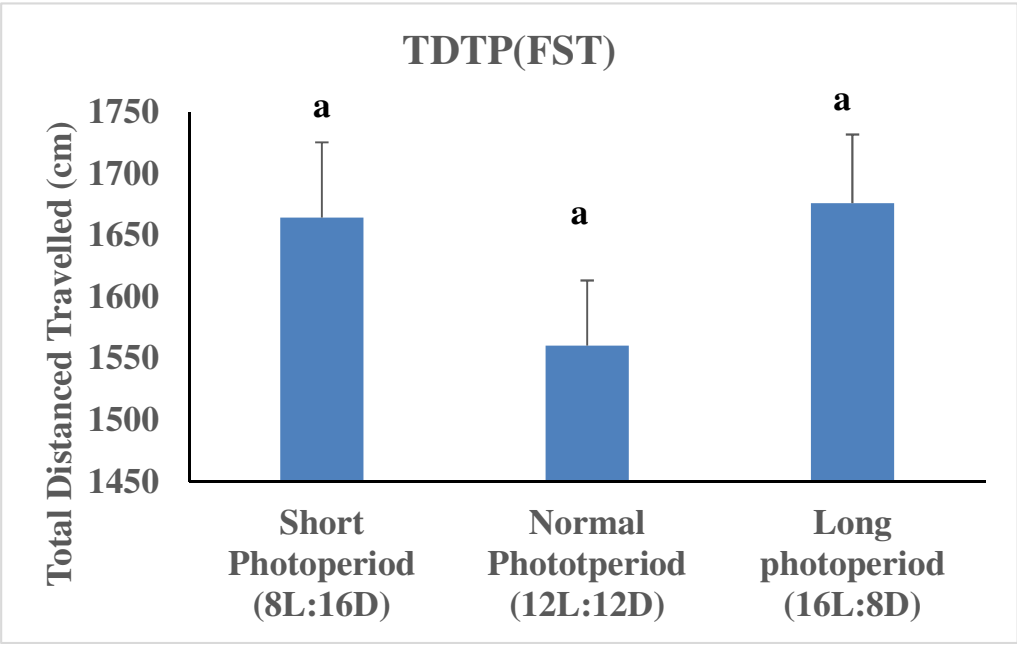

Figure 4. 39 Mean ± S.D. of the total distance travelled on the Porsolt of different photoperiod conditions

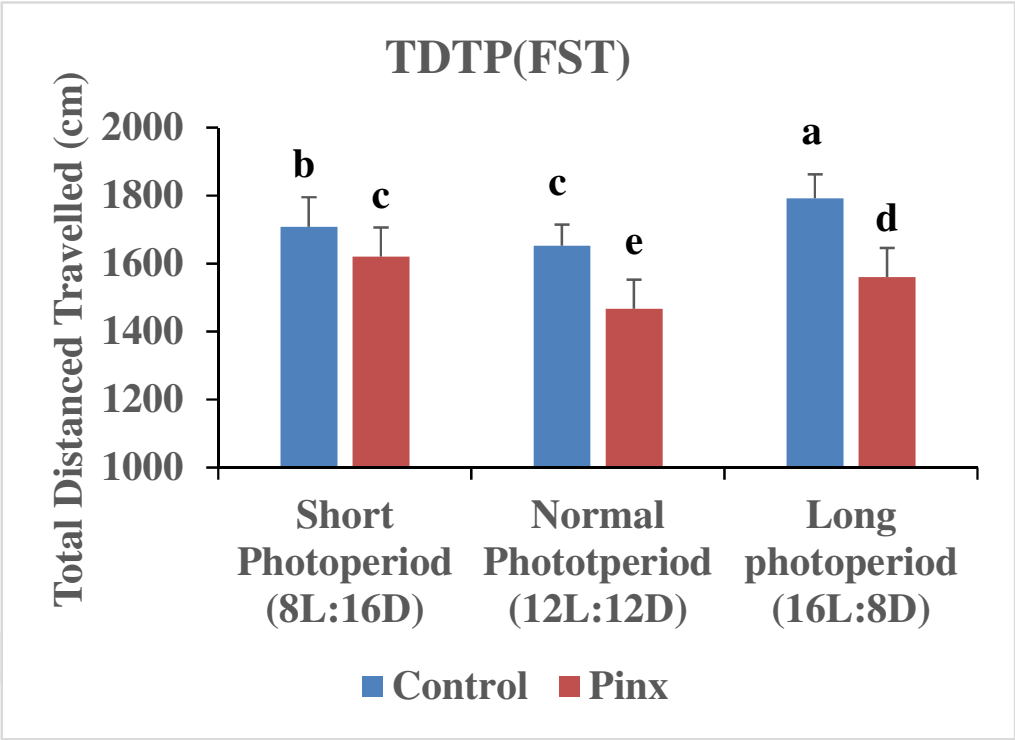

Figure 4. 40 Mean ± S.D. of the total distance travelled on the Porsolt of treatments (control and pinx), and different photoperiod conditions (short, normal, and long photoperiod)

The interaction effect between the measurement time and treatment was significant, Wilks' lambda = .83, F(3,67) = 4.70, p = .005, n^2 = .17. The subjects in the control group travelled more distance than those in pinx subjects at 6:00 h, 18:00 h, and 24:00 h. In other words, the control groups were significantly more active than pinx subjects at all tested measurement times (06:00, 12:00, 18:00, and 24:00 h) of the day. This means that pinx subjects were more depressive than subjects of controls condition (Figure: 4.41).

The interaction effect between the measurement time and photoperiod was also significant, Wilks' lambda = .75, F(6,134) = 3.42, p = .004, n^2 = .13. The subjects in the short and long photoperiod more active than those in normal photoperiod at 12:00 and 18:00 h. But the subjects of the short, normal and long photoperiods have shown similar behavior at other times (Figure 4.41).

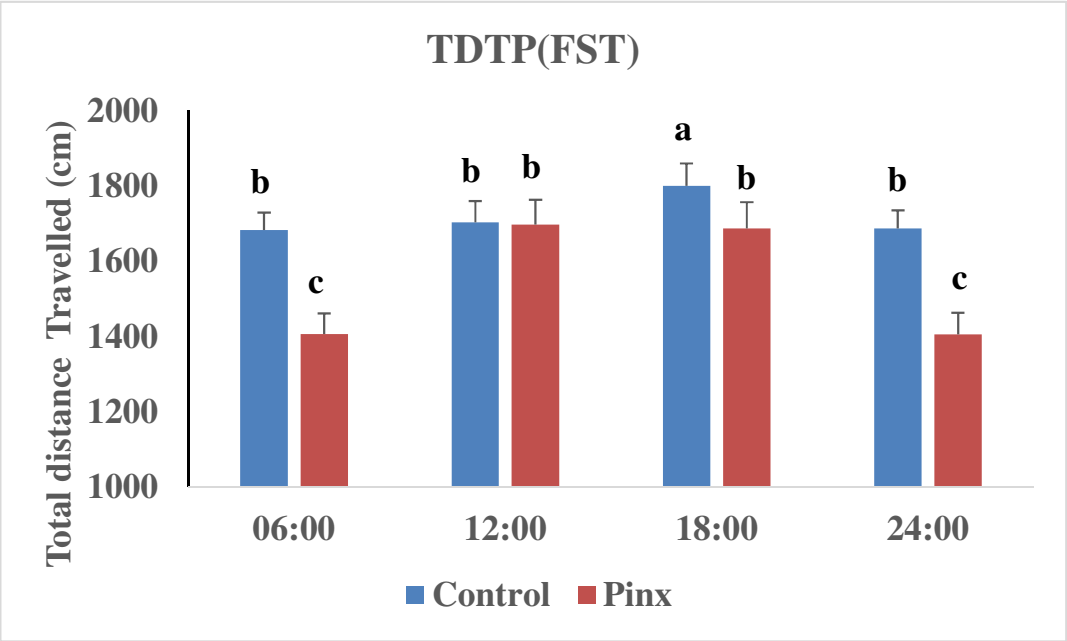

Figure 4. 41 Mean ± S.D. of the total distance travelled of control and pinealectoized subjects on the Porsolt in different measurement times (06:00, 12:00, 18:00, and 24:00 h) of the day

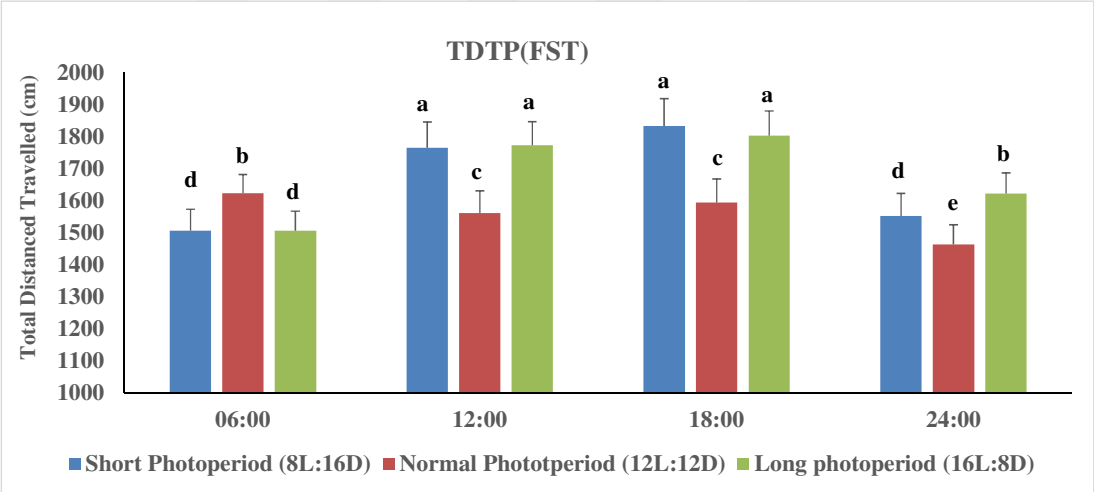

Figure 4. 42 Mean ± S.D. of the total amount of distance travelled of the four different measurement time (06:00, 12:00, 18:00, and 24:00 h) of the day with different photoperiod conditions

Lastly, the interactive effect among the measurement time, treatment and photoperiod was significant, Wilk's lambda = .69, F (6,134) = 4.48, p = .0001, η^2 = .17. Generally, the control subjects in the all tested photoperiods had the more total distance travelled performance than pinx subjects (Figure 4.43).

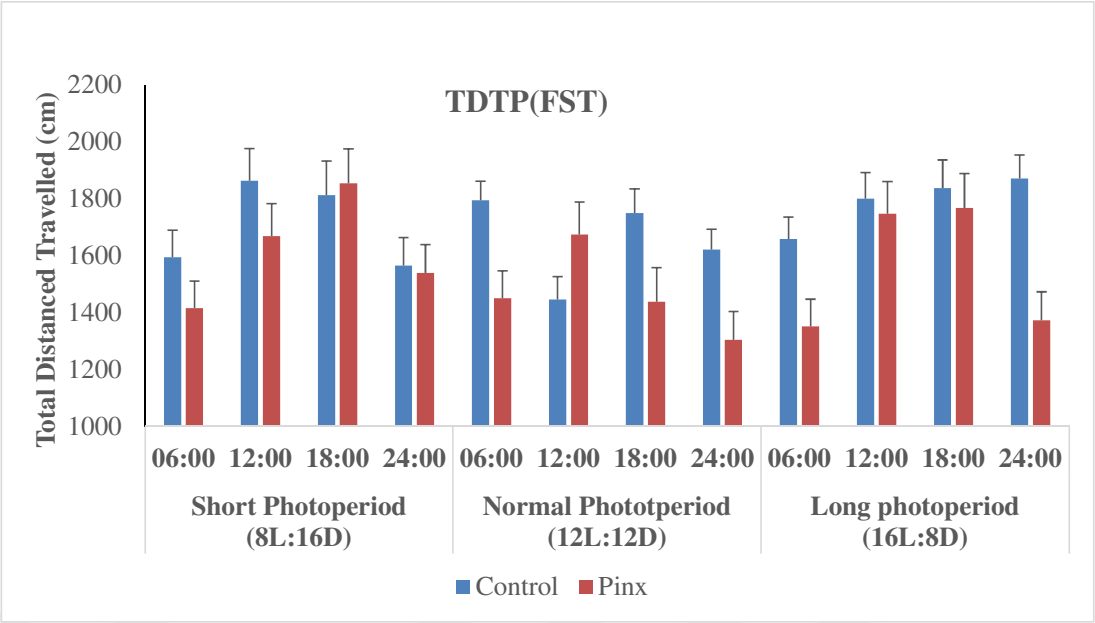

Figure 4. 43 Mean ± S.D. of the total distance travelled on the Porsolt for treatment (control and pinx), different photoperiod conditions, and measurement times (06:00, 12:00, 18:00, and 24:00 h)

4.2.2 Mobile Duration in the Porsolt (Force Swim Test) (MDP)

The main effect of the treatment was also significant, F(1,69) = 11.50, p = .001, η^2 = .14. The control subjects (M = 1.98) were more mobile than the pinealectomized subjects (M = 1.68). This means that pinx subjects were more depressive than control subjects (Figure 4.44). The main effect of the photoperiod was also significant, F(2,69) = 13.36, p = .0001, η^2 = .28. The subjects in the short photoperiod (M = 1.51) were less mobile than those in normal (M = 1.91), and long photoperiod (M = 2.08), with the last two significantly not different from each other (Figure 4.45). It means that long and normal photoperiod decrease depression like behavior on Wistar albino rats.

