LEPTIN INFUSION TO PREGNANT AND PINEALECTOMIZED SYRIAN HAMSTERS (*Mesocricetus auratus*) AND LOCOMOTOR ACTIVITY OF JUVENILES

by
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ABSTRACT

LEPTIN INFUSION TO PREGNANT AND PINEALECTOMIZED SYRIAN HAMSTERS (*Mesocricetus auratus*) AND LOCOMOTOR ACTIVITY OF JUVENILES

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A circadian rhythm generator suprachiasmatic nucleus (SCN) which synchronizes the daily variations in light intensity and integrates environmental light signals located in the hypothalamus. In rodents, circadian rhythms are synchronized to the outside environment through the maternal system which is in synchrony with those of their mothers during late embryonic and early neonatal development. Melatonin exerts a "chronobiotic" effect by acting directly on the SCN, which contain melatonin receptors and the endogeneous melatonin peak is phase-advanced with the direct application of exogenous melatonin into the SCN. Adipose tissue derived hormone leptin has receptors on the SCN and the activity rhythm is phase advanced by leptin treatment both *in vivo* and *in vitro*. In this thesis, the regulatory effects of leptin through maternal system on fetal SCN were investigated.

Long day housed (LD 14:10) pregnant hamsters were cannulated from back and subcutaneous leptin infusion has started at the 10^{th} day of pregnancy until the parturition. After parturition, 15 days of age juvenile Syrian hamsters were weaned from their mothers and placed in cages for their locomotor activity records. Body weights were recorded. Locomotor activities were recorded for 3 weeks by using Vital View Software. The results showed that the wheel running turns of juveniles (leptin infusion to mother between 00.00-01.00 h) was significantly higher in control group ($p<0.05$) and wheel running turns of this group (00.00-01.00 h) were not statistically different between control and pinealectomized animals. The wheel running turns of juveniles from mothers of leptin infusion between 12.00-13.00 h and between 13.30-19.30 h groups were not statistically different in both control and pinealectomized groups ($p>0.05$). Body weights of juveniles were statistically different between control and pinealectomized groups ($p<0.05$) in both 00.00-01.00 h and 13.30-19.30 h leptin infused groups, but not in 12.00-13.00 h leptin infused group ($p>0.05$).

In conclusion, the locomotor activities of juveniles were influenced by maternal leptin and SCN would possibly play an important role. Body weight of juvenile Syrian hamsters might also be controlled by maternal leptin in the postnatal period.

Keywords: SCN, Pineal gland, Leptin, Infusion, Maternal system, Syrian hamster

ÖZET

HAMİLE VE PİNEALEKTOMİLİ SURİYE HAMSTERLARINA

(*Mesocricetus auratus*)

LEPTİN İNFÜZYONU VE YAVRULARDA LOKOMOTOR AKTİVİTE

Çamsarı, Çağrı

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Hipotalamusta bulunan ve gün içi ritimlerin oluşmasını sağlayan suprakiazmatik nükleus (SCN), gün içerisindeki ışık yoğunluğundaki değişimlerin senkronizasyonundan ve çevresel ışık sinyallerinin entegre edilmesinden sorumludur. Kemirgenlerde geç embriyonik ve erken neonatal dönemde gün içi ritimler, annenin gün içi ritimlerine senkronize bir şekilde maternal sistem aracılığı ile çevresel şartlara senkronize edilir. Melatonin "kronobiyotik" etkisini melatonin reseptörlerinin bulunduğu SCN üzerine doğrudan etki ederek uygular ve içsel melatonin'in pik zamanı egzojen melatonin'in SCN'a doğrudan uygulanması ile safha kayması gösterir. Yağ dokudan salınan bir hormon olan leptin hormonu SCN üzerinde reseptörlere sahiptir ve aktivite ritimleri *in vivo* ve *in vitro* leptin uygulamalarında safha kayması gösterir. Bu tez çalışması ile leptin'in maternal sistem aracılığı ile fetal SCN üzerindeki düzenleyici etkisi incelendi.

Uzun fotoperiyotta (LD 14:10) tutulan hamile Suriye hamsterlar'a hamileliklerinin 10. gününden doğum gerçekleşene kadar sırtlarına yerleştirilen

kanüller vasıtasıyla deri altından leptin hormonu uygulaması yapıldı. 15 günlük yavru Suriye hamsterlar annelerinden ayrılarak lokomotor aktivite kayıtları için kafeslere yerleştirildi. Haftalık olarak vücut ağırlığı ölçümleri yapıldı. Vital View yazılımı kullanılarak lokomotor aktivite kayıtları 3 hafta süresince alındı. Sonuçlara göre gece 00.00-01.00 saatleri arasında leptin infüzyonu yapılan anne hamsterların yavrularının tekerlek dönüşlerinin kontrol gruplarında istatiksel olarak yüksek olduğu ($p<0.05$) ve aynı gurupta (00.00-01.00 saatlerinde leptin infüzyonu) kontrol ve pinealektomili hayvanların tekerlek dönüşleri arasında istatiksel olarak anlamlı bir fark bulunamadığı tespit edildi ($p>0.05$). Aynı zamanda 12.00-13.00 ve 13.30-19.30 saatleri arasında yapılan leptin infüzyonları gruplarında da yavruların tekerlek dönüşleri arasında hem kontrol hem de pinealektomi gruplarında istatiksel olarak anlamlı bir fark gözlenemedi ($p>0.05$). Kontrol ve pinealektomi grupları arasında 00.00-01.00 ve 13.30-19.30 saatleri arasında leptin infüzyonu yapılan annelerin yavrularının vücut ağırlıkları arasında istatiksel olarak anlamlı bir fark tespit edilirken ($p<0.05$), bu fark 12.00-13.00 saatleri arasında leptin infüzyonu yapılan grupta tespit edilemedi ($p>0.05$).

Sonuç olarak, yavruların lokomotor aktivitelerinin kontrolü maternal leptin tarafından gerçekleştirilmiştir ve bu etkide SCN'nin önemli bir rolü olabilir. Doğum sonrası dönemde yavru Suriye hamsterlarının vücut ağırlıklarının kontrolü de maternal leptin tarafından gerçekleştirilmiş olabilir.

Anahtar kelimeler: SCN, Pineal bez, Leptin, İnfüzyon, Maternal sistem, Suriye hamsteri

To My Parents

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

1. INTRODUCTION

1.1 Photoperiodism

Seasonal species that generally live in temperate latitudes breed only at restricted times of the year and exhibit additional physiological adaptations to changing environmental conditions such as the photoperiod, food availability/quality, ambient temperature or rainfall. Photoperiodism is the ability to establish the time of year and regulate annual biological rhythms by using the annual progression of day lenght change as a cue. Photoperiodic species show robust annual cycles of appetite, body weight and reproduction. The birth of young and lactation are timed according to the most favorable season for raising offspring, which occurs generally when resources are plentiful to support the energetic requirements of lactation and post-weaning growth of the young.

Photoperiod is used to regulate the sensitivity of reproduction and gonadal maturation in hamster species. Gonadal regression and loss of of sexual behavior is observed by exposing the hamsters to short photoperiods (Pospichal et al., 1991; Schilatt et al., 1993). An exposure of photoperiodic species to photoperiods which are greater than threshold (critical photoperiod) causes reproductive development, while photoperiods less than critical causes the prolongation of reproductive devolopment. For many rodent species critical photoperiods have been characterized as between 12 and 14 hours of light per day. A 12.5 hours of light per day is defined as the critical photoperiod for the Syrian hamsters and any photoperiod which is less than this causes gonadal atrophy whereas any photoperiod which is longer than this promote gonadal maintenance (Elliot, 1976; Hoffman, 1979). Critical photoperiod is

defined as 13 hours in Siberian hamsters. In Mongolian gerbil species, there are two critical photoperiods; the first is between 8-10 hours of light and second is between 22-24 hours of light (Karakaş and Gündüz, 2002). It has been shown that gonadal development of juveniles is also regulated by photoperiod in several species (Hoffmann, 1978, 1981; Johnson and Zucker, 1980). However, the prepubertal gonadal maturation and body weight gain in Syrian hamster are independent from environmental daylength.

Photoperiod-induced changes have been shown to be the main cue controlling some important variations such as energy metabolism. For example, in Siberian hamsters body weight is reversibly reduced by short-day exposure, however, in Syrian hamsters short-day exposure causes the increase of body weight which is mostly associated with an increase in fat mass.

1.2 Biological Rhythms and Regulation

All life on earth depends on the presence of the sun, which, due to the endless rotation of earth around its own axis, exposes all organisms on its surface to the daily change in light intensity. Consequently, to optimize their survival, organisms use sunlight to adjust their period of activities in 24 hour cycles determined by sunrise and sunset. Biological processes that repeat themselves every 24 hours are called daily or circadian rhythms. Biological rhythms are recurrent events within a biological system which means a biological process that takes place more than once usually many times throughout the system's life. For this purpose, light sensitive cellular clock mechanisms have evolved to anticipate the period of activity.

A highly localized brain clock in mammals was first suggested in 1972, with the demonstration that lesions ablating the suprachiasmatic nucleus (SCN)

abolish circadian rhythmicity in adrenal corticoid secretion, secretion of prolactin, melatonin, growth hormone, estrus cycling, locomotor activity, drinking and body temperature (Moore and Eichler, 1972; Stephan and Zucker, 1972; Meyer-Bernstein et al., 1999). It was demonstrated in these studies that the SCN is sufficient for sustained rhythms in metabolic, secretory and electrophysiological activity. Studies with transplantation of donor SCN tissue into the brains of arrhythmic, SCN-lesioned hosts restore circadian rhythmicity in behavior (Lehman et al., 1987; Ralph et al., 1990). It is important to note that the restored rhythms have the period of the donor SCN, which indicates that the transplanted tissue does not act by restoring host brain function but that the "clock" is contained in the transplanted tissue.

The circadian rhythm generator in the SCN is entrained to environmental rhythms by external cues called zeitgebers. One of the most important properties of circadian clock is the ability to become synchronized with the 24h light/dark (LD) cycle. The synchronization process is called entrainment and is the result of the ability of the clock to be reset by light. Photoperiod which is the change between day (light) and night (darkness), is the biologically most important zeitgeber. In adult mammals, including humans, light information is perceived by the retina which is then, conveyed by the retinohypothalamic tract (RHT) in the optic nerve to the brain. RHT makes monosynaptic neural projections which are terminate in the bilaterally paired suprachiasmatic nuclei (SCN) of the anterior hypothalamus, hosting the central pacemaker. It is known that the light/dark (LD) cycle is the major environmental synchronizing agent of the mammalian circadian clock, but it is also important to determine how "nonphotic" signals influence pacemaker activity. Overall activity-rest or sleep-wake state of the animal have clear phase shifting effects on the circadian pacemaker in response to variety of stimuli in species as

diverse as hamsters and humans (Mrosovsky et al., 1989; Van Reeth et al., 1994). Nonphotic signaling pathway involves both direct and indirect serotonergic projections from the midbrain raphe nuclei to the SCN. Intergeniculate leaflet of the lateral geneculate nucleus (IGL/LGN) of the thalamus is involved in the indirect pathway which integrates photic and nonphotic information that is utilized by the SCN to coordinate the temporal organization of circadian rhythms in mammals.

The SCN consists of different neurons containing different neuropeptides, such as vasopressin (VP), vasoactive intestinal peptide (VIP), gastrin-releasing peptide or somatostatin. GABA or glutamate are also contained by several of these neurons. In addition to these neuropeptides, neurons expressing cholecystokinin, calretinin, calbindin, and neurotensin were also demonstrated.

SCN makes GABAergic projections to the paraventricular nucleus (PVN) which forms a monosynaptic connection to the intermediolateral column in the upper thoracic spinal cord which gives rise to preganglionic sympathetic nerve fibers projecting to the superior cervical ganglion (SCG). Pineal gland receives postganglionic sympathetic nerve fibers from the SCG neurons which contain norepinephrine (NE) and neuropeptide Y (NPY) as neurotransmitters. NE which drives the rhythmic biosynthesis of melatonin, is released from the intrapineal terminals night by night (Drijfhout et al., 1996).

1.3 Pineal Gland and Melatonin

Pineal gland is a small pine-shaped structure located in the epithalamus of the brain. The main hormone secreted by the pineal gland is the melatonin, which was isolated and characterised from the bovine pineal, by the dermatologist Aaron Lerner in 1958 (Lerner et al., 1958). Melatonin is the neuroendocrine message of darkness and an important neuroendocrine hand of the clock, the secretion of which

is under the control of photoperiod and SCN. Melatonin, which is secreted in a rhythmic manner, has a role to transmit seasonal changes of daylenght, regulates seasonal physiology and behavior, and influences food intake, reproduction and pelage moulting (Barrett et al., 2003). Melatonin which is distributed via the systemic circulation to all peripheral and central structures, exerts its effects via the specific binding sites.

Melatonin, which is secreted at night, has a lipophilic structure. According to current concepts, the secretion of it is under the control of its formation. Since it has a lipophilic structure, it is not stored within the pinealocytes, but is immediately released into the blood stream and cerebrospinal fluid upon its formation. Depending on the species, intravenously administred radioactive melatonin rapidly disappears from the blood with a half-life of about 30 min. (Pang et al., 1993).

In mammals, the pineal gland and its hormone melatonin are clearly implicated as a central component of the photoperiodic mechanism. Pinealectomy which is the removal of the pineal gland, is one of the methods to investigate the effect of melatonin in photoperiodic animals. Pinealectomy eliminates melatonin hormone from the circulation and pinealectomized mammals are not able to exhibit species-specific seasonal responses to changes in photoperiod, although most circadian rhythms remain intact. But appropriately timed administration of melatonin to pinealectomized mammals can restore seasonal responses. The effect of pineal gland on body weight and lipid mass changes was reported in Syrian hamsters, Siberian hamsters and praire voles that pinealectomy may block the photoperiod related changes in body weight (Hoffmann, 1981).

Two hypotheses have been proposed from analysis of the endogenous melatonin patterns in different conditions and from studies with injections or chronic

infusions of exogenous melatonin. A suggestion that the duration of the nocturnal melatonin peak is positively correlated to the lenght of the night has come from the observation of the melatonin secretion pattern in sheep (Rollag and Niswender, 1976; Karsch et al., 1988), rat (Illnerova and Vanecek, 1980), Siberian hamster (Illnerova et al., 1984; Ribelayga et al., 2000), Syrian hamster (Skene et al., 1987; Maywood et al., 1993; Miguez et al., 1995) and European hamster (Vivien-Roels et al., 1992) which are raised in different photoperiodic conditions. Therefore "duration hypothesis" suggests that the photoperiodic information is transmitted from the environment to the body by a duration of the nocturnal melatonin peak. Gonadal regression was seen in intact hamsters kept in long photoperiod, which were injected acutely with melatonin at the end of the day or beginning of the night. But no effect was observed when the injection made at the end of the night or at the beginning of the day. Therefore "coincidence hypothesis" claims that the deciding factor for the appearance of a physiological effect is the coincidence of the injection of melatonin with a phase of sensitivity (Tamarkin et al., 1976; Reiter, 1987). Study of Pitrosky et al. (1995) in which the photoperiodic response of Syrian hamsters to melatonin were investigated, also supports the phenomenon of coincidence. In this study short-day type response were obtained for the reproductive axis by the infusion of two consecutive melatonin peaks, whose lenght from the beginning of the first peak to the end of the second peak corresponded to a short-day signal but whose total quantity of infused melatonin corresponded to a long-day signal. It can be concluded that on the basis of these findings that the interval between the first and the second melatonin peak determines the physiological response rather than the clock time when the double melatonin peak is applied.

Endogenous SCN clock uses the daily rhythm of melatonin to deliver the circadian message to melatonin target structures which are containing melatonin receptors. In addition, to affect the circadian clock, melatonin exerts a "chronobiotic" effect by acting directly on the SCN, which contain melatonin receptors (Vanecek et al., 1987). Melatonin uptake is determined in the SCN of nearly all species (Bittman, 1993) including humans (Reppert et al., 1988) as well as variety of other brain sites (Bittman, 1993). Melatonin receptors have been determined in the pineal gland of the newborn rat which become rare in 9-day-old rats and these receptors disappear in adults (Zitouni et al., 1995). Circadian rhythms of locomotor activity and melatonin synthesis are synchronized by pharmacological doses of exogenous melatonin in rats and hamsters with free-running circadian rhythms. It has been shown recently, the endogenous melatonin peak is phase-advanced and the amplitude of it is increased with the direct application of exogenous melatonin into the SCN by reverse microdialysis (Bothorel et al., 2002). Studies *in vitro* have demonstrated the metabolism, electrical activity and circadian rhythmicity of SCN is effected locally by the administration of melatonin (Cassone et al., 1988; Stehle et al., 1989; McArthur et al., 1991). Melatonin may exert its synchronizing properties indirectly on clock inputs or clock outputs, or directly on the clock via melatonin receptors (which were identified on VP-containing SCN neurons) (Song et al., 1999) or other binding sites. Circadian clock of the offspring is entrained by using this property of melatonin along with several circadian signals between the mother and fetus (Reppert et al., 1979; Reppert and Weaver, 1991).

1.4 Programming of the Fetal Suprachiasmatic Nucleus

Developing fetus relying on maternal rhythms, because of the projections of the ganglion cells to the SCN are made late in gestation in humans and during the

early postnatal period in the rat. Rhythmicity of the fetal SCN of rodents has been shown in the absence of a functional input pathway (Reppert and Schwartz, 1984a). The fetal SCN cells, which in response to the mother's response to the environment, are exposed to maternal hormone rhythms, such as melatonin (Zemdegs et al., 1988) and possibly to fetal pituitary hormone rhythms. Day length is communicated to the fetus via the changes in the pattern of melatonin secretion according to the maternal photoperiodic environment in the Siberian hamster which is manifested in the growth rate and testicular function in the developing neonate (Stetson et al., 1989).

According to the review of Seron-Ferre et al. (1993), ablation of maternal adrenal, pineal, thyroid or pituitary in the rat, has no effect upon the phase of neonatal circadian rhythms. However, in contrast to the rats, melatonin injections given during pregnancy to SCN lesioned hamsters entrain the pubs' postnatal activity rhythm.

Probably because of underdeveloped input/output pathways, humans are essentially noncircadian at birth (Swaab et al., 1990) in spite of the likelihood of a rhytmic SCN (Reppert and Schwartz, 1984b). This is evident by the absence of hormonal and body temperature rhythmicity and the lack of consolidated sleep (Rivkees and Hao, 2000) and these rhythms appear in humans around 9-12 weeks of age (Kennaway et al., 1992). In rodents, at birth, the SCN is rhythmic and can respond to light (Weaver and Reppert, 1995; Ferguson et al., 2000) but melatonin is synthesized from the rat pineal gland at a low, nonrhythmic rate until day 5, the rhythmicity of which is apparent when the pineal gland receives sympathetic input from the superior cervical ganglion (SCG) (Yuwiler et al., 1977). Melatonin production is stimulated by norepinephrine, the release of which is promoted by SCN signals, at the pineal β adrenergic receptors at night.

1.5 Leptin Hormone

Lipid metabolism is regulated by numerous hormones and anorectic adipose tissue hormone leptin, which is discovered approximately a decade ago, is one of the potent regulator of lipid metabolism (Zhang et al., 1994). Leptin is a 167 amino acid protein whose amino terminal signal sequence is cleaved during secretion and it circulates as a 146 amino acid peptide. Leptin is produced mainly by adipose tissue (Zhang et al., 1994), but also by placenta (Masuzaki et al., 1997), stomach (Bado et al., 1998) and skeletal muscle (Wang et al., 1998), bone marrow adipocytes (Laharrague et al., 1998) and may be avian liver (Taouis et al., 1998; Freidman-Einat et al., 1999). Leptin is secreted by white adipose tissue (WAT) and its plasma concentration is correlated with total fat mass. Leptin is functioning as a satiety factor in the regulation of body weight (Casanueva and Dieguez, 1999; Ahima et al., 2000), and acts at the hypothalamic level (Zhang et al., 1994; Cone, 1999). Therefore, this hormone circulates through the organism as an internal signal indicating the size of body fat stores. Leptin secretion is pulsatile and shows a circadian rhythm that varies in timing between different laboratory rodent species (Ahima et al., 1996). Adipose tissue leptin mRNA content and serum leptin concentrations in mice and rats, decrease in the light phase and increase in the dark phase (Ahima et al., 1998; Pickavance et al., 1998; Shimokawa and Higami, 1999; Licinio et al., 1998). In contrast, there is a decrease in the dark phase and an increase in the light phase for the serum leptin concentrations in Syrian hamsters (Gündüz, 2002). Circadian rhythmicity has not been detected for the leptin release in sheep (Tokuda et al., 2000; Daniel et al., 2002).

The regulatory mechanism must control the total body weight content by informing central integrator as afferent, or input signal, and an efferent, or output

signal should be generated to respond the needs for maintainance of homeostatis. To inform brain about the lipid stores of body, white adipose tissue is innervated by sensory nerves (Bartness and Bamshad, 1998; Bartness et al., 2000), and it is suggested that this mechanism involves the adipose derived factor, leptin (Campfield et al., 1995). A primary role for leptin is in the communication of information about adipose tissue energy stores and energy flux, providing prompt feedback to brain centres involved in the regulation of energy balance (Ahima and Flier, 2000). Studies have shown that leptin-treated animals have high rate of adipose tissue loss and also leptin injection directly into WAT, increases the firing rate of sensory afferent nerves (Niijima, 1998, 1999), which causes the increase in sympathetic efferent nerve firing rates to WAT (Wade and Bartness, 1984; Niijima, 1999). These data suggest that, a negative feedback system may use leptin and sensory nerves to regulate body fat stores.