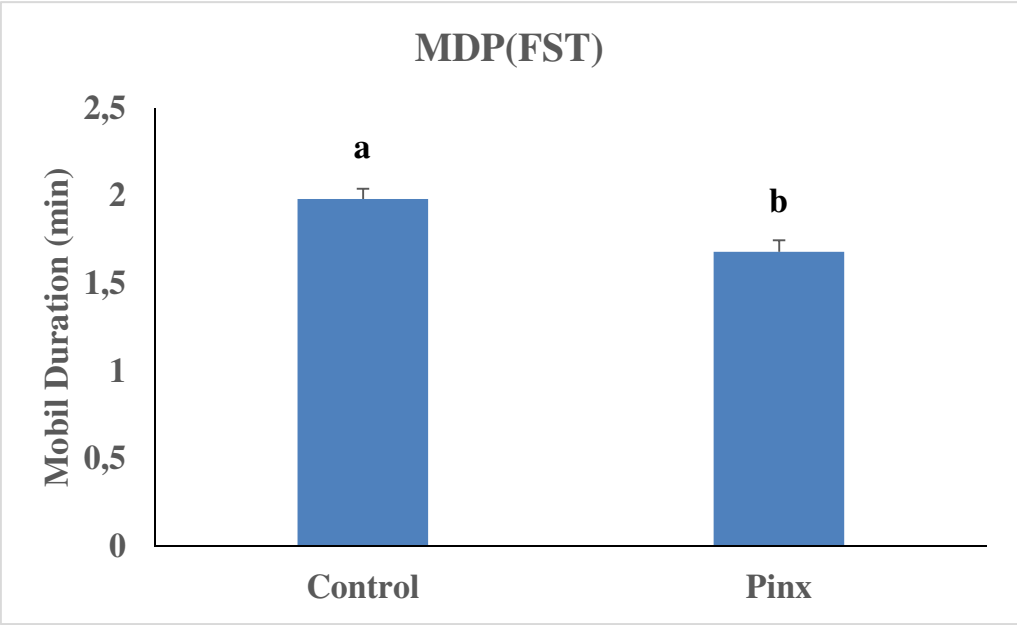

Figure 4. 44 Mean ± S.D. of the mobility duration on the Porsolt of treatment conditions (control and pinx)

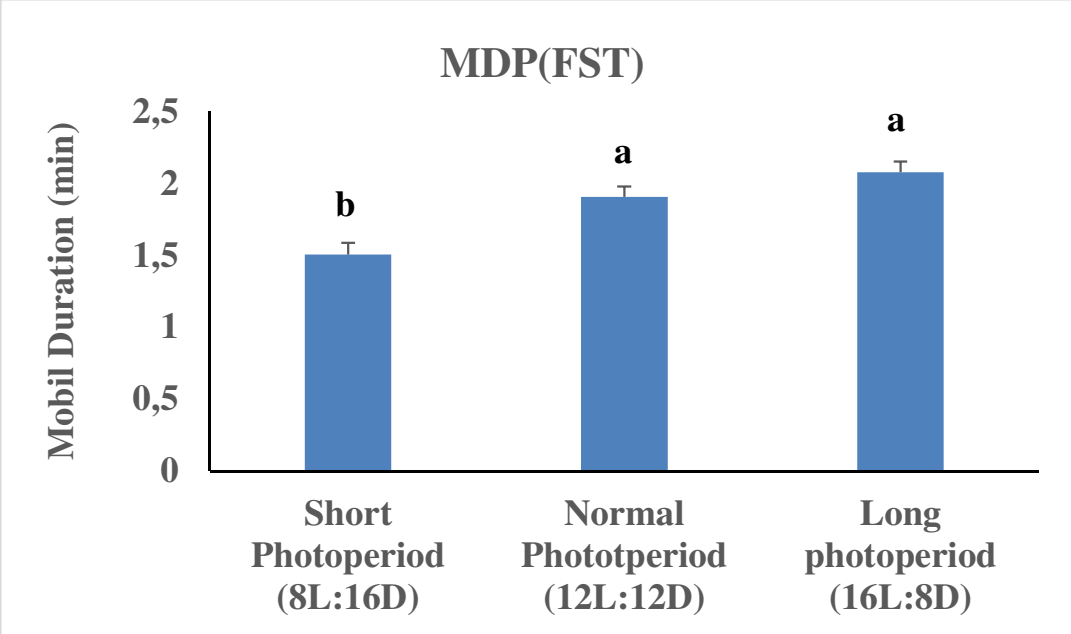

Figure 4. 45 Mean ± S.D. of the mobility duration on the Porsolt of different photoperiod conditions

The main effect of the measurement time was significant, F(3,67) = 31.57, *p* = .0001, n^2 = .59. The subjects at noon time (12:00 h) (M = 2.16), were more mobile than these in the morning (M = 1.69), evening (M = 1.67) and at night (M = 1.79). This means that the tested subjects were less depressive at noon (12:00 h) than at night, evening, and in the morning, respectively (Figure 4.46).

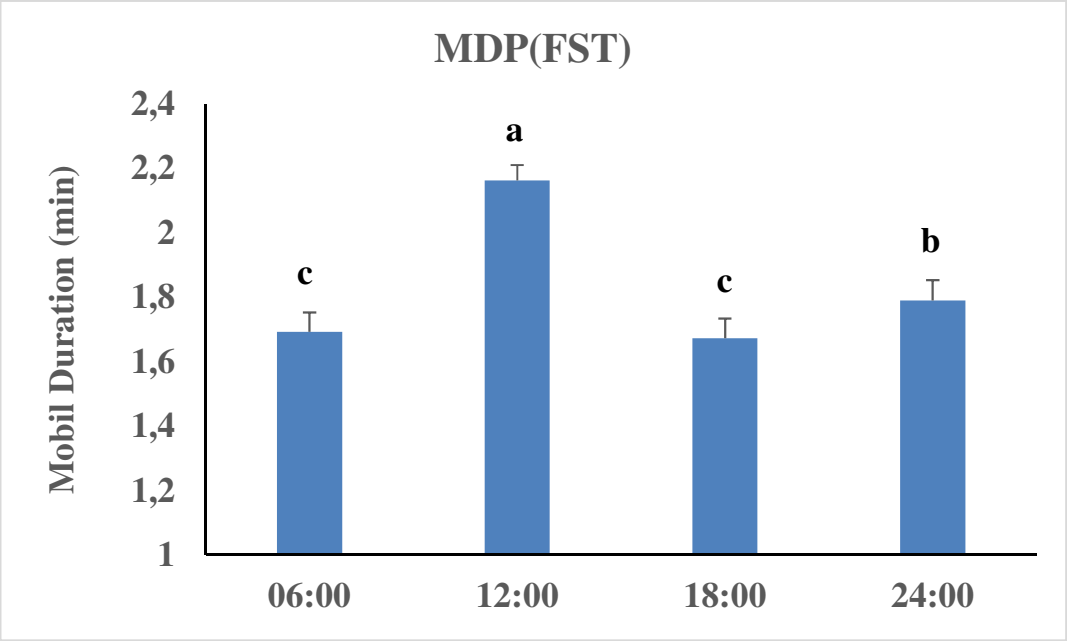

Figure 4. 46 Mean ± S.D. of the mobility duration on the Porsolt in different measurement times (06:00, 12:00, 18:00, and 24:00 h) of the day

In addition, the interaction effect between the measurement time and the treatment was significant, Wilks' Lambda = .38, F(3,67)= 35.82, p = .0001, η^2 = .62. This interaction effect reflected the fact that the control subjects were more mobile in the morning, at noon and in the evening than pinealectomized subjects but this trend was reversed at night (see in the figure 4.47).

The interaction effect between the measurement time and photoperiod also was significant, Wilks' Lambda = .58, F(6,134) = 6.9, p =.0001, η^2 = .24. This interaction effect showed that the subjects were more mobile at noon (12:00 h) all tested photoperiods (Figure 4.48).

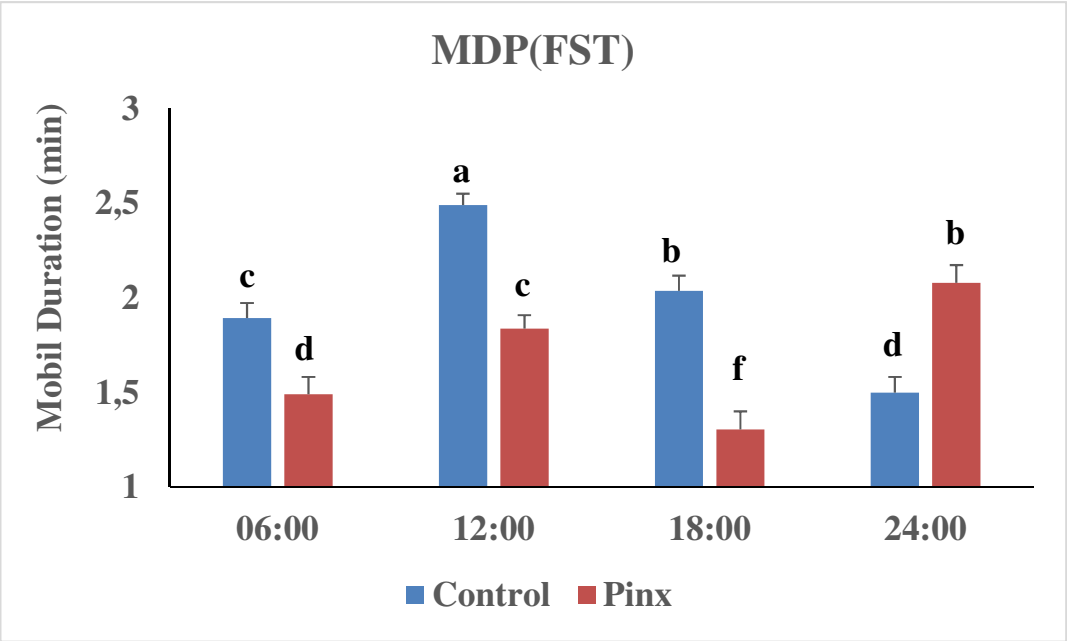

Figure 4. 47 Mean ± S.D. of the mobility duration of control and pinealectomy on the Porsolt in different measurement times (06:00, 12:00, 18:00, and 24:00 h) of the day

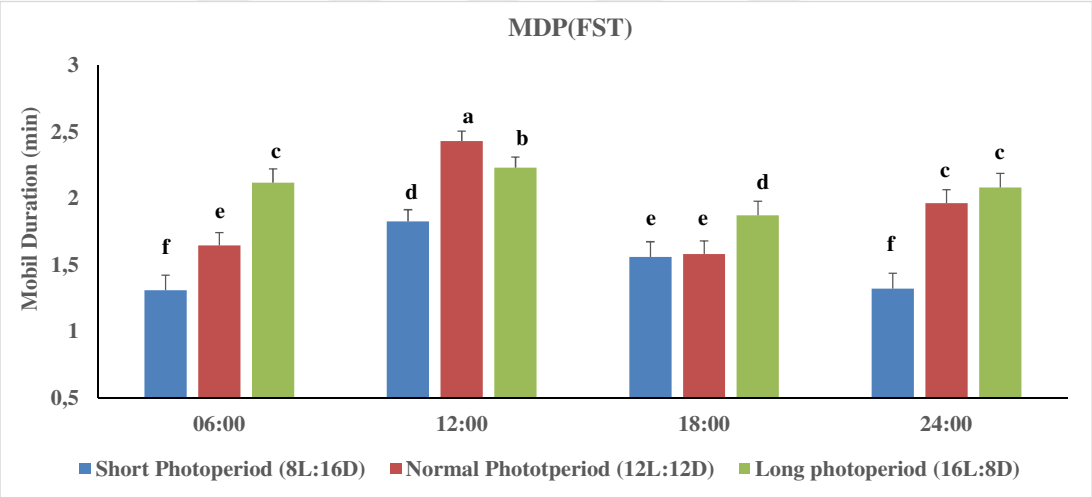

Figure 4. 48 Mean ± S.D. of the mobility duration of the four different measurement time (06:00, 12:00, 18:00, and 24:00 h) of the day with different photoperiod conditions

The lastly, the interaction effect among the measurements time, treatment and photoperiod was significant, Wilks' Lambda = .50, F(6,134) = 9.27, p = .0001. η^2 = .29. In general, the subjects in the pinx condition were less mobile of control conditions in short, normal and long photoperiod but the subjects in the pinx condition were mobile than those control condition at 06:00 h and 24 h for short period, and at 24:00 h for normal and long photoperiods (Figure 4.49).

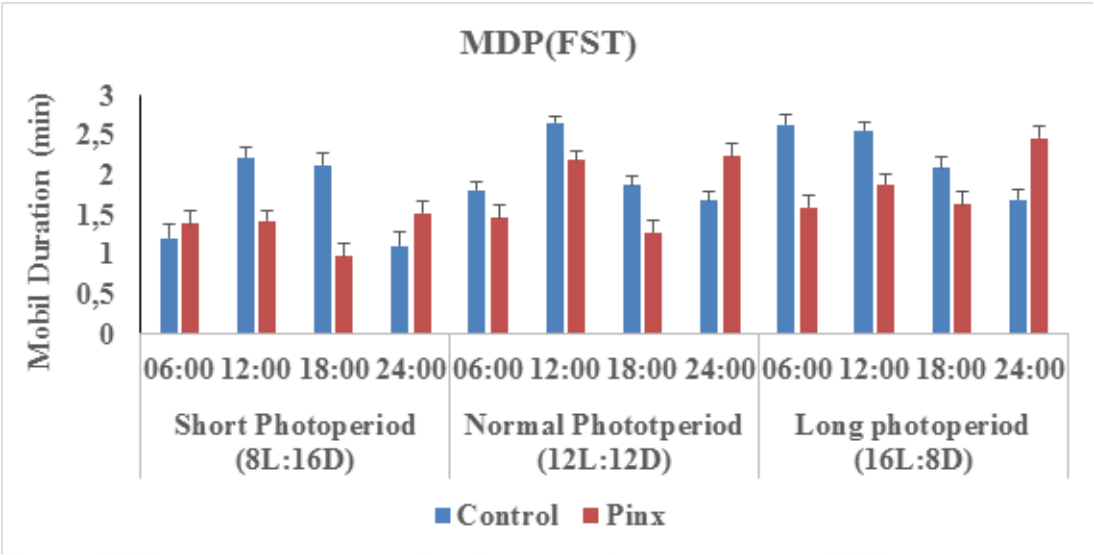

Figure 4. 49 Mean ± S.D. of the mobility duration on the Porsolt for treatment (control and pinx), different photoperiod conditions, and measurement times (06:00, 12:00, 18:00, and 24:00 h)

4.2.3 Mobile Frequency in the Porsolt (Force Swim Test) (MFPFST)

The main effect of the photoperiod was significant, $F(2,69) = 8.72$, $p = .0001$, $\eta^2 = .20$. The mobility frequency of the subjects in the long ($M = 826.93$) and normal photoperiods ($M = 823.99$) had higher mobile frequency than those in short photoperiod ($M = 695.64$). This means that the subjects kept in the long and normal photoperiod were less depressive than in short photoperiod (Figure 4.50).

The main effect of the treatments was not significant, $F(1,69) = 43$, $p=.706$, $\eta^2 = .002$. (Figure 4.40). It means that pinealectomy condition had not significant effect on the mobile frequency of Wistar albino rats (Figure 4.51).