There are numerous brain lesion and electrical stimulation studies have implicated paraventricular nucleus (PVN) plays a role in the control of food intake and adiposity (Brobeck, 1948; Reynolds, 1963; Sclafani, 1971; Gold, 1973; Cox and Powley, 1981; Vilberg and Keesey, 1984; Kirchgessner and Sclafani, 1988; Cook et al., 1995, 1997). There is a connection via sympathetic nervous system (SNS) outflow from brain to WAT (Bamshad et al., 1998) and brown adipose tissue (BAT) (Bamshad et al., 1999) in the paraventricular nucleus (PVN). Interestingly studies in Siberian hamsters with PVN lesions indicated that there is a recovery of total body fat after lipectomy, which is the removal of fat pads, (Mauer and Bartness, 1997; Wood and Bartness, 1997) suggests that an intact PVN is not essential for body fat regulation. The finding that the lipectomy-induced food hoarding activity of Siberian hamsters is blocked by the PVN lesions (Wood and Bartness, 1997) has led

to the development of an alternate hypothesis that the PVN may integrate internal and external fuel sources. Most recently arcuate nucleus neurons, which contain neuropeptide Y (NPY) as a neurotransmitter, have been suggested as a canditate brain site for the adipostat control of body fats (Cowley et al., 1999). These neurons have leptin receptors as an input signal involved in the conveying of the information on the size of body fat stores, and as an output component they are part of the SNS outflow to WAT and BAT (Bamshad et al., 1998, 1999).

Single gene mutations causing either the suppression of normal leptin production or the expression of dysfunctional leptin receptors. These mutant genes causing the severe obesity due to hyperphagia, hypometabolism and preferential fat storage and are usually insulin resistant. An animal model which bears a mutation of the *ob* gene is a valuable tool for studying the biochemical messages between fat stores and the reproductive axis. The *ob/ob* mouse, which has a mutation in the gene encoding leptin hormone, is infertile and has severly impaired spermatogenesis probably because of an insufficient hypothalamic-pituitary drive and consequently low circulating gonadal steroids (Zhang et al., 1994). The administration of leptin to *ob/ob* mice, result with the weight gain of the uterus and ovaries in the female and of the seminal vesicles and testes in the male, and restores fertility in both sexes (Chehab et al., 1996; Mounzih et al., 1997). The *db/db* mouse, which has a mutation in the gene encoding leptin receptor, shows alterations of reproductive function similar to those of the *ob/ob* mouse, but because the defect is at the receptor level, leptin treatment is unable to either modify the appetite of the animals or to restore fertility. Mutations of the genes encoding leptin and its receptor have also been found in humans with similar phenotypic characteristics shown in mice. Homozygous missense mutations of leptin receptor encoding gene in adult patients

results with severe early-onset obesity, failure to enter puberty, primary amenorrhoea in females and clinical features hypogonadism in males (no beard, scanty pubic and axillary hair, small penis and testes) with a prepubertal pattern of gonadotropin secretion. Leptin deficient nine-year-old patient were treated with recombinant leptin for 12 months led to a marked reduction in weight and fat mass, and the appearance of a nocturnal pulsatile secretory pattern of gonadotropins, which is characteristic of early puberty (Farooqi et al., 1999).

Initiating factors for puberty in rat was proposed by Kennedy as the body weight and food intake (Kennedy and Mitra, 1963). He demonstrated that the body weight is correlated more significantly for the onset of puberty than with age. These findings were extended by Frisch to women which indicated that the maintenance of a minimum weight for height is important for the continuity of ovulatory cycles, this is accounted for mostly by fat storage. And the results for the delayed puberty in ballet dancers and interrupted ovulatory cycles in marathon runners showed that the opposite effect occurs in under-nourished states. The critical fat hypothesis was proposed based on these results which indicates that in order for menstruation to proceed, girls must reach a lean to fat ratio threshold accounted for by approximately 24% of fat content (Frisch, 1990). An alternate hypothesis was proposed based on the availability and oxidation of metabolic fuels. This hypothesis suggested that there would be a central mechanism detecting the degree of glucose and free fatty acid oxidation, resulting in stimulation of GnRH effector neurons, and triggering or inhibiting the reproductive axis, depending on the nutritional status of the organism. Both hypotheses agree that peripheral signals are sensed centrally.

A leptin surge has been detected in rodents (Ahima et al., 1998) and non-human primates before puberty (Plant and Durrant, 1997). In humans, leptin levels

rise throughout childhood, reach a peak in the early stages of puberty and then decline in males, whereas they increase steadily during pubertal development in females. It is important to note that the arcuate nucleus (ARC), which is a hypothalamic region where intense leptin binding occurs and from where it is thought leptin initiates its effects on adipose mass regulation, also contains GnRH-containing neurons. This indicates the dichotomous actions of leptin on energy metabolism and reproduction, at least anatomically.

During pregnancy, especially in the second and third trimesters, there is an increase in serum leptin levels both in animal models and humans (Chehab, 1997; Chien et al., 1997; Masuzaki et al., 1997), and drops sharply after delivery. But this increased levels of leptin during pregnancy cannot be attributed solely to gestational weight gain and the increase in BMI (Chien et al., 1997; Hardie et al., 1997). Instead, the secretion of placenta-derived leptin (Masuzaki et al., 1997; Dotsch et al., 1999) and the soluble form of its receptor into the maternal cirulation (Lewandowski et al., 1999), as well as changes in the levels of hormones that might stimulate leptin secretion (e.g. insulin, estrogens and human chorionic gonadotropin, hCG) (Sivan et al., 1998), are the main factors responsible for this increase. There is not a decrease of food intake or an increase of energy-expenditure (Holness et al., 1999) in pregnancy-induced hyperleptinemia. It is suggested that placenta-derived leptin might be an important growth factor for the fetus and/or a signal of energy status between mother and fetus (Hassink et al., 1997; Hoggard et al., 1997; Wauters et al., 2000). In addition, the reduced fertility during the period of lactation might be because of the drop in leptin levels after delivery.

1.6 Interaction of Leptin with Suprachiasmatic Nucleus

It has been determined that visceral adiposity, and leptin and insulin levels are decreased by melatonin without altering food intake (Wolden-Hansen et al., 2000). Further evidence that the leptin secretion is increased by pinealectomy has led to the suggestion that melatonin may exert an inhibitory effect on leptin release (Canpolat et al., 2001; Gündüz, 2002) and the circadian pattern of leptin release could not be affected by pinealectomy (Baydaş et al., 2001). As it was mentioned previously, melatonin receptors have been localized on the SCN. Siberian hamsters with SCN lesioning lose their body weight response to timed infusions of short day melatonin, it has been suggested that the SCN may be important to photoperiodic regulation of body weight (Bartness et al., 1991). Leptin receptors have been identified in the SCN of the rat (Guan et al., 1997). Studies show that the white adipose tissue receive sympathetic innervation which is originated in the SCN (Bartness et al., 2001). A reciprocal connection has also been determined by neuroanatomical tracing studies between the hypothalamus arcuate nucleus (ARC), which is crucial for leptin signaling to the central nervous system, and the SCN (Bouret et al., 2004; Buijs et al., 2006; Dawson et al., 1997; Yi et al., 2006). It is suggested that the leptin-induced lipolysis is controlled by SCN. In the study of Shen et al. (2007) leptin injection increased the plasma free fatty acids (FFA) in sham operated and intact rats, whereas the effect of leptin on lipolysis disappeared completely in SCN-lesioned rats has led to the suggestion that the effects of leptin on lipolysis is executed through autonomic nerves and controlled by the SCN.

It has been shown that the diurnal rhythmicity of circulating plasma leptin is eliminated through SCN lesions (Kalsbeek et al., 2001; Karakaş and Gündüz, 2006). Studies have indicated the role of the autonomic nervous system in the

regulation of leptin secretion. Administration of α-methyl-*p*-tyrosine or 6-hydroxydopamine causes the reduction of noradrenaline release from sympathetic nerve endings which induces hyperleptinemia (Rayner et al., 1998; Sivitz et al., 1999). In contrast, plasma leptin levels and leptin mRNA expression is decreased by β-adrenergic receptor agonist (Trayhurn et al., 1995; Li et al., 1997). This regulation of leptin secretion by sympathetic activity resembles the regulation of melatonin release by the pineal gland which has a pronounced day/night rhythm due to the nocturnal release of noradrenaline from the sympathetic nerve endings in the pineal gland (Roseboom et al., 1996). It has been demonstrated by both anatomical and (electro)physiological techniques that the sympathetic input to the pineal gland is controlled through SCN by contacting with PVN neurons which possess descending projections to the preganglionic neurons in the spinal cord (Hermes et al., 1996; Kalsbeek et al., 1999, 2000; Teclemariam-Mesbah et al., 1999). It is hypothesized by Kalsbeek et al. (2001) that the circadian rhythm of plasma leptin level is controlled by a similar neural pathway which is supported by the retrograde virus tracing labelling from the white adipose tissue to the neurons in spinal cord, PVN and SCN (Bamshad et al., 1998). A removal of SCN results in increased leptin levels has led to the suggestion that, similar to the rhythm in melatonin release, SCN control of leptin release is mainly inhibitory (Kalsbeek et al., 1996, 2000).

1.7 Syrian Hamster

Syrian hamsters have been prefered as a laboratory model species because of their strong photoperiodic characteristics that illuminate effect various mechanisms of the day length. However, body weights and gonadal development of prepubertal individuals of this species are independent from ambient photoperiod (Gaston and Menaker, 1967).

Estrous cycles of Syrian hamsters display a 4-day cycle which is comparable to easy to monitor to the estrous cycles of other laboratory animals. When adult females which are on the 4^{th} day of estrous cycle, being introduced to a sexually experienced male, show stereotypical estrous behavior (lordosis). On the night of 4^{th} day ovulation occurs which is followed by a conspicious vaginal discharge on the next day (Orsini, 1962).

When gestational period is compared hamster has the shortest in eutherian mammals, which is about 373 hours for one month-old mated hamster while 402 hours for 14 months of age. But there is a sharp drop in mating and deliveries after 14 months of age.

It has been thought that annual breeding cycles and sexual maturation process is regulated by photoperiod in seasonal mammals and the timing of the prepubertal events can be modulated for environmental conditions such as nutrition, temperature and noise. The reproductive system of adult Syrian hamster is quite sensitive to photoperiod which is inactivated when daylength is less than 12.5 hours of light per day. But it is not clear whether the prepubertal sexual maturation process of juvenie Syrian hamsters is under photoperiodic control. Syrian hamsters born in spring and summer have been reported to reach puberty more rapidly than those born in the fall under conditions of decreased daylength (Czyba, 1968). Gonadal weights of male Syrian hamsters which were blinded at birth is similar to those of sighted controls at 6 weeks of age has led to the suggestion that early sexual development is independent from photoperiodic regulation.