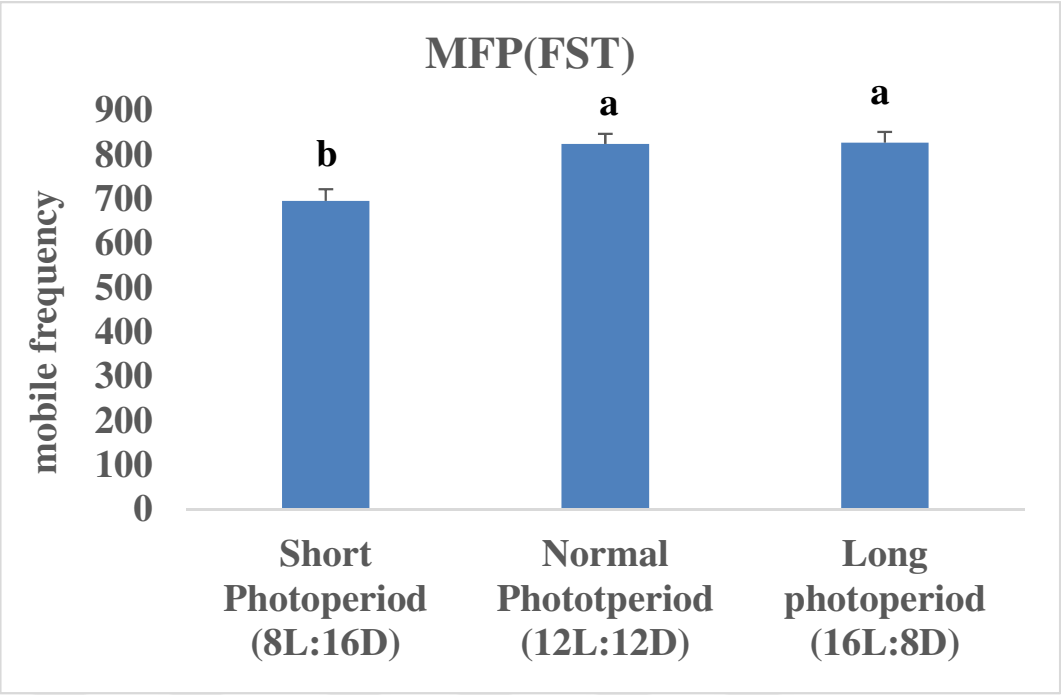

Figure 4. 50 Mean ± S.D. of the mobility frequency on the Porsolt for different photoperiod conditions

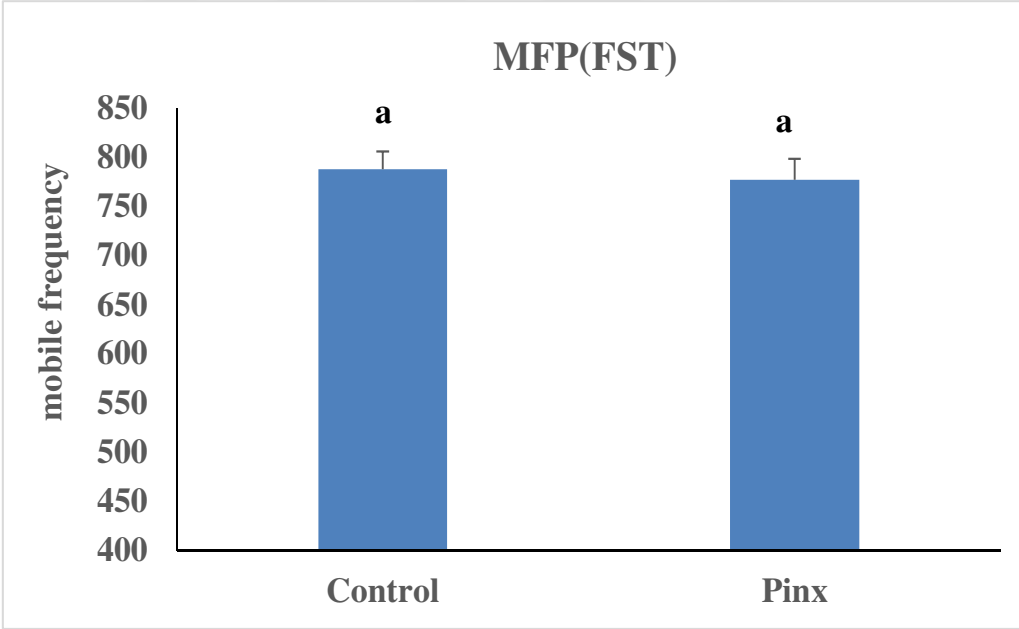

Figure 4. 51 Mean ± S.D. of the mobility frequency on the Porsolt of treatment conditions (control and pinx)

The main effect of the measurement time was significant, Wilks' Lambda = .75, F(3,67) = 7.39, p = .0001, η^2 =.25. The subjects at noon (12:00 h) were more mobile (M = 842.75) than those at night (24:00 h) (M = 780.32), in the evening (18:00 h) (M = 774.94) and in the morning (06:00 h) (M = 730.75). This means that

the subjects were less depressive at noon (12:00 h) than those at night, evening, and morning times, respectively (Figure 4.52).

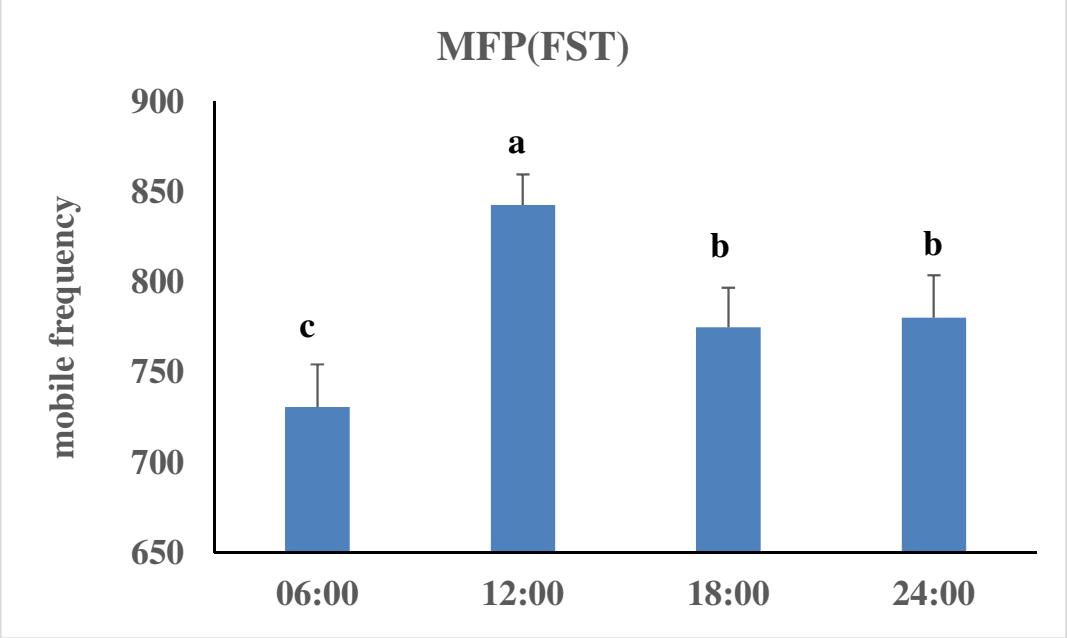

Figure 4. 52 Mean ± S.D. of the mobility frequency on the Porsolt in different measurement times (06:00, 12:00, 18:00, and 24:00 h) of the day

In addition, the interaction effect between the measurement time and the treatment was significant, Wilks' Lambda = .75 F(3,67) = 7.37, p = .0001, n^2 = .25. This interaction effect reflected the fact that the mobility frequencies of control subjects had greater than pinealectomized subjects at noon and in the evening times, whereas pinealectomized subjects had higher mobility frequency than control subjects at night (24 h) (Figure 4.53).

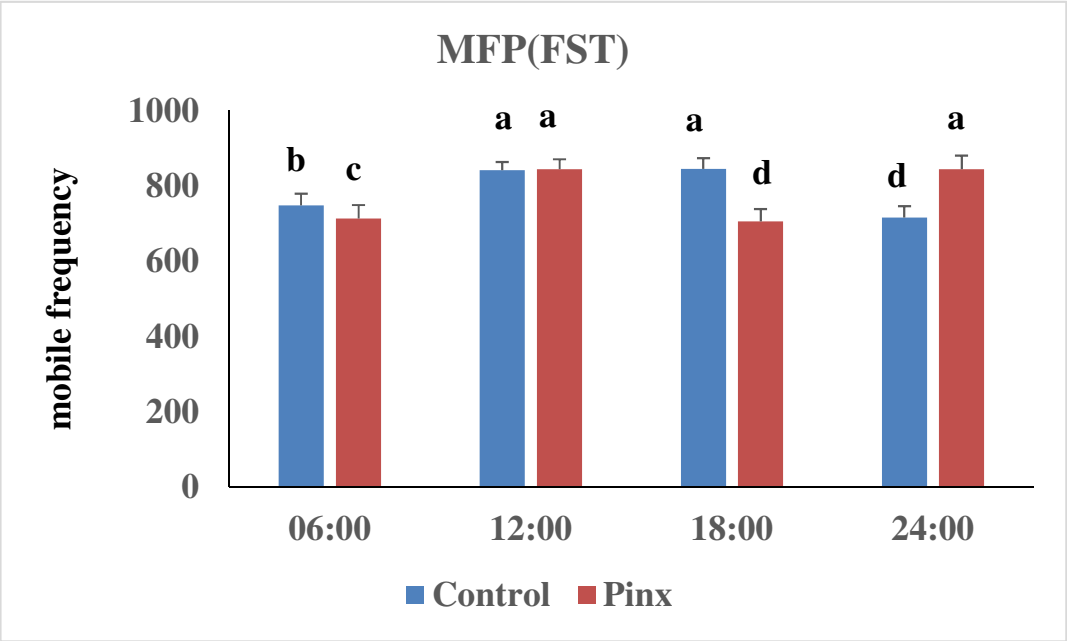

Figure 4. 53 Mean ± S.D. of the mobility frequency of control and pinealectomy on the Porsolt in different measurement times (06:00, 12:00, 18:00, and 24:00 h) of the day

The interaction effect between the measurement time and photoperiod were significant, Wilks' Lambda = .82 F(6,134) = 2.27, p = .04, η^2 = .09. This interaction effect showed that the subjects were more mobile at noon time (12:00 h) in normal and long photoperiod than short photoperiod. This means that the subjects in the short photoperiod were more depressive than those in the long and normal photoperiods (Figure 4.54).

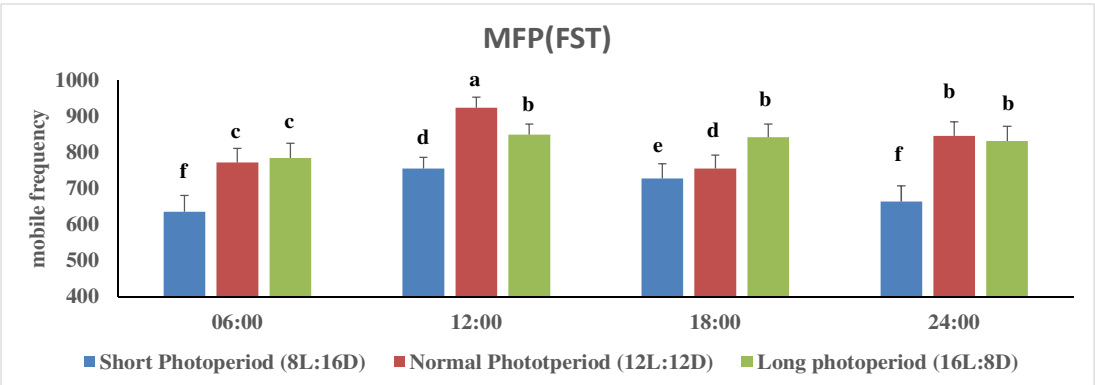

Figure 4. 54 Mean ± S.D. of the mobility frequency on the Porsolt of the four different measurement time (06:00, 12:00, 18:00, and 24:00 h) of the day with different photoperiod conditions

The lastly, the interaction effect among the measurement time, treatment and photoperiod were significant, Wilk's lambda = .74, F (6,134) = 3.59, *p* = .002, η^2 =

.14. The subjects in the pinx condition were less mobile than those in the control conditions for short, normal and long photoperiod but the subjects in the pinx condition were mobile than those control condition at 06:00 h and 24:00 h for short photoperiod, at 12:00 h, 24:00 h for normal and at 24:00 h for long photoperiod (Figure 4.55).

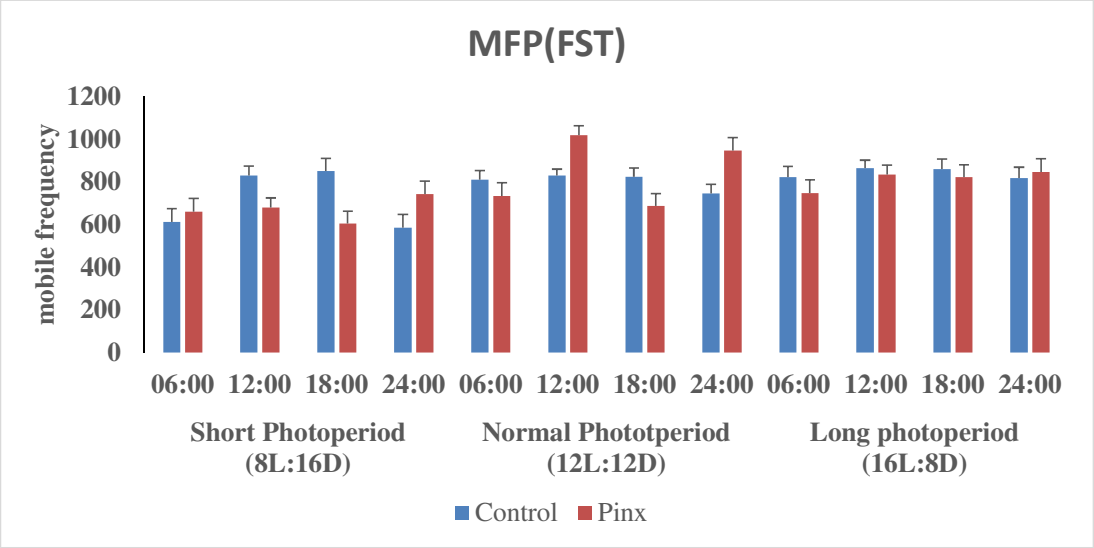

Figure 4. 55 Mean ± S.D. of the mobility frequency on the Porsolt for treatment (control and pinx), photoperiods, and measurement times (06:00, 12:00, 18:00, and 24:00 h)

4.2.4 Center Point Moving Duration in Porsolt (CMDP)

The main effect of the treatment was significant, $F(1,69) = 25.28$, $p = .0001$, $n^2 = .27$. The control subjects ($M = 4.42$) showed more movement than the pinealectomized subjects ($M = 4.20$). This means that pinx subjects were more depressive than control ones (Figure 4.56). The main effect of the photoperiod was significant, $F(2,69) = 4.12$ $p = .02$, $n2 = .11$. The subjects in the short photoperiod ($M = 4.23$) were less mobile than those normal ($M = 4.32$) and long ($M = 4.39$) photoperiod. This means that the subjects exposed to long and normal photoperiod were less depressive than subjects that exposed to short photoperiod (Figure 4.57).