Photoperiodic changes precisely control body weight and fat content of hamster species. Body weights are reduced in long photoperiods and increase in short photoperiods in Syrain hamsters which is opposite to other counterparts, such as

Siberian hamsters. It has been suggested that leptin may adjust these changes in body fat and weight, but the mechanism of which is not well known (Karakaş and Gündüz, 2002).

1.8 Objectives

It has been well defined that suprachiasmatic nucleus (SCN) have both leptin and melatonin receptors and it was demonstrated that exogeneous application of melatonin and leptin phase advances the SCN. But the effect of maternal leptin for the programming of the brain of juveniles by the way of maternal system has not been clear on the literature.

The aim of this study was to determine the effect of exogenous leptin on the locomotor activity of juveniles which was administered during the pregnancy of melatonin deprived Syrian hamsters.

2. MATERIALS AND METHODS

2.1 Animal Care

Syrian hamsters (*Mesocricetus auratus*) were used in this experiment. All hamsters were born in our laboratory colony at the Abant Izzet Baysal University. Hamsters were kept in a light:dark (LD) cycle of 14:10 (lights off at 20.00 h) beginning from the parturition. All juveniles were weaned at the 15 days of age. Animals were maintained in plastic cages (16x31x42 cm) with pine shavings as bedding with food pellets (Purina Rodent Chow, Bolu, Turkey) and tap water provided *ad libitum.* The procedures used in this study were carried out in accordance with the Animal Scientific Procedure Act of 1986 and approved by the Institutional Animal Care and Use Committee. Room temperature was generally maintained at 22±2 ^{0}C in air-ventiled rooms and all lighting was provided by cool-white fluorescent tubes controlled by automatic programmable timers; light intensities at the animal's eye level exceeded 200 lux.

2.2 Anesthesia

Adult female Syrian hamsters were anesthetized subcutaneously with ketamine (20 mg/kg body weight, Sigma Chemical Company, St. Luis, Missouri, USA) and intraperiotoneally with pentobarbitol (32.5 mg/kg body weight, Sigma Chemical Company, St. Luis, Missouri, USA) before surgery. The depth of anesthesia was monitored by frequent testing of leg reflexes and muscle tonus.

2.3 Pinealectomy

Pinealectomy was performed according to the method of Hoffman and Reiter (1965), aspiration was used to control hemorrhaging. To stabilize the head,

anesthetized hamsters were placed in a stereotaxic apparatus during the pinealectomy. After the head was shaved the surgical area was sterilized with 70% ethanol, an incision was made in the scalp and muscle attachments were removed from the dorsal skull. After drying the skull, an incomplete circular cut was made with a dental drill on the lambda (λ) region, and a piece of cranium covering the pineal gland was folded anteriorly. A fine-tipped forceps was used to extend into the confluence of the sinuses to grasp and remove the pineal gland. After removal of the pineal gland, the bone flap was replaced, and a small square of absorbable gelatin sponge (Gelfoam, Up John, Kalamazoo, MI) was applied to the skull surface to help promote clotting. Finally, the scalp was sutured surgically. Control groups were exposed to the same operation conditions like pinealectomy groups, but forceps was kept closed during the insertion so that no tissue was removed. Thus, similar stress conditions were created for both of the groups. After surgery, the incision was treated with Newskin adhesive to prevent any contamination.

2.4 Cannulation

After the pinealectomies were performed, hamsters were allowed for a 1 week recovery period. Then, both the pinealectomized and sham-pinealectomized female hamsters were allowed for mating. Pregnancy was confirmed by checking the estrus cycle. At the 10^{th} day of their pregnancy they were cannulated for leptin infusion.

The method of cannulation and infusion was based on that described by Carter and Goldman (1983a, 1983b). A simple flow-thru swivel for infusion into unrestrained animals was designed according to the method of Brown et al. (1976). Animals which were under ketamine and pentobarbitol anesthesia, were fitted with a subcutaneous catheter under the dorsal skin of the back for the infusion of leptin.

2.5 Leptin Infusions

4 μg/kg of leptin were used in this experiment. Stock leptin solution were diluted in sterile saline (0,9 % NaCl) to the desired concentrations which were kept at 4 ^{0}C prior to use. 0,1 ml of leptin were infused subcutaneously to each pregnant female hamsters whether at 3 different times of each day starting with 10th day of pregnancy until the parturition. Infusion rates of leptin were 0,1ml/h for 1 hour infusions and 0,017ml/h for 6 hour infusions and leptin infusion in dark periods were done under dim red light. Syringes were refilled every time just before the infusion started and cannulas were checked at least twice daily and replaced when necessary.

2.6 Wheel Running Activity

After the parturition, infusions of leptin were ceased and animals were allowed lactation for 15 days. During this time period animals were fed with food pellets and food and water was accessible *ad libitum*. During lactation period the light-dark cycle was LD 14:10 (lights off at 20.00 h) and room temperature was maintained at 22±2 ^{0}C in air-ventiled rooms.

At the 15 days of age, pups were weaned and housed in plastic cages with food and water provided *ad libitum*. At the 20 days of age animals were placed individually in polypropylene cages, each equipped with a running wheel that closed a microswitch mounted on the outside of the cage with each revolution. Similarly, the light-dark cycle was LD 14:10 (lights off at 20.00 h) and room temperature was 22±2 ^{0}C. Food pellets and fresh water were provided *ad libitum*. Counts of wheel revolutions were stored in 10 min bins for 21 days. The graphic display and analysis of activity records were done using Vital View Software (Mini Mitter Company, Bend, Oregon, USA).

Body weights of juveniles were recorded each week during one month.

2.7 Experimental Design

This study was designed to determine the locomotor activity of juveniles borned to pregnant with/without pinealectomized Syrian hamsters. This study was performed in the long photoperiod (LD 14:10) and all juveniles were maintained at the same light-dark cycle.

A total of 30 adult female hamsters were divided into 2 main groups as sham-pinealectomy (Control, n=15) and pinealectomy (Pinx, n=15) groups. Those groups were comprised of 3 subgroups according to the time of leptin infusions during their pregnancy. Those subgroups receiving subcutaneous leptin infusions at different times are summarized as;

Group 1 (infused at 12.00-13.00 h, n=5 for control and n=5 for pinx)

Group 2 (infused at 00.00-01.00 h, n=5 for control and n=5 for pinx)

Group 3 (infused at 13.30-19.30 h, n=5 for control and n=5 for pinx)

2.8 Statistics

Wheel revolutions and body weights were analyzed using one-way analysis of variance followed by Tukey's multiple comparison test (ANOVA: GraphPad Prism 5 for Windows). Differences between means within or between groups were determined by student's t-tests. Values were considered statistically significant at $p<0.05$. Data are presented as mean ± S.E.M.

3. RESULTS

3.1 Group1 (*leptin infusion between 12.00-13.00 h)*

Wheel running activity of juvenile Syrian hamsters were recorded for 21 days in LD 14:10. When actograms were compared there were no phase-advance or phase-delay observed in both control and pinealectomy groups (Fig 1, Fig 3). When period lenghts were compared it was 24:05 hours for control and 24:00 hours for pinealectomy group (Fig 2, Fig 4).

When the wheel running activity of light and dark periods were compared between control and pinealectomy groups, there were not statistically significant difference observed ($p>0.05$) (Fig 5). However, there was a tendency of higher wheel running activity during dark period in pinealectomy group.

If body weights are compared between control and pinealectomy groups, body weights were not statistically different ($p>0.05$) between control and pinealectomy groups, except in first week. In first week, body weights were statistically different ($p<0.01$) between control and pinealectomy groups (Fig 6).

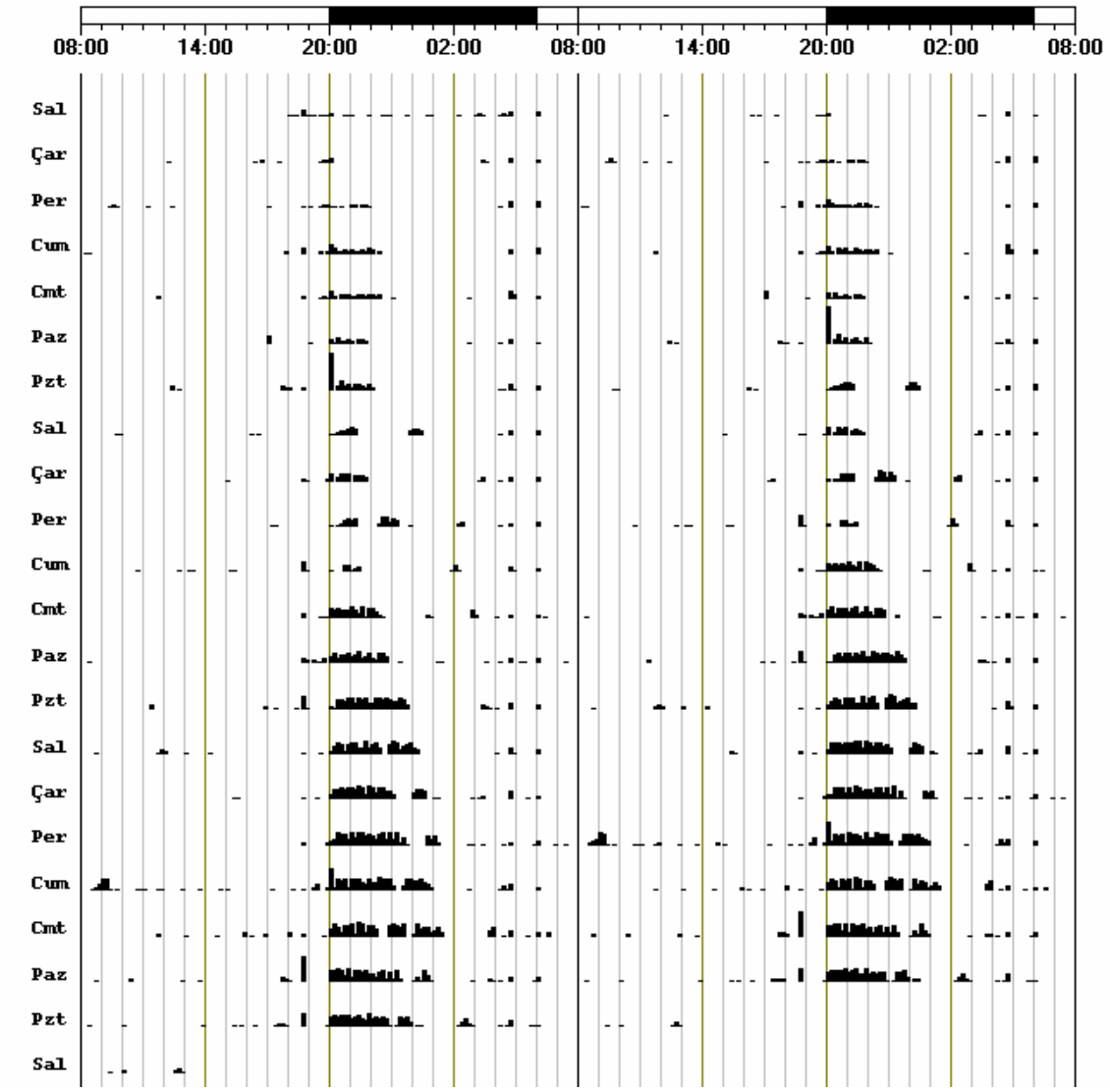

Figure 1: A representative actogram of one juvenile Syrian hamster from control group (12.00-13.00) in LD 14:10. Open bars represent the day time and dark bars represent the night time. Y axis indicates the days of the week.

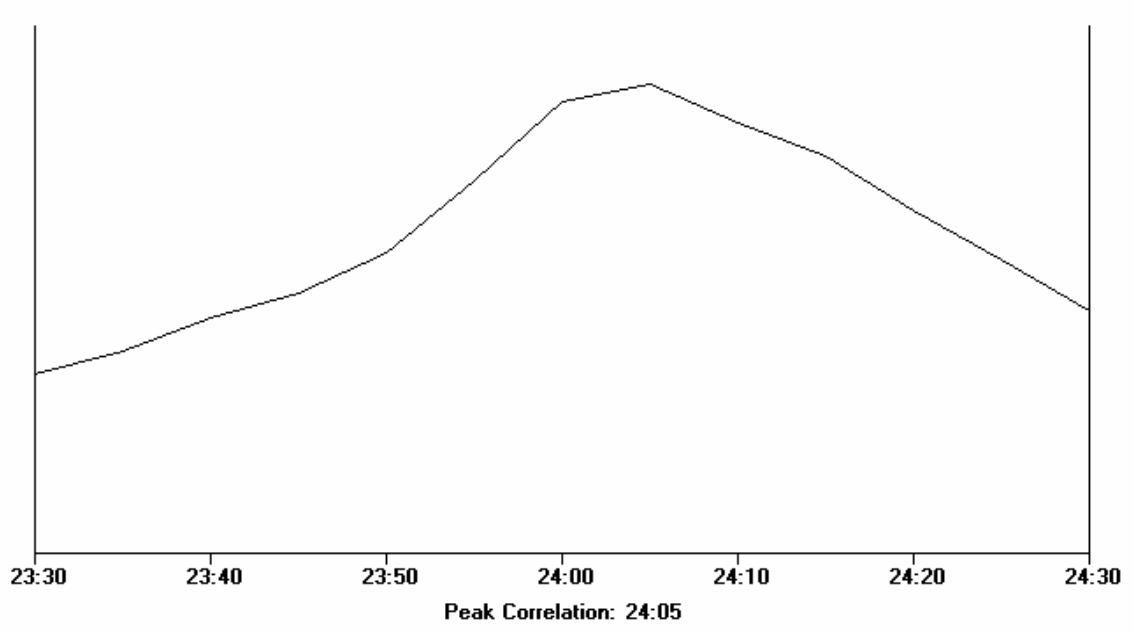

Figure 2: A representative periodogram of one juvenile Syrian hamster from control group (12.00-13.00) in LD 14:10.

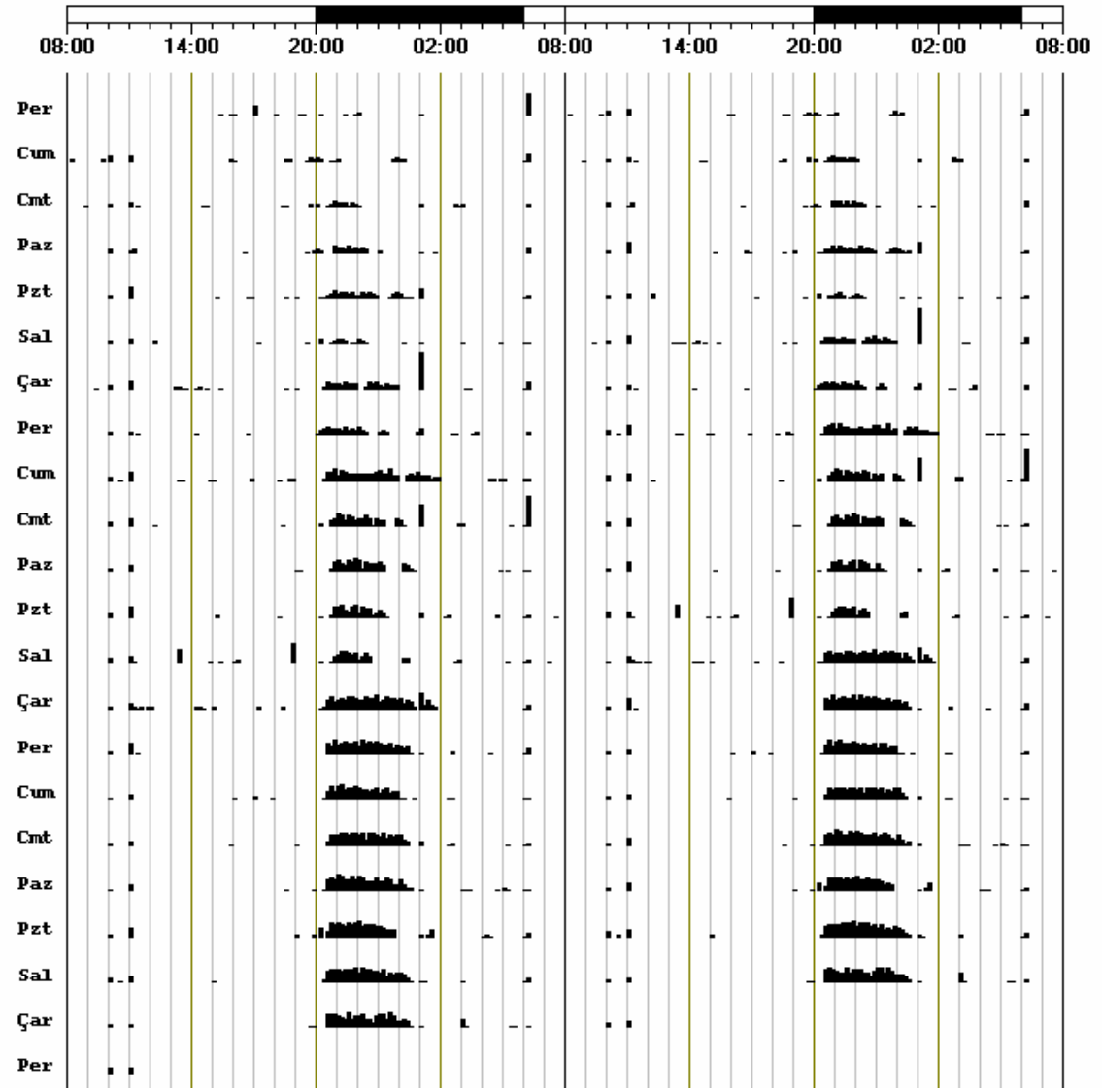

Figure 3: A representative actogram of one juvenile Syrian hamster from pinealectomy group (12.00-13.00) in LD 14:10. Open bars represent the day time and dark bars represent the night time. Y axis indicates the days of the week.

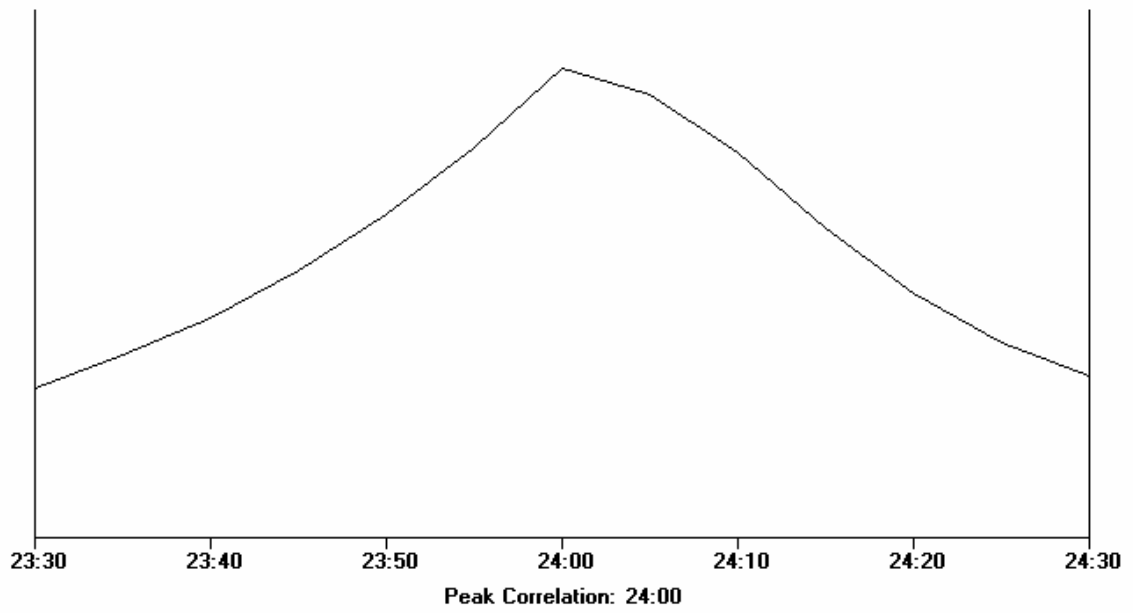

Figure 4: A representative periodogram of one juvenile Syrian hamster from pinealectomy group (12.00-13.00) in LD 14:10.