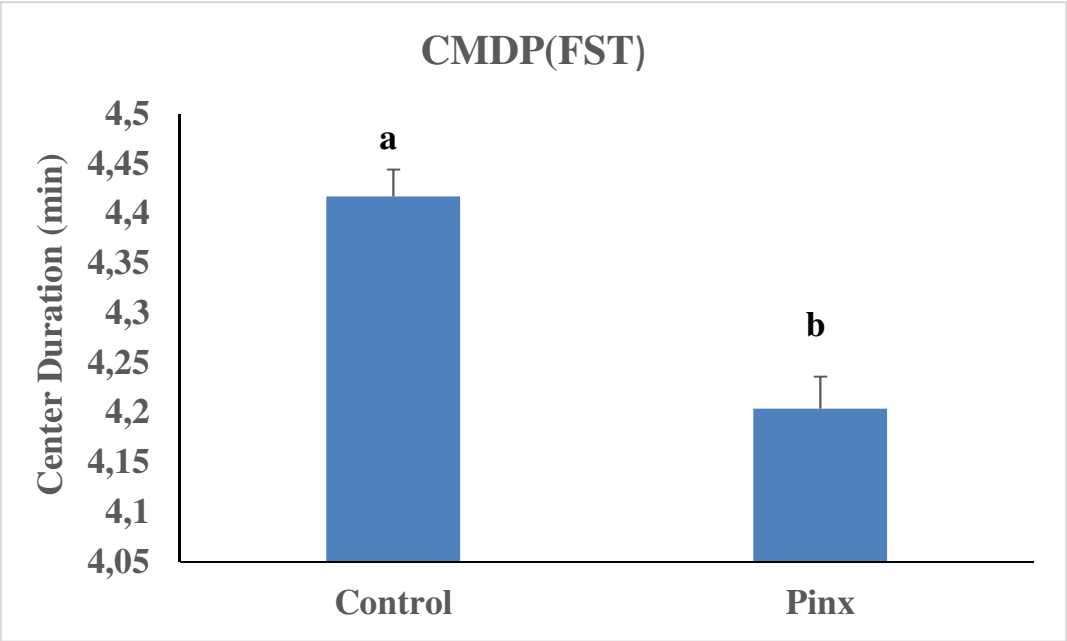

Figure 4. 56 Mean ± S.D. the center moving duration on the Porsolt of treatment conditions (control and pinx)

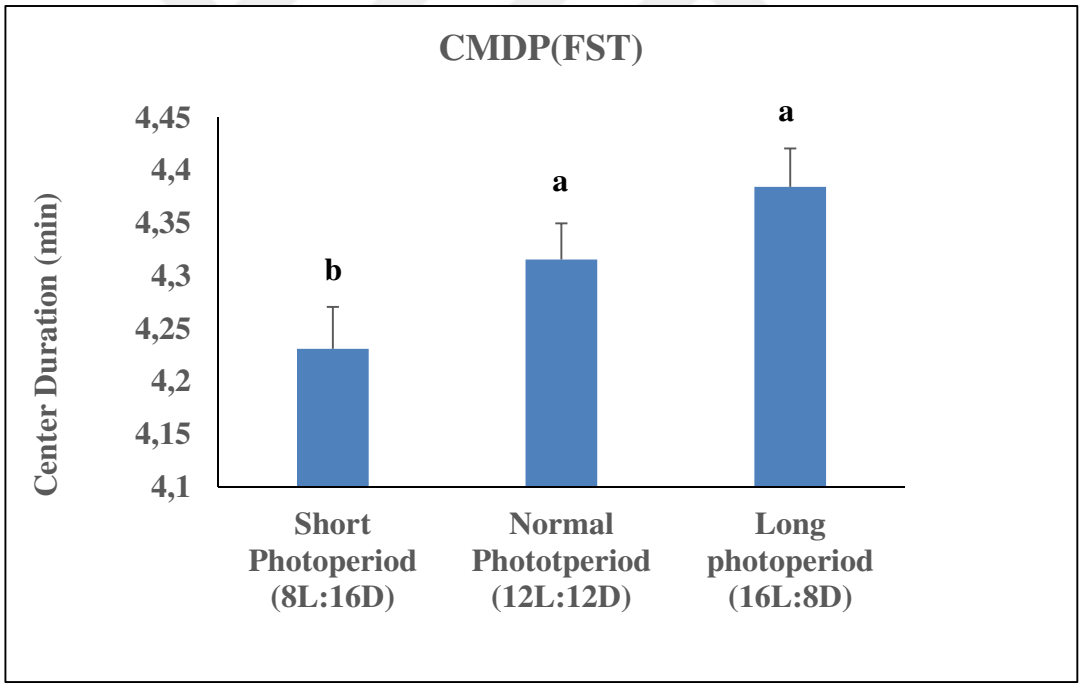

Figure 4. 57 Mean ± S.D. the center moving duration on the Porsolt of different photoperiod conditions

The main effect of the measurement time was significant, Wilks' Lambda = .45 F(3,67) = 27.66, p = .0001, η2 = .55. The subjects showed more movement at noon time (12:00 h) (M = 4.44) than those at night (M = 4.21 min), in the evening

(M = 4.34) and morning (M = 4.23) times. This means that tested subjects were less depressive at noon time (12:00 h) than at night (24:00 h), in the morning (06:00 h), and in the evening (18:00 h) times, respectively (Figure 4.58).

In addition, the interaction effect between the measurement time and photoperiod was significant, Wilks' Lambda = .67 F(6,134) = 4.97, p = .0001, n^2 = .18. This interaction effect showed that the subjects were more mobile at noon time (12:00 h) under all tested photoperiod. This means that the subjects were less depressive at noon times at normal (M = 4.47 min), long (M = 4.45 min) and short (M = 4.40 min) photoperiod, respectively (Figure 4.59).

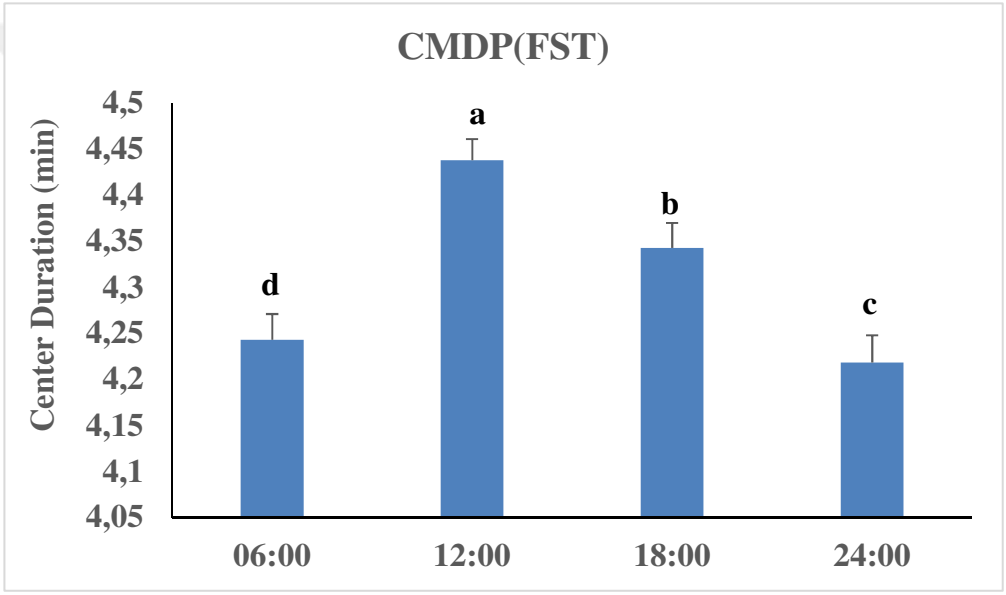

Figure 4. 58 Mean ± S.D. the center moving duration on the Porsolt in different measurement times (06:00, 12:00, 18:00, and 24:00 h) of the day

The interaction effect between treatment and photoperiod was not significant, F (2, 69) = .53, *p* = .59, n^2 = .02 (data not shown).

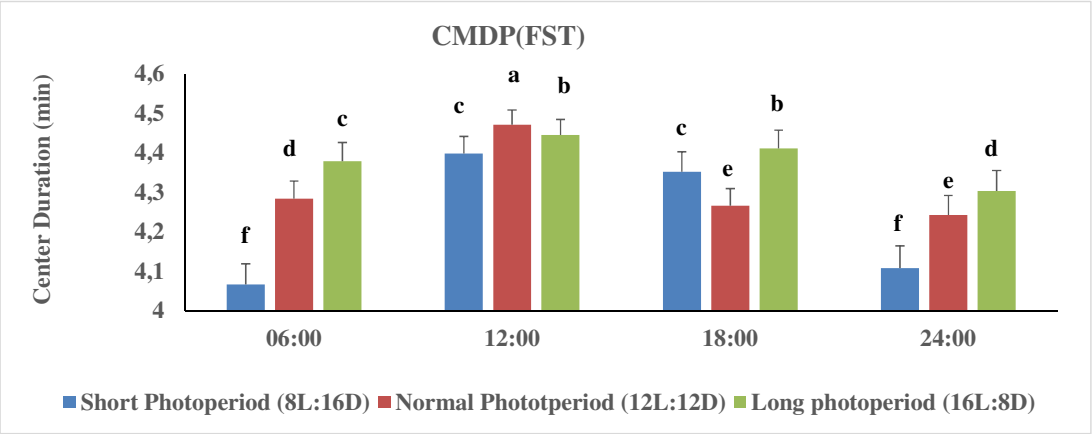

Figure 4. 59 Mean ± S.D. of the center moving on the Porsolt of the four different measurement times (06:00, 12:00, 18:00, and 24:00 h) of the day with different different photoperiod conditions

The interaction effect between the measurement time and the treatment was significant, Wilks' Lambda = .56 F(3,67) = 17.62, p = .0001, η^2 = .44. This interaction effect reflected the fact that the control subjects showed more movement than those pinealectomized subjects. This means that pinx subjects were more depressive than controls (Figure 4.60).

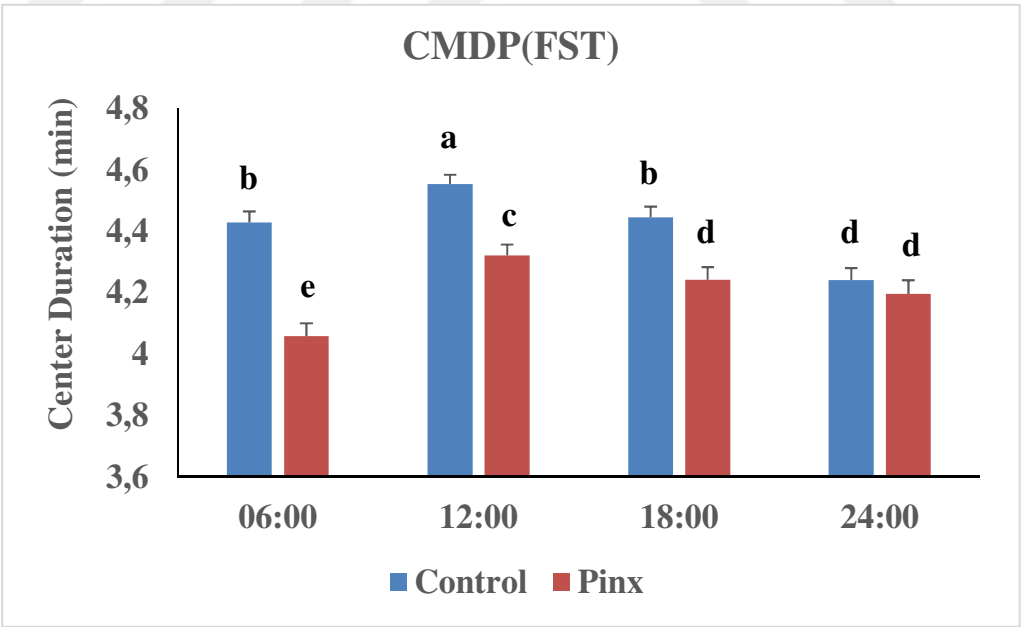

Figure 4. 60 Mean ± S.D. of the center moving on the Porsolt of the four different measurement time (06:00, 12:00, 18:00, and 24:00 h) of the day with treatments (control and pinx)

The lastly, the interaction effect among the measurement time, treatment and photoperiod was significant, Wilks' Lambda = .65, F(6,134) = 5.34, p = .0001, n^2 = .19. The subjects in the pinx condition were less mobile than controls at short, normal, and long photoperiods. This means that the subjects in the short photoperiod were more depressive than those in the long and normal photoperiods (Figure 4.61).

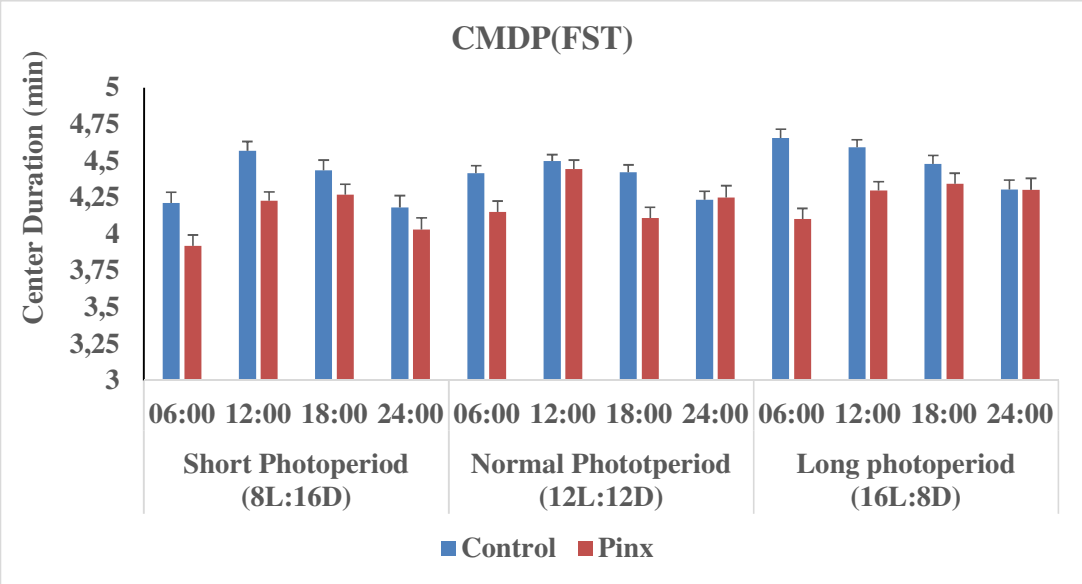

Figure 4. 61 Mean ± S.D. of the center moving duration on the Porsolt for treatments (control and pinx), different photoperiod conditions, and measurement times (06:00, 12:00, 18:00, and 24:00 h)

4.2.5 Velocity in PORSOLT (VELP)

The main effect of the treatment was significant on the velocity in force swimming test (FST), F(1,69) = 28.05, *p* = .0001, n^2 =.29. The control subjects (M = 411.54 cm/min) were faster than the pinealectomized subjects (M = 338.23). This means that pinx subjects were more depressive than control condition (Figure 4.62).

The main effect of the photoperiod was also significant, *F*(2, 69) = 4.08, *p* = .02, n^2 = .11. The subjects under long photoperiod were faster (M = 401.77) than short (M = 365.45) and normal photoperiod (M = 357.45). This means that the subjects under short and normal photoperiod were more depressive than long photoperiod (Figure 4.63).

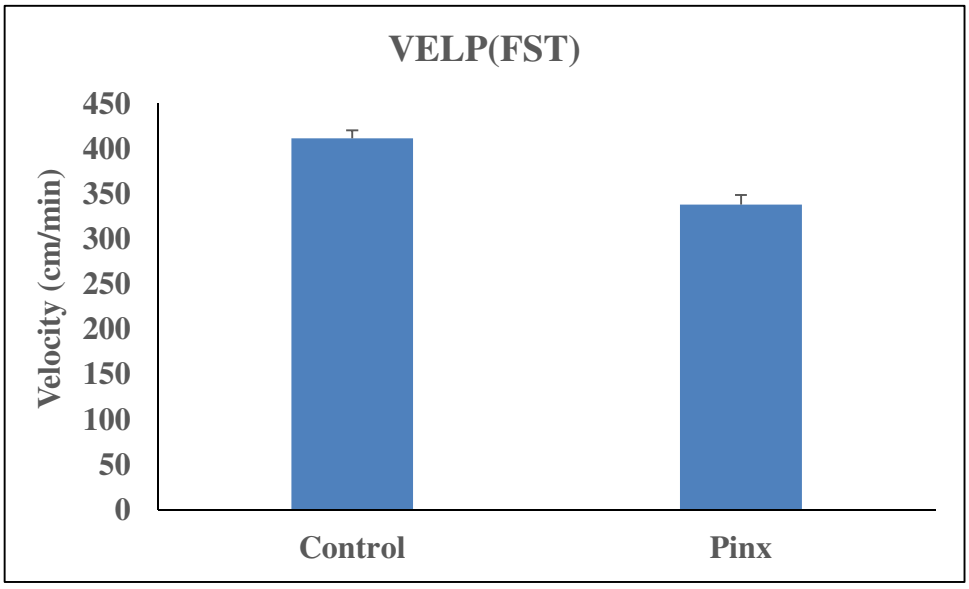

Figure 4. 62 Mean ± S.D. of the velocity on the Porsolt of treatment conditions (control and pinx).

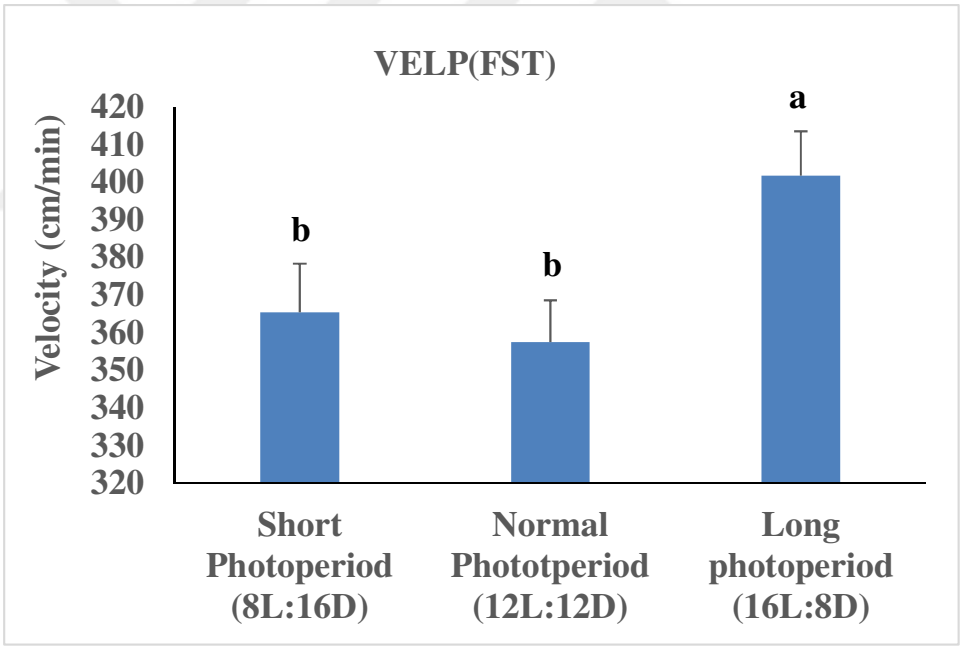

Figure 4. 63 Mean ± S.D. of the velocity duration on the Porsolt of different photoperiod conditions

The main effect of the measurement time was significant, Wilks' Lambda =.52, F(3,67) = 24.26, p = .0001, $\eta 2$ = .52. The subjects were faster at noon (12:00 h) (M = 410.50) than those at night (24:00 h) (M = 349.06), evening (M = 384.84), and morning (M = 355.13) times. This means that the subjects at noon (12:00 h)

were less depressive than in the evening, morning, and at night times, respectively (Figure 4.64).

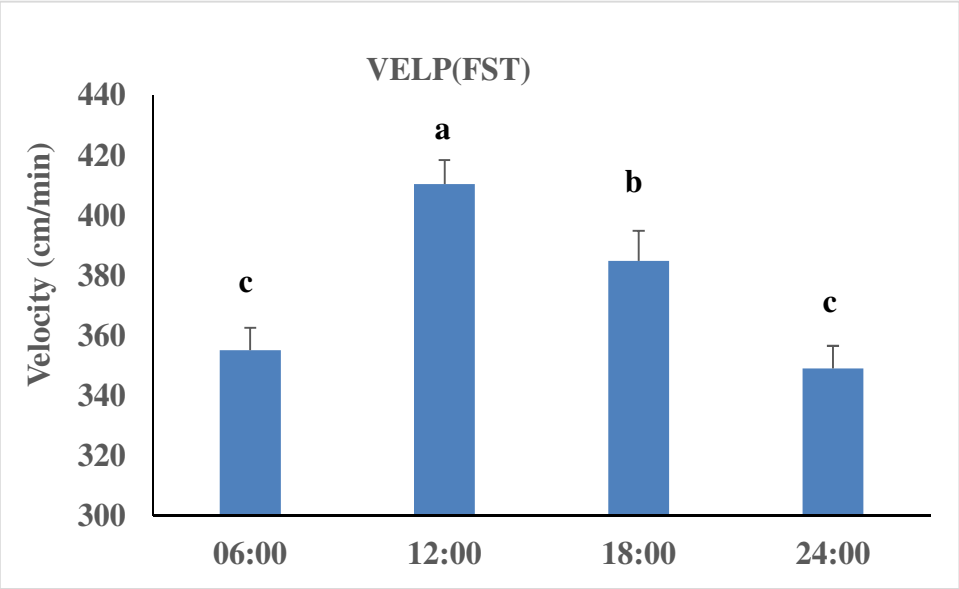

Figure 4. 64 Mean ± S.D. of the velocity duration on the Porsolt in different measurement times (06:00, 12:00, 18:00, and 24:00 h) of the day

The interaction effect between the measurement time and photoperiod was significant, Wilks' Lambda = .61, F(6, 134) = 6.34, p = .0001, η^2 = .22. The interaction effect reflected the fact that the subjects at noon (12:00 h) had higher velocity than in those other measurement times (06:00, 18:00 and 24:00). The subjects under long photoperiod were less depressive than short and normal photoperiod at all the tested measurement times (Figure 4.65).

The interaction effect between the measurement time and the treatment was significant, Wilks' Lambda = .61, F(3,67) = 14.20, p = .0001, η^2 = .39. This interaction effect reflected the fact that the control subjects showed more velocity than pinealectomized subjects. This means that the subjects under control condition were less depressive than pinealectomized subjects (Figure 4.66).

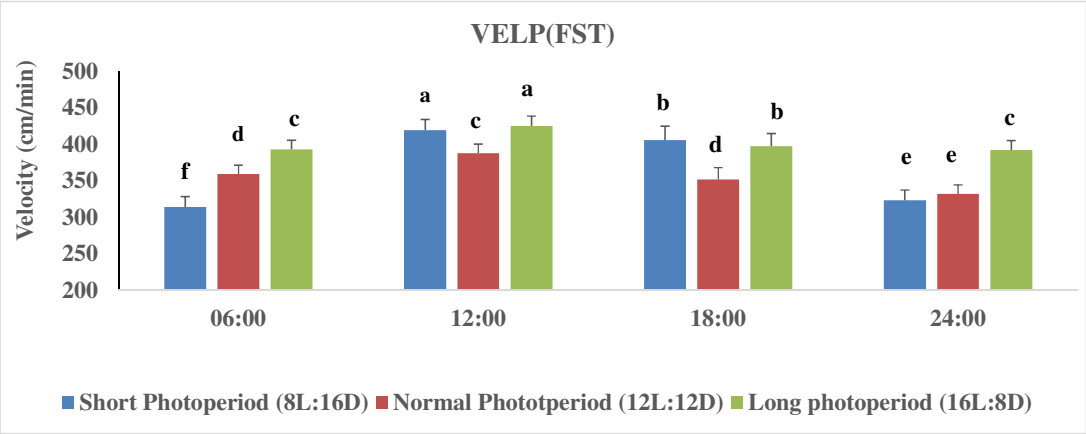

Figure 4. 65 Mean ± S.D. of the velocity on the Porsolt of the four different measurement time (06:00, 12:00, 18:00, and 24:00 h) of the day with different photoperiod conditions

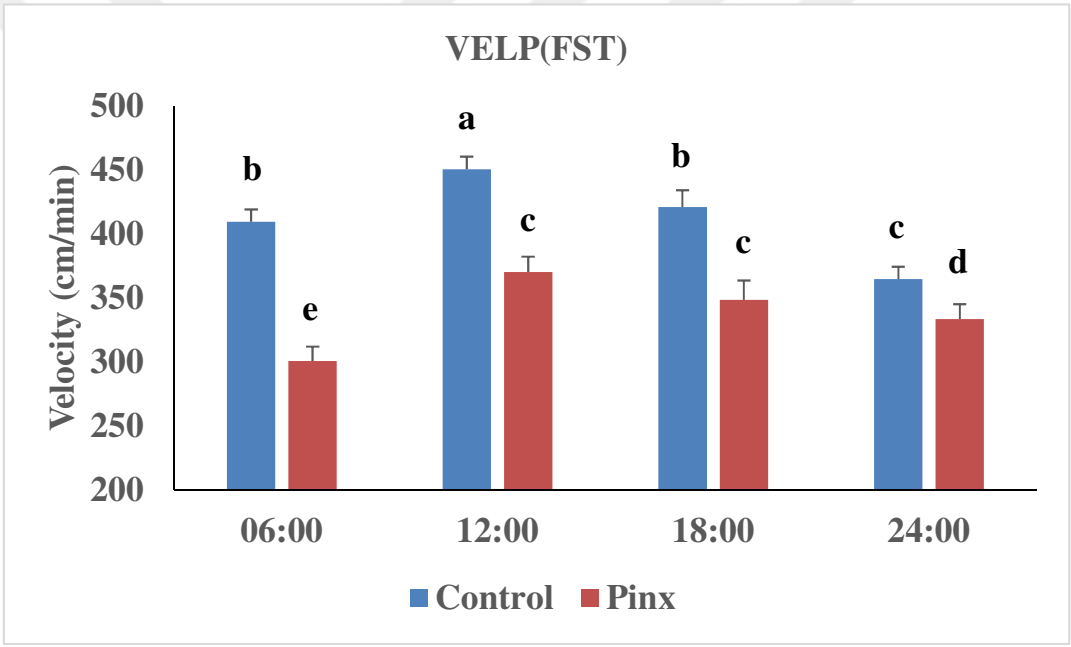

Figure 4. 66 Mean ± S.D. of the center moving duration of control and pinealectomy on the porsolt in different measurement times (06:00, 12:00, 18:00, and 24:00 h) of the day

The lastly, the interaction effect among the measurement time, treatment and photoperiod was also significant, Wilk's lambda = .48, F (6,134) = 9.76, p = .0001, η^2 = .30. The subjects in the control condition were more mobile than subjects in pinx condition under all tested photoperiod types (short, normal and long photoperiod) (Figure 4.67).

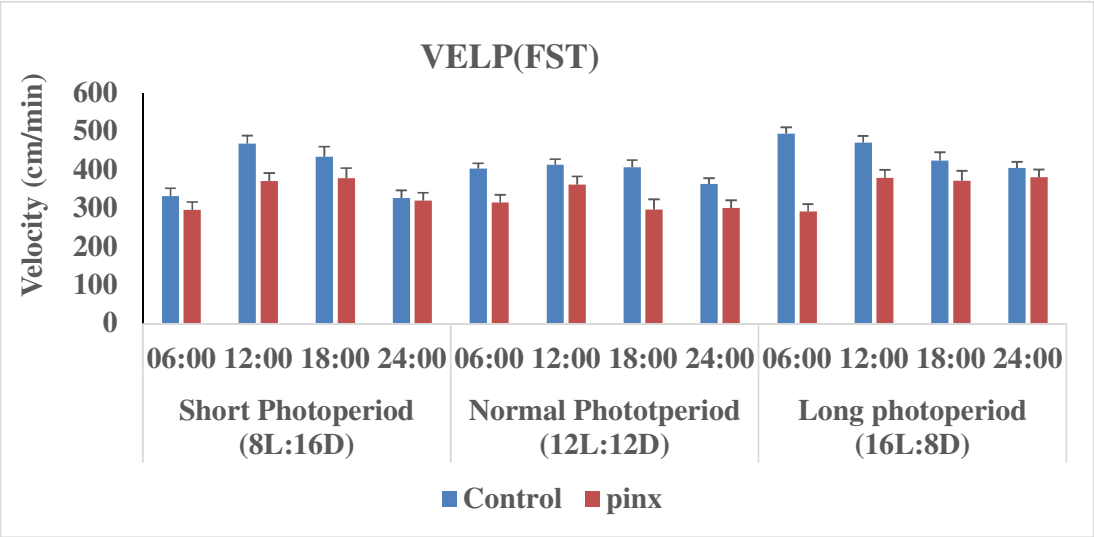

Figure 4. 67 Mean ± S.D. of the velocity on the Porsolt for treatment (control and pinx), different photoperiod conditions, and measurement times (06:00, 12:00, 18:00, and 24:00 h)

4.3 Results of Aggression Study

4.3.1 Grooming (GMG)

The main effect of the photoperiod was significant on grooming, F (2, 54) = 22.27, p = .0001, η^2 = .45. The subjects in the short photoperiod (M = 24.75) showed more grooming behavior than those in normal (M = 39.75), and long photoperiod (M = 33.73), the last two being significantly different from each other (Figure 4.68).

The main effect of the treatment was also significant, F (1, 54) = 52.25, p = .0001, η^2 = .50. The control subjects (M = 39.42) showed more grooming than the pinealectomized subjects (M = 26.78) (Figure 4.69).

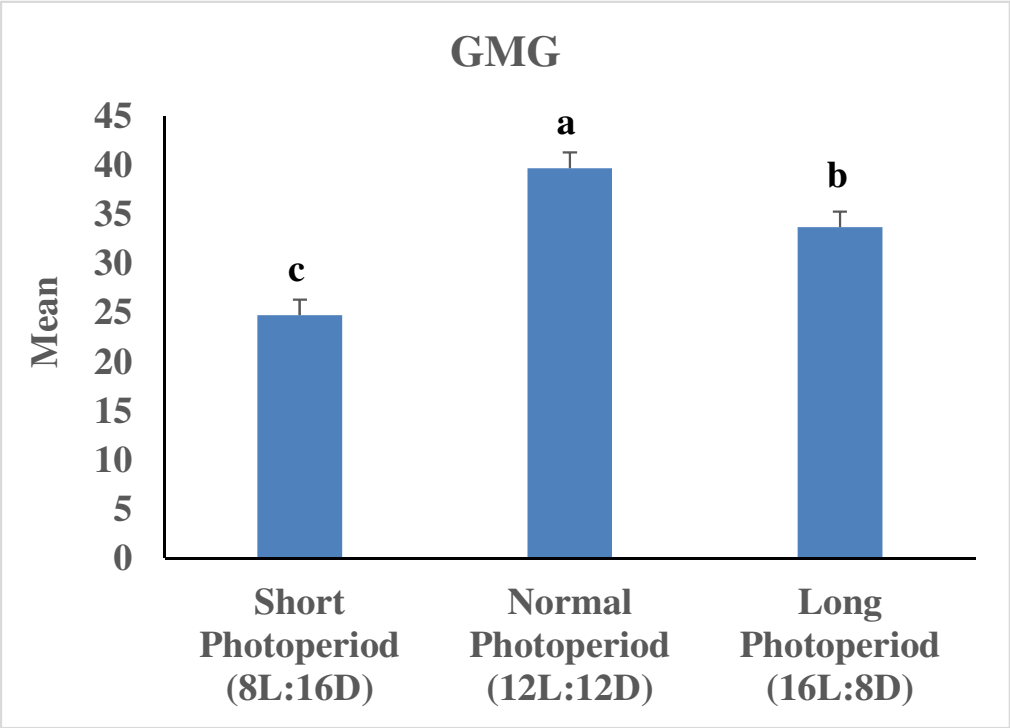

Figure 4. 68 Mean ± S.D. of the amount of the grooming behavior for five min for aggression test in different photoperiod conditions