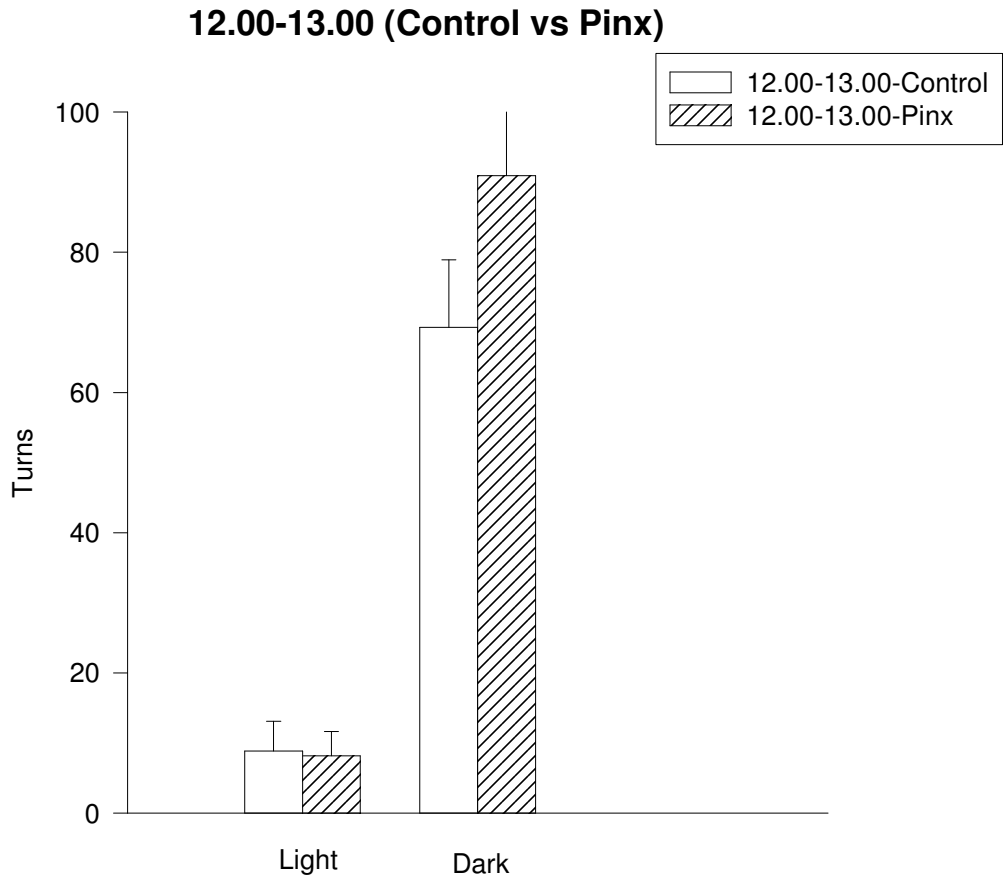

Figure 5: Comparison of running wheel turns of the juvenile Syrian hamsters between control and pinealectomy groups in light and dark period. Data are given as mean ± S.E.M. (Pinx: pinealectomy; 12.00-13.00 indicates the leptin infusion times).

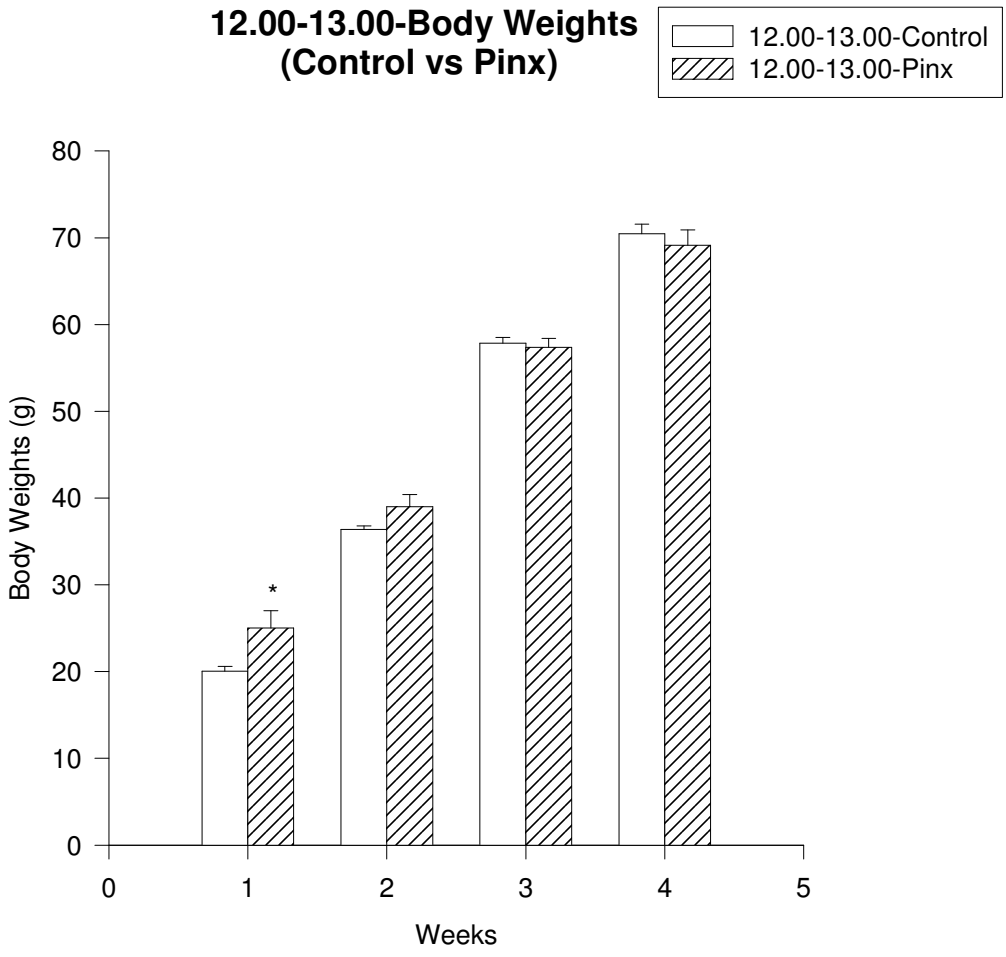

Figure 6: Comparison of body weights of the juvenile Syrian hamsters between control and pinealectomy groups. * indicates statistically significant difference ($p<0.01$). Data are given as mean ± S.E.M. (Pinx: pinealectomy; 12.00-13.00 indicates the leptin infusion times).

3.2 Group2 (*leptin infusion between 00.00-01.00 h)*

Activity records were similar to those of group 1, which were entrained to normal light:dark cycle and activities were started when the lights off (Fig 7, Fig 9). Period lenght is also similar to group 1 which is 24:05 hours for control and 24:00 hours for pinealectomy group (Fig 8, Fig 10).

There were not statistically significant difference observed for the running wheel activites between groups ($p>0.05$) (Fig 11), but body weights were statistically different between control and pinealectomy groups which were higher in pinealectomy group in all weeks ($p<0.001$) (Fig 12).

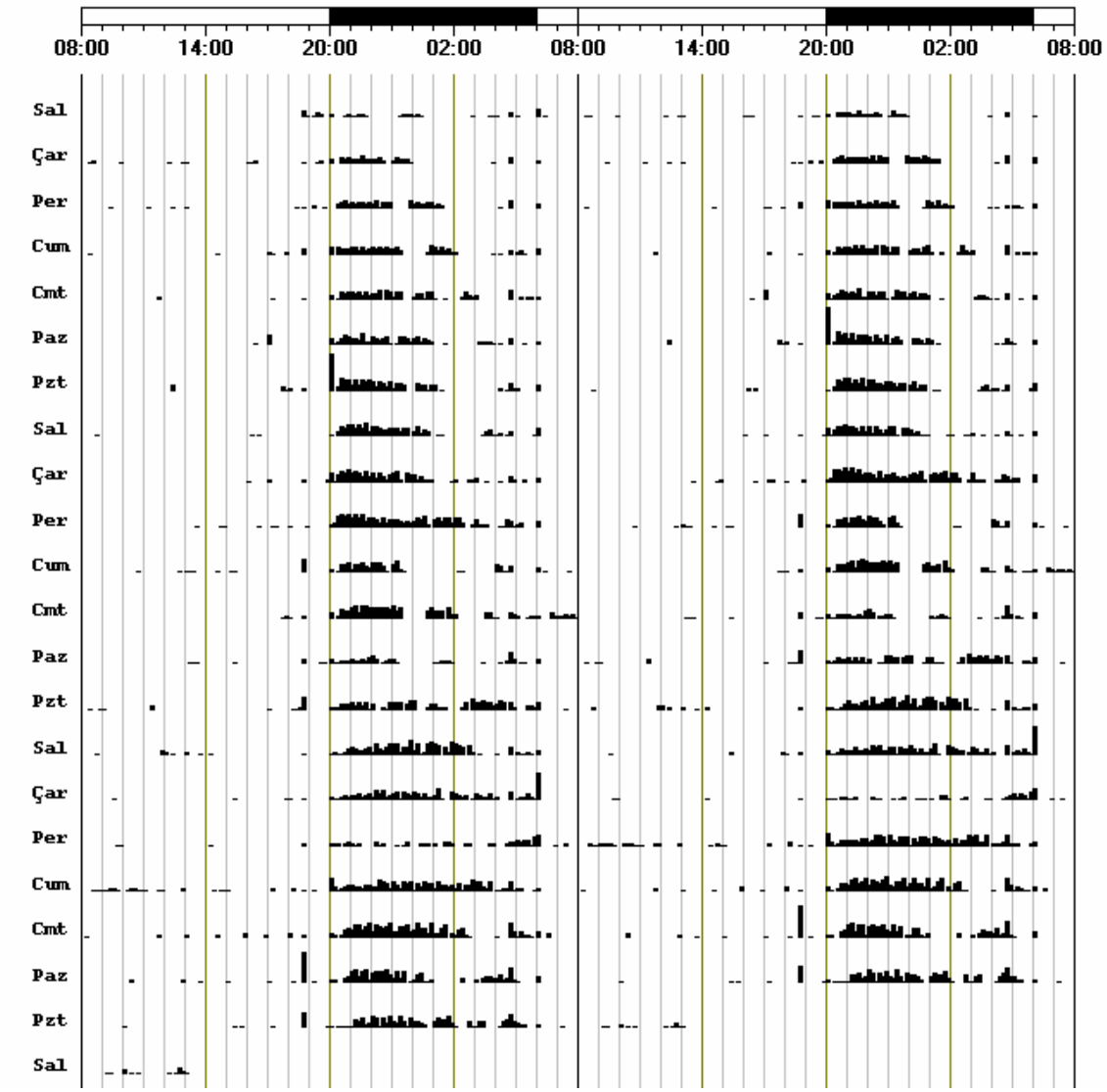

Figure 7: A representative actogram of one juvenile Syrian hamster from control group (00.00-01.00) in LD 14:10. Open bars represent the day time and dark bars represent the night time. Y axis indicates the days of the week.