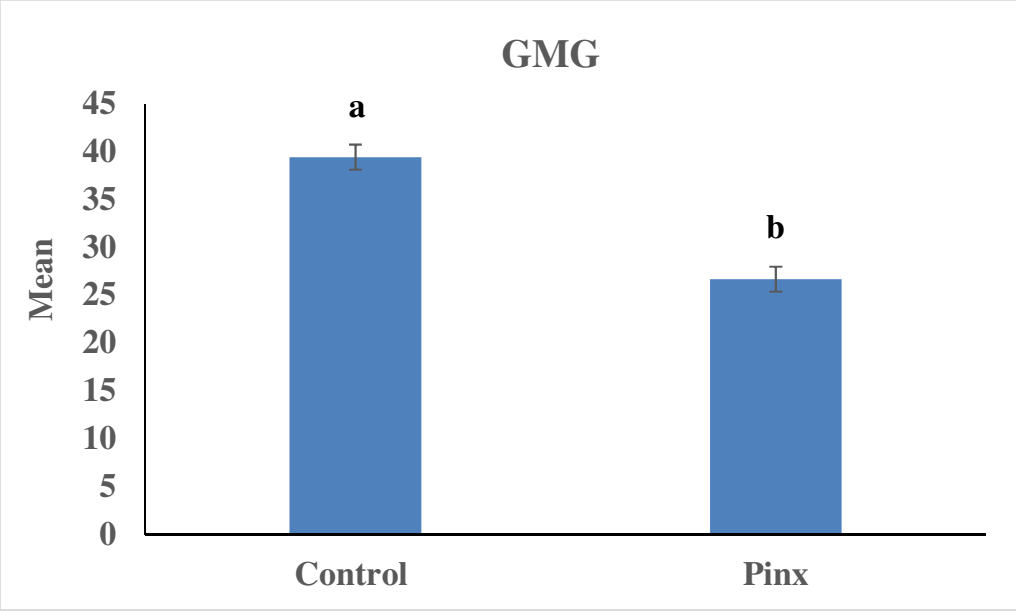

Figure 4. 69 Mean ± S.D. of the amount of the grooming behavior for five min rooming for aggression test in treatment (conrol and pinx)

4.3.2 Aggressive Posture (AGP)

The main effect of the photoperiod was significant on aggressive posture, F(2,54) = 33.10, p = .0001, η^2 = .55. The aggressive posture was the highest in short photoperiod (M = 13.2), the middle in the normal photoperiod (M = 5.55) but the lowest in long photoperiod (M = 1.55). The subjects under short photoperiod more aggressive than those under normal and long photoperiod conditions (Figure 4.70). The results showed that the subjects under the long photoperiod had the least aggressive behavior.

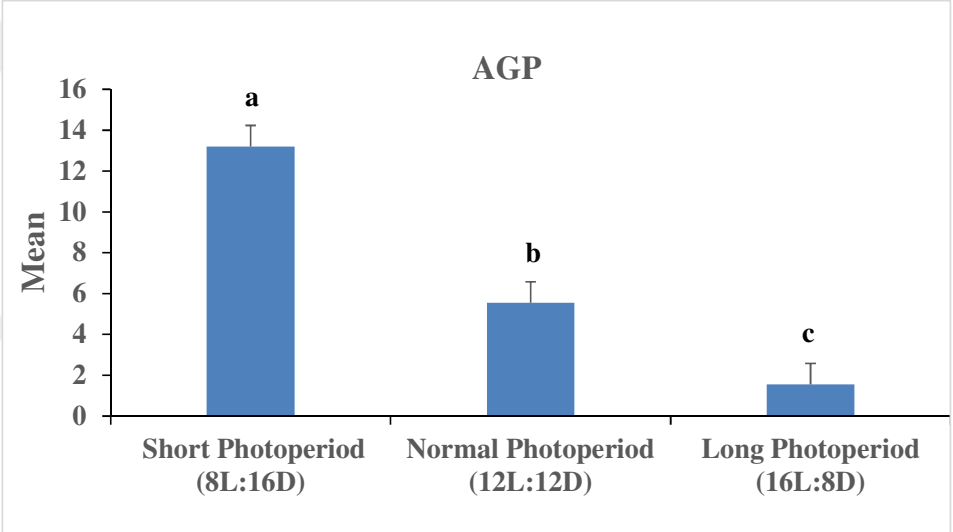

Figure 4. 70 Mean ± S.D. of the amount of the aggressive posture for aggression test in different phototperiod conditions

The main effect of the treatment was not significant, F(1,54) = 33.10, p = .87, η^2 = .001 (Figure 4.71).

The interaction effect between photoperiod and treatment was not significant, F(2,54) = 1.09, p = .342, η^2 = .04 (Figure 4.72).

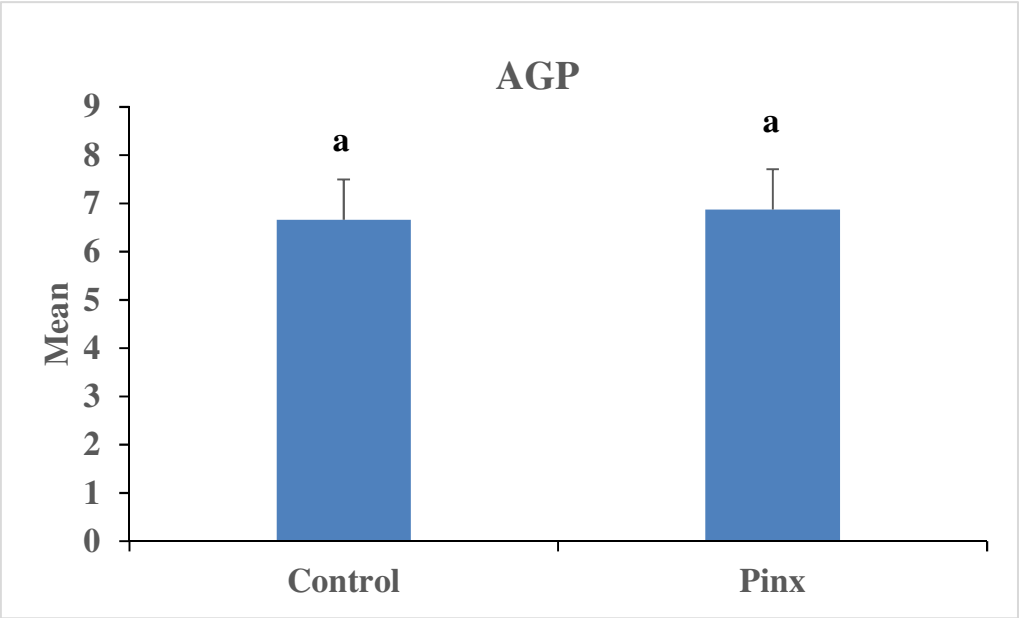

Figure 4. 71 Mean ± S.D. of the amount of the aggressive posture for aggression test in treatment (conrol and pinx)

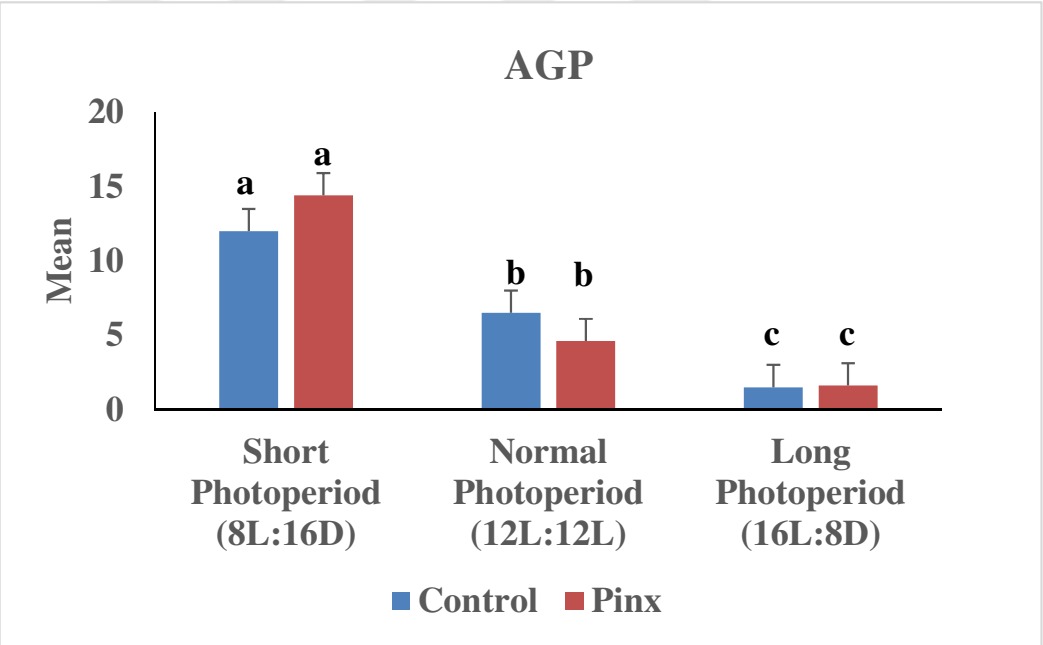

Figure 4. 72 Mean ± S.D. of the amount of the aggressive posture for aggression test in different photoperiod conditions with treatment (control and pinx)

4.3.3 Push and Pull Behavior (PHPLB)

The main effect of the photoperiod was significant on push and pull behavior, F (2, 54) = 23.08, p = .0001, n^2 = .46. The push-and-pull behavior was the highest in short photoperiod (M = 40.72) and long photoperiod (M = 37.83), and the lowest in normal photoperiod (M = 26.20). There was no significant difference between the normal and long photoperiod (Figure 4.73).

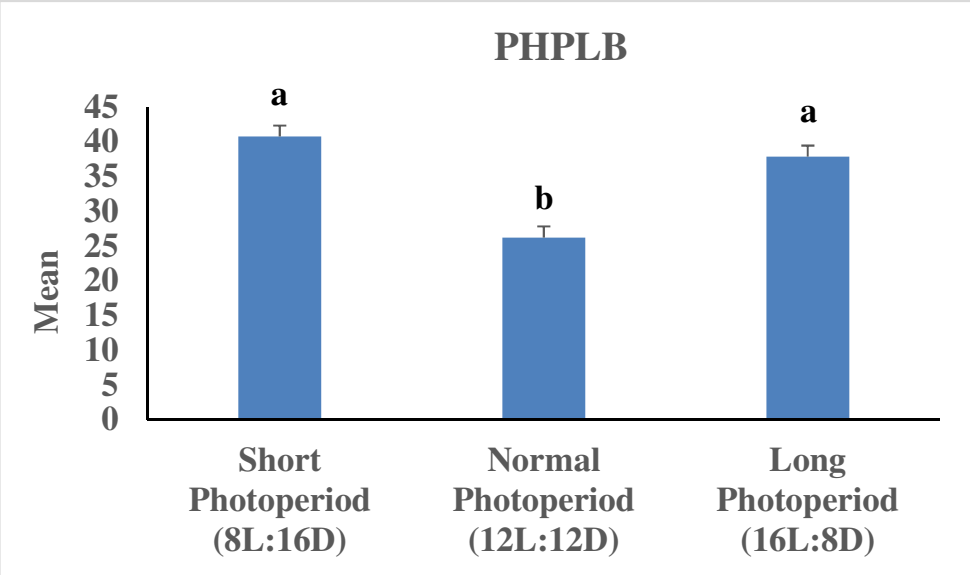

Figure 4. 73 Mean ± S.D. of the amount of the push and pull behavior for aggression test in different photoperiod conditions

The main effect of the treatment was not significant, F(1, 54) = 3.59, *p* = .87, n^2 = .06 (Figure 4.74).

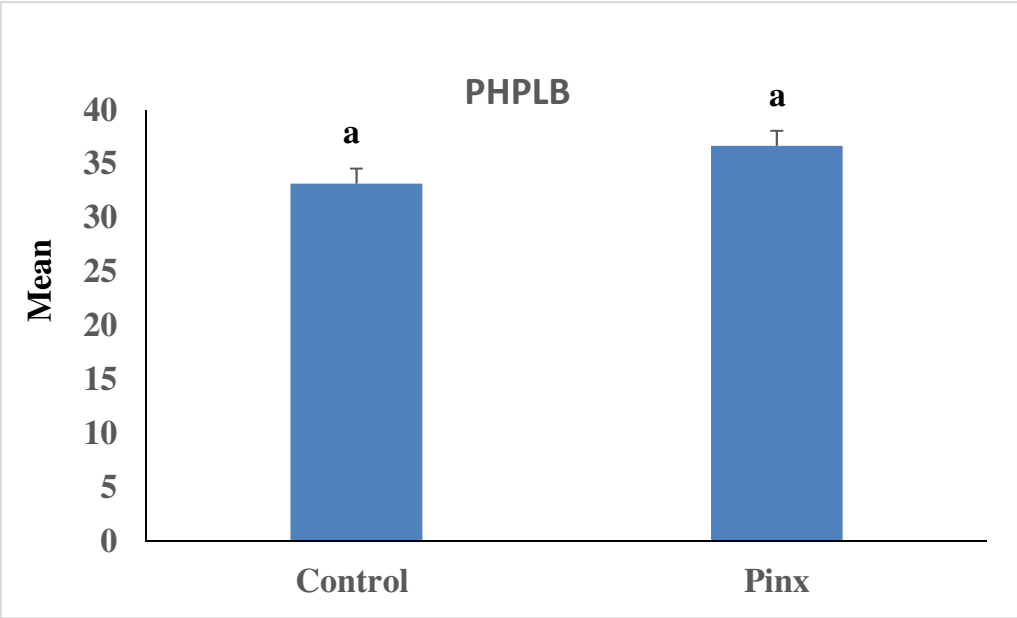

Figure 4. 74 Mean ± S.D. of the amount of the push and pull behavior for aggression test of treatments (conrol and pinx)

4.3.4 Attack to other subjects (AOS)

The main effect of the photoperiod was significant on attacking behavior to other subjects, $F(2,54) = 14.68$, $p = .0001$, $\eta^2 = .35$. The attacking to other subjects was the highest in short photoperiod (M = 9.70) and lower in long (M = 2.73) and normal photoperiod (M = 1.4), the last two being not significantly different from each other (Figure 4.75).

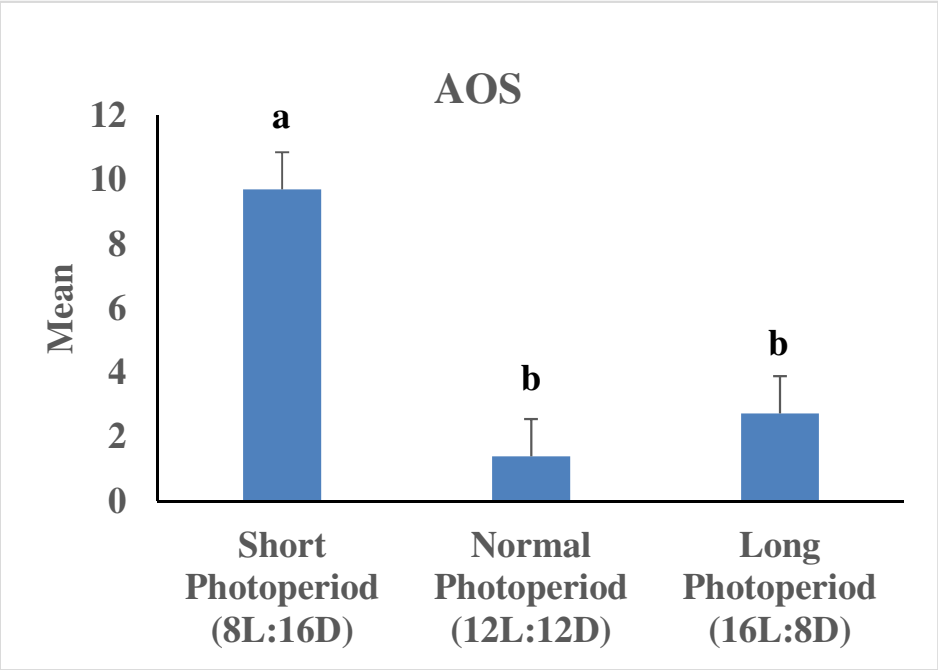

Figure 4. 75 Mean ± S.D. of the amount of the attack to other subjects for aggression test in different photoperiod conditions

The main effect of the treatment was not significant, $F(1,54) = 1.63$, $p = .21$, $n^2 = .03$ (Figure 4.76).

The interaction effect between treatment and photoperiod was significant, $F(2,54) = 3.46$, $p = .039$, $n^2 = .11$. The pinx subjects in the short photoperiod had the highest on attacking behavior than o all other conditions (Figure 4.77).

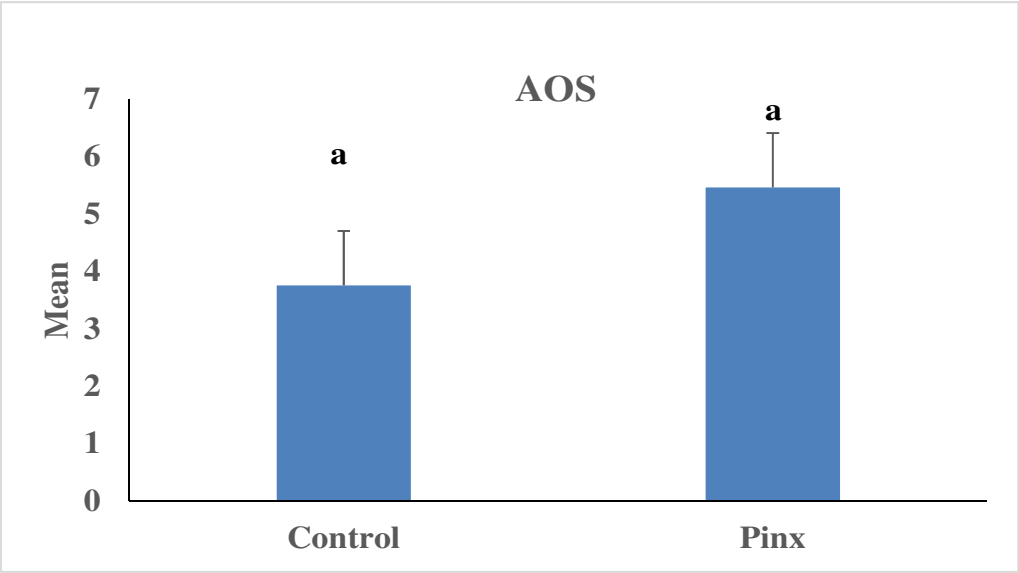

Figure 4. 76 Mean ± S.D. of the amount of the attack to other subjects for aggression test of treatments (control and pinx)

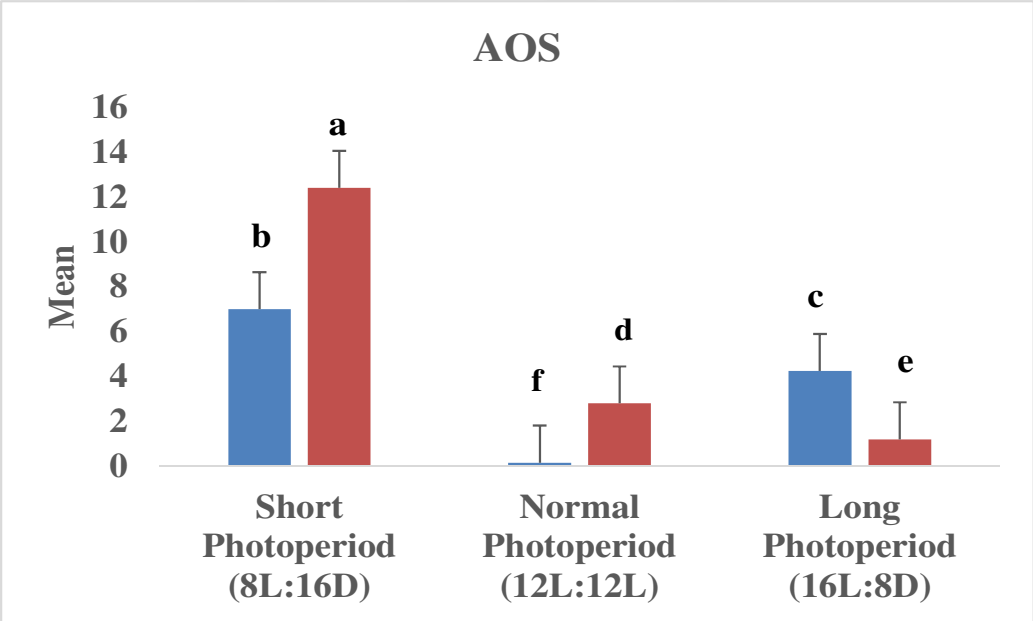

Figure 4. 77 Mean ± S.D. of the amount of the attack to other subjects for aggression test in treatments (control and pinx) under different photoperiod conditions

4.3.5 General Aggression (GEAG)

The main effect of the photoperiod was significant on general aggression behavior of the subjects, F(2, 54) = 29.16, p = .0001, η^2 = .52. The subjects in the short photoperiod (M = 88.37) showed more aggression than those in the normal (M

= 72.90) and those in the long (M = 75.83) photoperiods, the last two being not significantly different from each other (Figure 4.78).

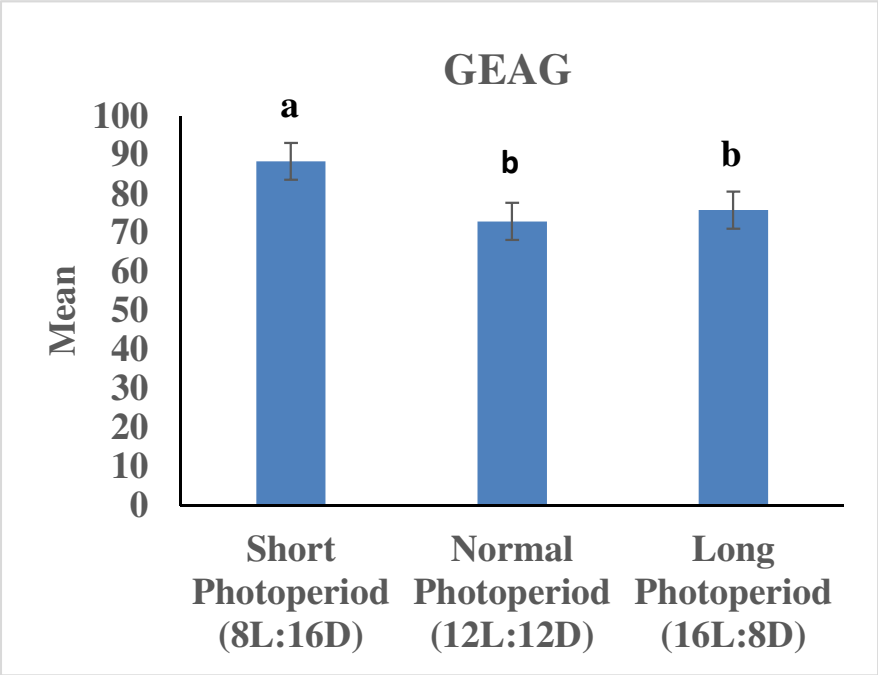

Figure 4. 78 Mean ± S.D. of the amount of the general aggression for aggression test in different photoperiod conditons

The main effect of the treatment was also significant on the general aggression (total aggression over 20 minutes), $F(1,54) = 20.37$, $p = .0001$, $\eta^2 = .27$. The control subjects (M = 83.00) showed more total aggression than the pinealectomized subjects (M = 75.07) (Figure 4.79).

The interaction effect between treatment and photoperiod was significant on the general aggression, $F(2,54) = 7.41$, $p = .0001$, $\eta^2 = .22$. This interaction effect indicated that the control subjects showed more aggression in the short photoperiod (M=87.75) than long (M = 83.25) and normal (M = 78.00) photoperiod, respectively (Figure 4.80).

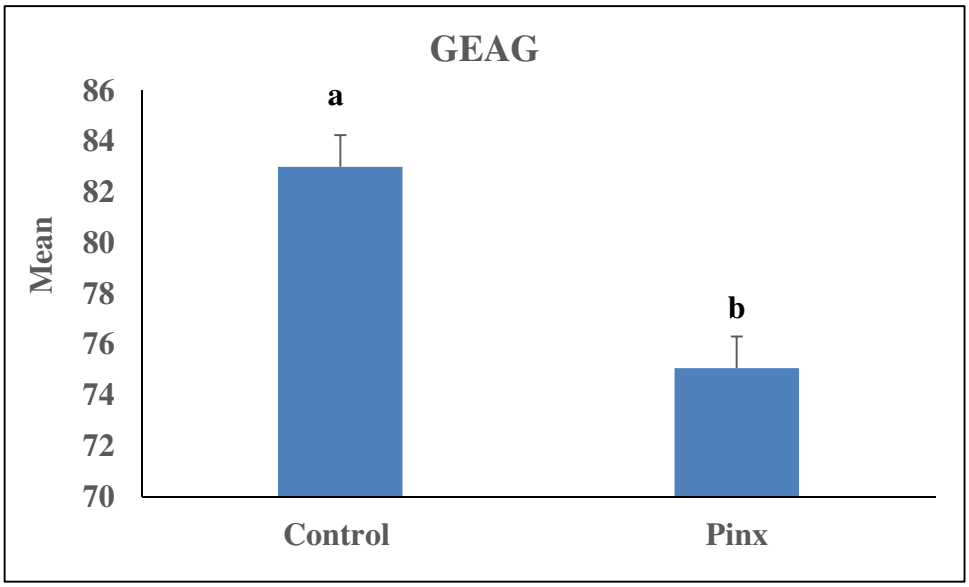

Figure 4. 79 Mean ± S.D. of the amount of the general aggression for aggression test in treatments (conrol and pinx)

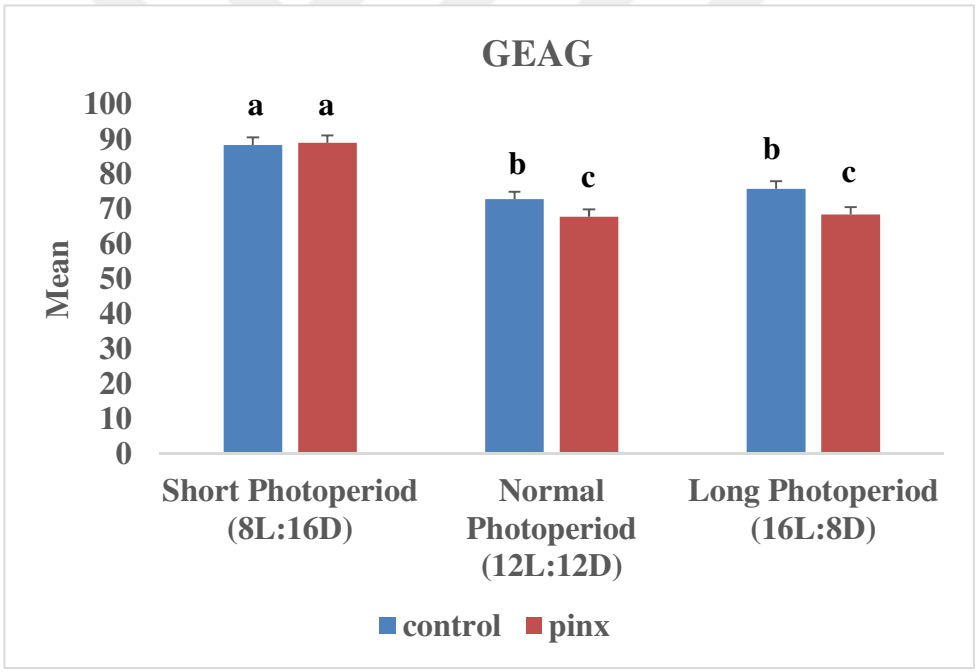

Figure 4. 80 Mean ± S.D. of the amount of the general aggression for aggression test in treatments (control and pinx) under different photoperiod conditions

5. DISCUSSIONS

This experiment was mainly showed to shed on lights on one important methodological flaw concerning the effects of different day-length [short (8L:16D), normal (12L:12D), and long (16L:16D) photoperiod], different measurement times of the day (6:00, 12:00, 18:00 and 24:00 h), and pinealectomy on anxiolitic, depressive and aggressive-like behaviours of male Wistar rats because some of the previous studies were conducted when the animals were in their active period (Einat et al. 2006; Ashkenazy et al. 2009; Ashkenazy- Frolinger et al. 2009; Kaya et al., 2011), while others were not (Nava and Carta 2001; Prendergast and Nelson 2005; Pyter and Nelson 2006; Benabid et al. 2008) in the literature. The outcomes of this study, which provided an extensive research paradigm with regard to the effects of different day-length, different measurement times of the day, and pineal gland emerged the thee principal findings that: (1) in open field, pinealectomized subjects in the normal photoperiod at 24:00 h were the least anxious of all other conditions for most of the measurement parameters, (2) in Porsolt forced swim test, control subjects in the long photoperiod at 12:00 h were the least depressive of all other conditions for most of the measurement parameters, and (3) in the intruder-resident test, the subjects in the short photoperiod were more aggressive than those in the normal and long photoperiod.

5.1 Anxiety-Like Behaviors (In the Open Field)

The open field test measures of motor activities such as spontaneous activity of animals and it is also used to detect the anxiety-like behaviours in rodents (Benabid et al. 2008; Kaya et al., 2011; Karakaş et al., 2011). The total distance travelled, the total number of entries to the edge and centre the of the open field, the time spent in the edge and centre of the open field, mobility, mobil frequency, and velocity are generally used parameters measured in open field test in the literature (Pyter and Nelson 2006; Karakaş et al., 2011; Pehlivan Karakaş et al., 2016). In this maze, if the anxiety of the animal is high, the time spent in the edge and the number of the entries to the edge of the open field are increasing and the total distance

travelled, the time spent in the centre, mobile duration, and velocity are decreasing. The total distance traveled, the total number of the entries into the centre, mobility, and velocity provides a built-in control measure for general hyperactivity, relaxing, non-anxiolytic or sedation (Kaya et al., 2011).

In our study, the pinealectomy subjects travelled more distance than those in the control condition. This means that pinealectomy decreases anxiety like behaviors. This may be due to fact that the presence of the melatonin increases some behaviours such anxiety like behaviours. Similarly to our finding, the exogenous melatonin hormone administration increased anxiety-like behaviors in rats (Karakas and Coskun, 2012). In addition to this information, the effect of pinealectomy on anxiety-like behaviours has not been fully elucidated in previous studies. In other words, in similar studies on animals of the same species, the effect of the pineal gland on anxiety-like behaviours showed different results. For example; Kaya et al. (2011) and Karakas et al. (2011) reported that pinealectomized rats had no significant effect on anxiety-like behaviours, while in another study it was reported that pinealectomy caused anxiety in rats (Nenchovska et al, 2014). These opposite outcomes suggested that the pineal gland is not effective alone or has partially effect on anxiety-like behaviours (Juszczak et al., 1996; Karakas et al., 2011; Kov´acs et al., 1974; Nenchovska et al., 2014).