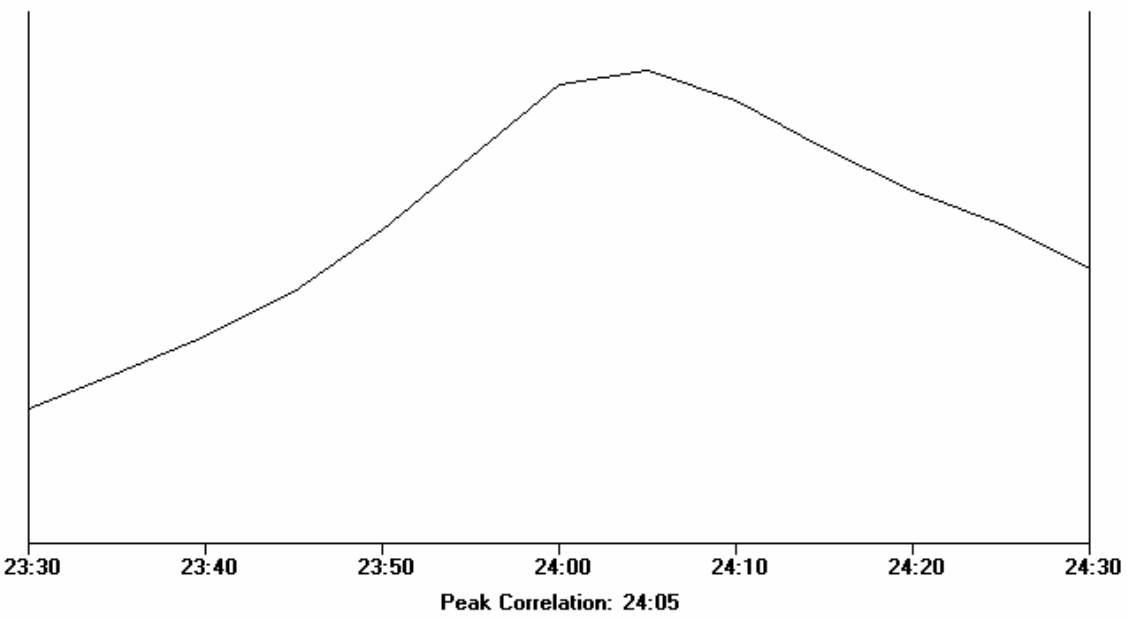

Figure 8: A representative periodogram of one juvenile Syrian hamster from control group (00.00-01.00) in LD 14:10.

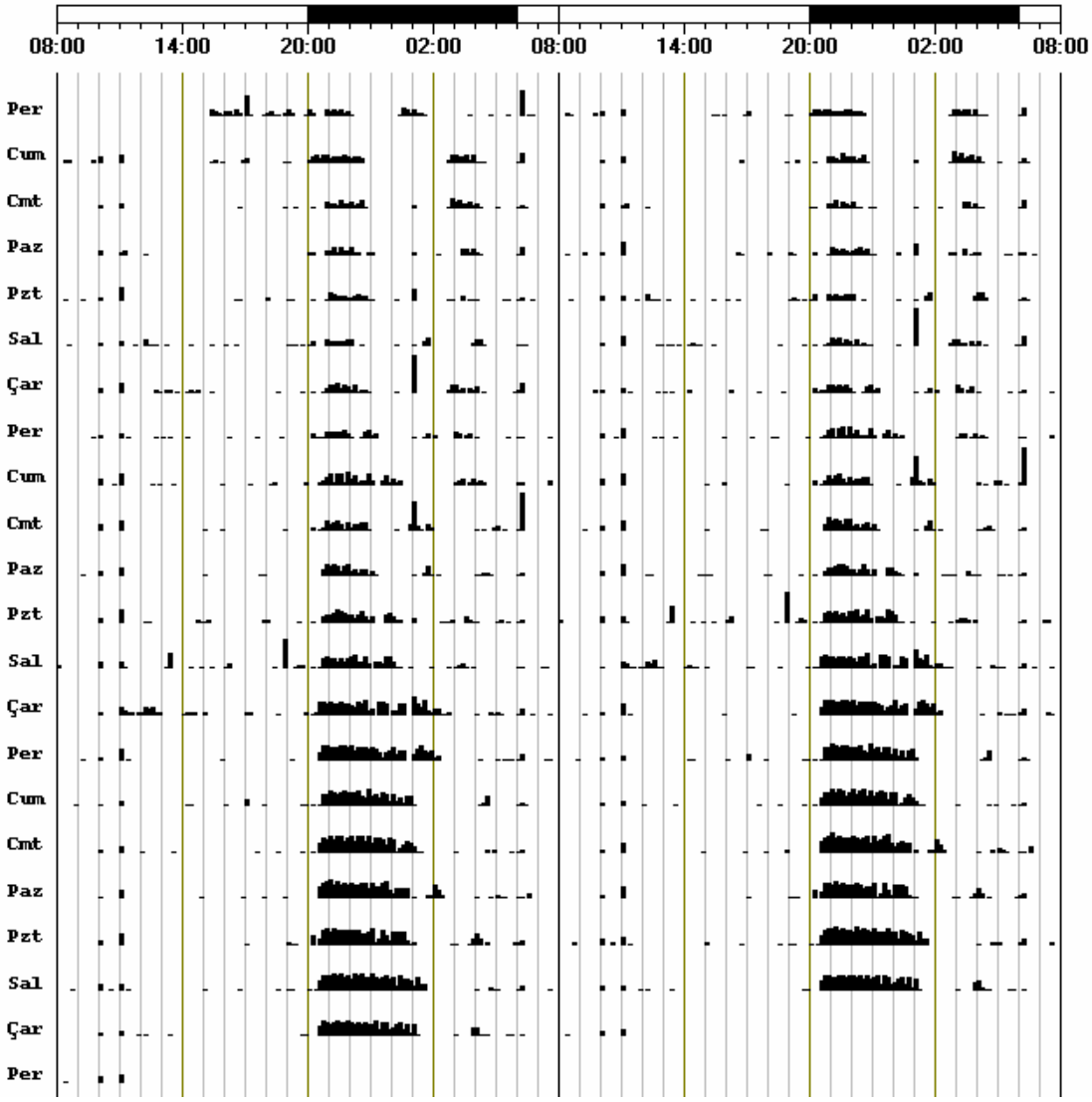

Figure 9: A representative actogram of one juvenile Syrian hamster from pinealectomy group (00.00-01.00) in LD 14:10. Open bars represent the day time and dark bars represent the night time. Y axis indicates the days of the week.

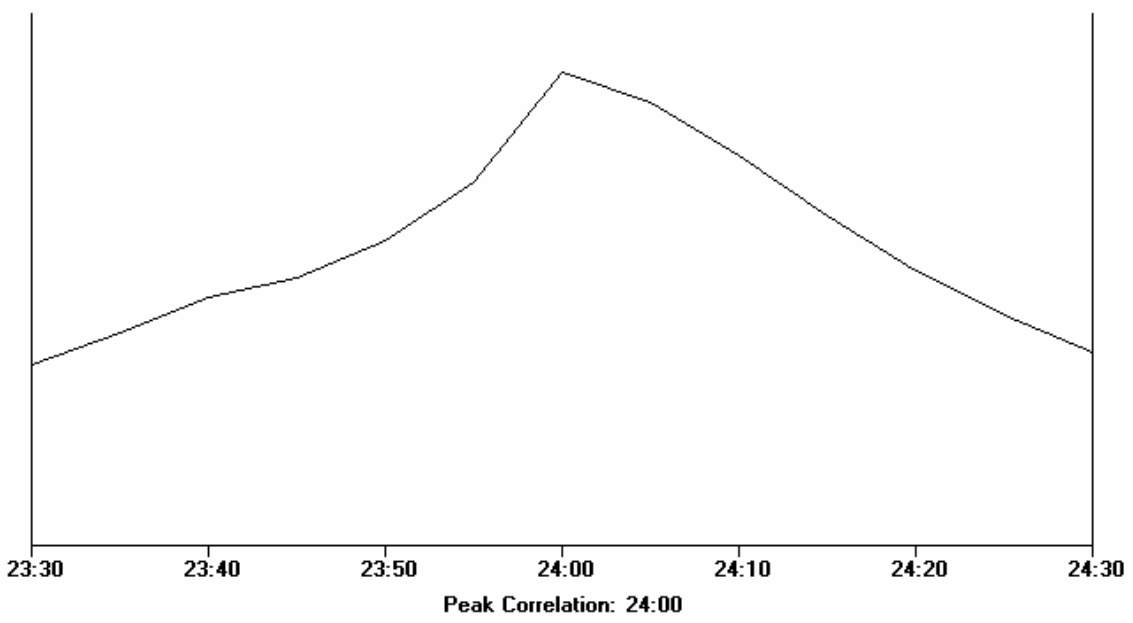

Figure 10: A representative periodogram of one juvenile Syrian hamster from pinealectomy group (00.00-01.00) in LD 14:10.

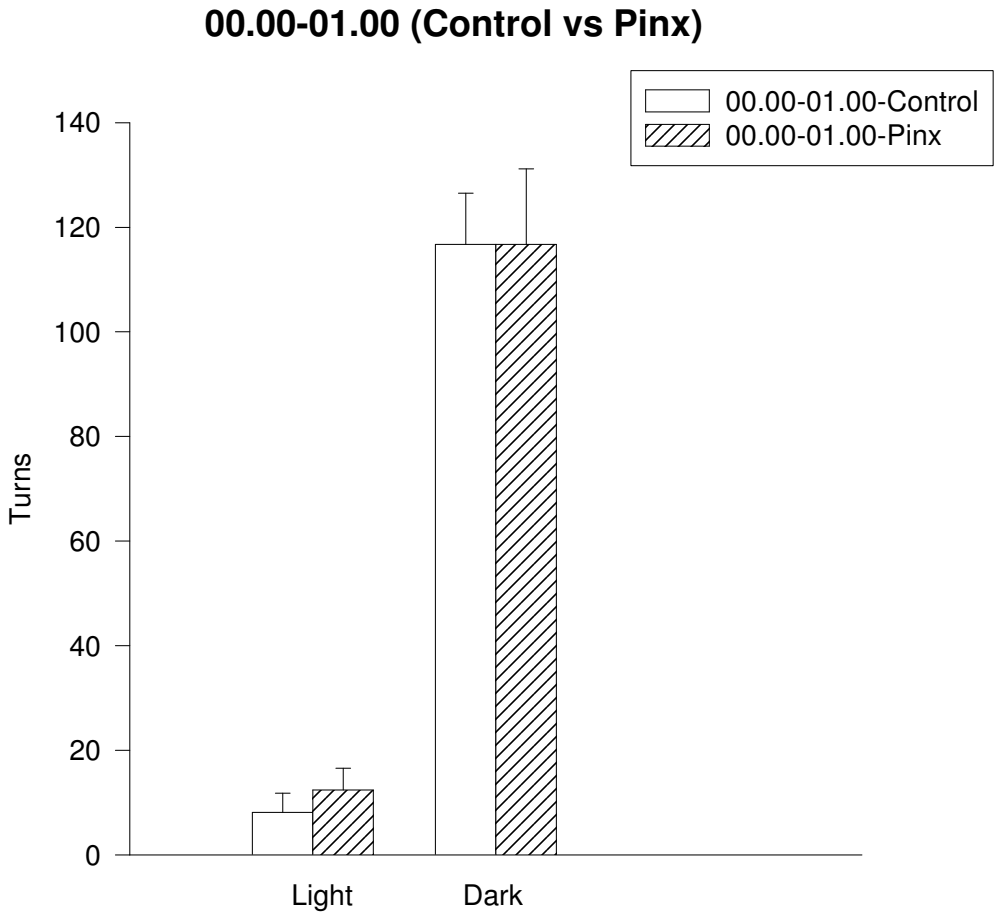

Figure 11: Comparison of running wheel turns of the juvenile Syrian hamsters between control and pinealectomy groups in light and dark period. Data are given as mean ± S.E.M. (Pinx: pinealectomy; 00.00-01.00 indicates the leptin infusion times).