The subjects under the normal photoperiod travelled more distance than those under the short and long photoperiod, with the last two being not significantly different from each other. This means that normal photoperiod makes animals less anxious than long and short photoperiod. This suggest that normal circulation of melatonin leads to an decrase in anxiety like behavior. This finding is in line with the previous reserach finding (Kaya et al., 2011).

The measurement time of the day was also found to be effective on the total distance travelling. In contrary, Kaya et al. (2011) reported that the pinealectomy was not significant on the total distance travelled under normal photoperiod in the open field test. The animals were more travelled at midnight (24:00 h) followed by 18:00, 12:00, and 6:00 h, with the last two being not significant different from each other. Especially at night and in the evening times, the animals travelled more distance than morning and noon times. In other words, anxiety is less evident at night and in the

evening. Our results showed that the effect of the measurement time was consistent with that of Kaya et al (2011) study, indicating that animals were more active at 24:00 h than 6:00 h. In addition to this finding, another consistent result is that our study and the former study consistently showed that animals were the least active in the moring (at 6:00 h). However, in their study the effect of treatment (pinealectomy and control) was not significant on all measures in the open field, whereas our research showed that pinealectomized subjects were more active than control subjects. This may be due to a lack of effective pinealectomy operation in the previous study that was implemented by the research assistant. Here all pinealectomy operiations were made by an experienced faculty member.

We also found an interaction effect between measurement time and photoperiod, indicating that the subjects in the normal photoperiod at night (24:00 h) travelled the most distance of all other conditions. This may suggest that both melatonin rhtym and circadian rthym play an important role in regulating anxiety like behaviors.

Our findings showed that pinealectomy was effective in the amount of the time spend at the edge of the open field, but photoperiod and measurement time were not significant. However, Kaya et al. (2011) found that pinealectomy and measurement time were not effective in the amount of the time spend at the edge of the open field. The difference between our study and Kaya et al. (2011) study is that pinealectomy was effective in the amount of the time spend at the edge of the open field. Kaya et al. (2011) found that pinealectomy and measurement time were not effective in the total entry to the edge of the open field. We found that photoperiod was effective, indicating that the subjects in the normal photoperiod stayed longer than those in the short and long photoperiod. However, this measure or parameter does not reflect anxiety like behaviors.

Kaya et al. (2011) found that pinealectomy and measurement time were not effective in the amount of the time spend at the center of the open field. The difference between our study and our findings showed that treatment was only effective in the amount of the time spend at the center of the open field, indicating that pinealectomized subjecst spend less time than controls at the center. This means that pinealectomized subjects were more anxious than controls. It is well known fact

that melatonin has a sedative effect. The absence of melatonin in the pinealectomized subjects may lead to a higher level of anxiety than controls. We also found an interaction effect between measurement time and photoperiod, indicating that the subjects in the normal photoperiod at night (24:00 h) spend the most time of all other conditions. This may suggest that both melatonin rhtym and circadian rthym play an important role in regulating anxiety like behaviors at the center of open field.

The pinx subjects were mobile than control subjects at 24:00 h for the normal photoperiod. This means that the removal of the pineal gland may interact with the normal photoperiod. In two cases, the release of melatonin hormone is less evident or absence. This effect was only evident at 24:00 h. This also suggest that the circadian rhytm play important role. This time is a time when the melatonin is secreted at the high level. Such internal activation may make animals more active and anxiety-free. We also found that the subjects in the normal photoperiod were more mobile than those in the short and long photoperiod, suggesting that the regular melatonin rhytm play important role in decreasing anxiety like behaviors. The animals were more mobile at midnight (24:00 h) followed by 18:00, 12:00, and 6:00 h, with the each one being significantly different from each other. Especially at night and in the evening, the animals were more active than morning and noon. In other words, anxiety is less evident in the evening and at night. This suggest the role of circadian rthym in regulating anxiety like behaviors. The interaction effect between measurement time and treatment was also significant, indicating that the pinealectomized subjects at the 24:00 h were the most mobile of all other conditions. Melatonin is secreted and released in a circadian fashion, high levels at night and very low levels at day time (Arendt, 1988; Karakas and Coskun, 2012). This high release of melatonin at night may make animals more active and anxiety-free. Kaya et al. (2011) also found that measurement time was effective in mobility, indicating that the subjects at the 24:00 h were the most mobile of all other conditions. However, they found no significant effect of treatment.

The pinx subjects were more frequently mobile than control subjects at 24:00 h for the normal photoperiod. We also found that the subjects in the normal photoperiod were more mobile than those in the short and long photoperiod, suggesting that the regular melatonin rhytm play important role in decreasing anxiety like behaviors. The animals were more mobile at midnight (24:00 h) followed by

18:00, 12:00, and 6:00 h, with the each one being significantly different from each other. Especially at night, the animals were more frequently mobile than morning and noon. The interaction effect between measurement time and treatment was also significant, indicating that the pinealectomized subjects at the 24:00 h were the most mobile of all other conditions.

The pinealectomized animals were faster than control ones at 24:00 h under the normal photoperiod. We also found that the subjects in the normal photoperiod were more mobile than those in the short and long photoperiod, suggesting that the regular melatonin rhytm play important role in decreasing anxiety-like behaviors. The animals were more mobile at midnight (24:00) followed by 18:00, 12:00, and 6:00 h, with the last two being not significantly different from each other. Especially at night, the animals were faster than evening, noon and morning. In other words, anxiety is less evident at night. This suggest the role of circadian rthym in regulating anxiety like behaviors. The interaction effect between measurement time and photoperiod was also significant, indicating that the subjects in the normal photoperiod at the 24 :00 h were the fastest of all other conditions. The results of open field test showed that the tested rats generally active at night and normal photoperiod. These outcomes were expected for nocturnal animals. Kaya et al. (2011) also found that measurement time was effective in velocity, indicating that the subjects at the 24:00 h were the fastest of all other conditions. However, they found no significant effect of pinealectomy.

Melatonin is a small amount or more increases the anxiety. In other words, the presence of more or less melatonin in the blood plasma is a condition that disrupts the animal's internal system or rhythm. Karakas et al. (2011) argue that changes in melatonin lead to more rhythm disturbances and that rhythm disruption is a more important factor than circadian rhythm in understanding the effect of melatonin release. On the other hand, in normal photoperiod, the release of melatonin in the blood plasma of the animal is regular and normal. In this case this normal level of melatonin might decrease anxiety.

So far, measurements from animals have not been taken at different measurement times of the day and different photoperiods except for this study. Our finding the results of open field in rats consistently lead that at night (24:00) and

under normal photoperiod (12L:12D) are more mobile than day. Rats are nocturnal animals, so they are more active during the night. Applications (i.e., photoperiod and pinealectomy) make these animals more active at night. This should be explained in detail in the future. There may be a possible reason that pinealectomized animals are more active in the evening and at night than the animals under control. Decreased melatonin release as a result of pineal gland removal may be due to circadian rhythm. Although the animals have been tested in red light, the release of melatonin in the evening and at night is higher than in daylight. This may affect circadian rhythm results.

Taken together these findings suggest that the normal photoperiod may interact with the removal of the pineal gland in which, the release of melatonin hormone is low or absence. Since normal concentration of melatonin is secreted in normal photoperiod, the known sedative effect of melatonin may have occurred. We found that in the pinealectomy condition, anxiety was low. This suggests that absence of melatonin reduces anxiety-like behaviors. On the other hand, melatonin may remain in the blood for a longer period in short photoperiod, which may increase anxiety-like behaviors. This emphasizes the importance of homeostasis. This effect was only evident at 24:00 h.

5.2 Depression-Like Behaviors (Porsolt)

In our study, we investigated that the depressive-like behaviors (learned helplessness) of the subjects in a forced swim test (Porsolt et al., 1977) and found that the control subjects were less depressive than the pinealectomy subjects in total distance travelled, mobil duration, center movement duration and velocity. These findings were in line with previous research findings (El Mrabet et al., 2012) that demonstrated that the pinealectomy significantly increased immobility time in animals of both sex although the effects were more evident in females (El Mrabet et al., 2012). The control group is less depressive because the melatonin is produced endogenously and is known to be an important neurotransmitter that reduces the level of depressive-like behaviour (Pehlivan Karakaş et al., 2016). The subjects in the long photoperiod and control condition were less depressive, because the short-term presence of melatonin in the blood reduces depression-like behaviors. A research

showed that melatonin deficit (pinealectomy) led to depressive-like behaviour, which was evident for thee months (Nenchovska et al., 2014). The measurement time was also effective, indicating that the subjects at noon (12:00 h) were less depresive than those at other tested times. This result shows that measurement time is very important in depression detection experiments. In other words, it indicates that depression is low at noon, when melatonin is at a low level. In dark cycle serotonin converts to melatonin, whereas in light melatonin convers to serotonin (Kırım et al., 2006)

The subjects in short photoperiod is more depressive because short photoperiod increases the hormone melatonin. On the other hand, internal reduction of melatonin though pinealectomy increases depression. The anxiety tool can be variable. An interesting finding is that the removal of endogenous melatonin though pinealectomy increases depression but decreases anxiety-like behaviours. This can be explained by different pathways depression and anxiety. Melatonin is effective in several psychiatric conditions including depression (Ramírez-Rodríguez et al. 2014, Stefanovic et al. 2016, Taniguti et al. 2018) and anxiety (Karakaş et al. 2011, Ochoa-Sanchez et al. 2012, Zhang et al. 2017). Some scholars have suggested that the antidepressant action of melatonin is related to enhanced hippocampal neurogenesis (Crupi et al. 2010) and an increase in serotonergic system triggered by melatonin is probably related to its antidepressant property (Ramírez-Rodríguez et al. 2014). However, the melatonin mechanisms for anxiety-like behaviors are still unkown and usually explained from serotoninergic system (Rebai et al. 2017, Zhang et al. 2017, Taniguti et al. 2018).

The future researchs should investigate what kinds of mechanisms of pathways are effective by removal of endogenous melatonin. Another explanation is that receptors in lymbic system play an important role in a sense that melatonin may be more related to depression than anxiety. Put it differenty, depressive-like behaviours may be more sensitive to melatonin than anxiety-like behaviors.

5.3 Aggression-Like Behaviours (Introdure-Resident)

Aggression is complex social behaviors that are displayed by virtually all organisms. This behavior serves a wide range of adaptive functions (acquiring and maintaining food or mates) in both sexes and requires integration of both environmental and physiological factors (Clutton-Brock, 2009).

This research examined the effects of photoperiod (short, normal and long photoperiods) and pinealectomy on the aggressive behaviors of Wistar albino rats. Pinealectomy was not significant on the aggresive-like behaviours but short photoperiod caused to high level of aggression. Most consistent results in the current study is that the subjects in the short photoperiod were the most aggressive of all other photoperiod conditions in terms of aggressive posture, push and pull behavior, attact to the other, and general aggression. However, no significant difference was observed with the animals in the pinx group and those in the control group. This suggests that melatonin does not have an effect on aggression alone and that different mechanisms play an important role. For instance, melatonin produced in the gonads and adrenal glands may play an effective role, even if the pineal gland is removed. Increased melatonin concentration in short photoperiod may activate mechanisms such as testosterone that increase aggression (Chichinadze K and Chichinadze N, 2008).

In some studies, in the literature, serotonin is known to have a role in emotions, and its deficiency causes aggressive and depression-like behaviours (Seo et al., 2008, Özenoğlu and Ünal, 2015). Aggression and depression-like behaviors may be related to serotonin level and its mechanism. We can associate the aggression with the longer duration of melatonin in the blood because serotonin duration and concentration will be less under short photoperiod.

These results can be explained from two perspectives. First, a high level of melatonin due to short photoperiod may inhibit serotonin level in which, in turn, leads to a high level of aggression. In the literature, there is research support that a high-level melatonin leads to a low level of serotonin (Kırım et al., 2006). Serotonin synthesized in the presence of light and tryptophan amino acid in the pineal gland,

and melatonin is synthesized enzymatically in the pineal gland with darkness (Kırım et al., 2006). Second, a high level of melatonin due to short photoperiod may increase adrenal steroids in which, in turn, lead to a high level of aggression. There is some evidence that a high level of testosterone enhances aggression, indicating a positive relationship between gonadal steroids, (e.g., particularly testosterone) and aggression in males and females of many vertebrates, including birds, lizards and mammals (Lincoln, Guinness, Short, 1972; Moore, 1988; Wingfield et al., 1987). For instance, some studies have found that male hamsters in short photoperiods tended to show more aggression than those in long one (Bronson and Heideman, 1994; Garrett and Campbell, 1980; Jasnow et al., 2002). So far, an effective explanation was provided by Jasnow et al (2002), who found that high agressive behaviors occured in hamsters exposed to short photoperiod. This finding may be due to the longer duration of melatonin under short photoperiod.

The other mechanisms underlying the effects of melatonin on aggression was explained from the role of the adrenal steroids such as the adrenal androgen dehydroepiandrosterone (DHEA) for female aggression on the basis of melatonin secretion induced by short and long photoperiod (Soma et al., 2016). For instance, some other steroid hormones appeared to play important roles in the regulation of aggression in both males and females (Boonstra et al., 2008; Crews, 1984; Crews and Moore, 1986; Gutzler et al., 2009; Hau et al., 2004; Moore et al., 1985; Scotti et al., 2009). There is research suggestion that short-day patterns of melatonin induce a suite of traits including gonadal regression and decreases in sex steroids (Hardeland, 2009). Or gonadol hormones such as testesterone may be decreased by increased melatonin level under short potoperiod (Yılmaz et al., 2000). This plausible relationship is still unknown in the literature and needs a detailed research in the future.

6. CONCLUSIONS

In conlusion, this research aimed to investigate the effects of different photoperiod (short-8L:16D, normal-12L:12D, and long 16L:8D), different measurement times of the day (6:00, 12:00, 18:00 and 24:00 h), and pinealectomy on anxiety, depression and aggression-like behaviors in male Wistar albino rats by using open field, Porsolt forced swim test, and intruder-resident test. The findings showed that: (1) in open field, pinealectomized subjects in the normal photoperiod at night (24:00 h) were the least anxious of all other conditions for most of the measurement parameters, (2) in Porsolt forced swim test, control subjects in the long photoperiod at noon (12:00 h) were the least depressive of all other conditions for most of the measurement parameters, and (3) in the intruder-resident test, the subjects in the short photoperiod were more aggressive than those in the normal and long photoperiod.

Taken together these findings suggest that the normal photoperiod may interact with the removal of the pineal gland in which, the release of melatonin hormone is low or absence. Since normal concentration of melatonin is secreted in normal photoperiod, the known sedative effect of melatonin may have occurred. We found that the anxiety-like behaviors of the pinealectomized animals were low but depression-like behaviors were high. This suggests that an absence or low dose of melatonin reduces anxiety-like behaviors. On the other hand, long duration of melatonin in the blood under short photoperiod, this may increase anxiety-like behaviors and aggression-like behaviours. In addition, the presence of the pineal gland, in other words, normal secretion of melatonin, is important in reducing depression-like behaviors.

The future researchs should investigate what kinds of mechanisms of pathways are effective by removal of endogenous melatonin. Another explanation is that receptors in lymbic system play an important role in a sence that melatonin may be more related to depression than anxiety. Put it differenty, depressive-like behaviours may be more sensitive to melatonin than anxiety-like behaviors.

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