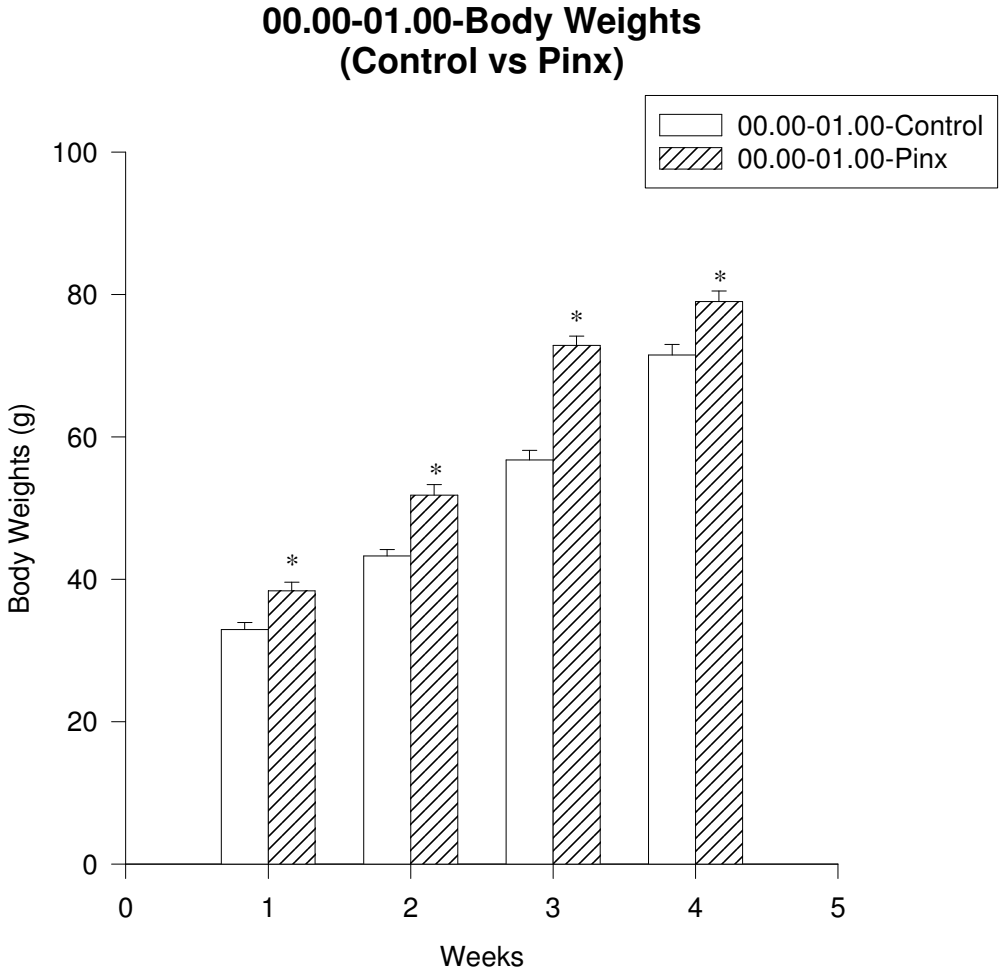

Figure 12: Comparison of body weights of the juvenile Syrian hamsters between control and pinealectomy groups. * indicates statistically significant difference ($p<0.001$). Data are given as mean ± S.E.M. (Pinx: pinealectomy; 00.00-01.00 indicates the leptin infusion times).

3.3 Group3 (*leptin infusion between 13.30-19.30 h)*

Activity records were entrained to normal light:dark cycle and activities were started when the lights off (Fig 13, Fig 15). Period lenght is similar to group 1 and group 2 which is 24:05 hours for control and 24:00 hours for pinealectomy group (Fig 14, Fig 16).

When the wheel running activity of light and dark periods were compared between control and pinealectomy groups, there were not statistically significant difference observed ($p>0.05$) (Fig 17). However, there was a tendency of higher wheel running activity during dark period in pinealectomy group.

If body weights are compared between control and pinealectomy groups, body weights were statistically different ($p<0.001$) and animals in pinealectomy group had higher body weights than control in all weeks (Fig 18).

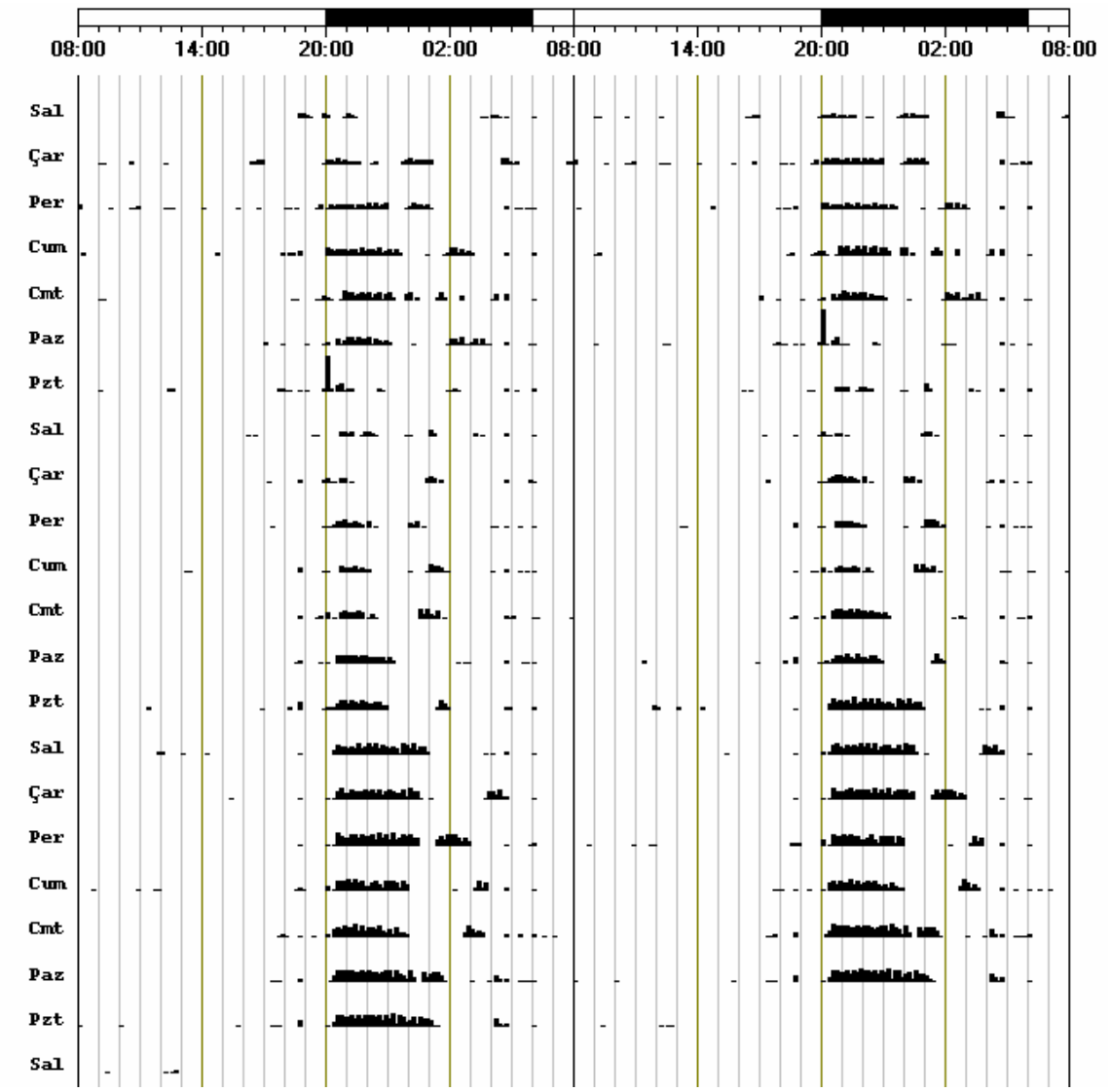

Figure 13: A representative actogram of one juvenile Syrian hamster from control group (13.30-19.30) in LD 14:10. Open bars represent the day time and dark bars represent the night time. Y axis indicates the days of the week.

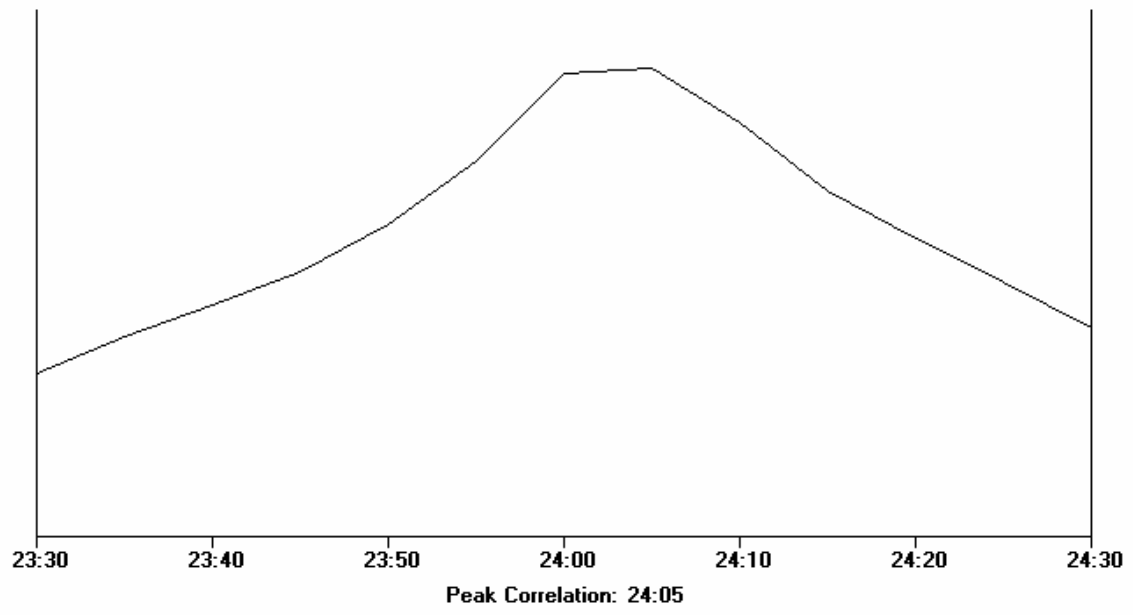

Figure 14: A representative periodogram of one juvenile Syrian hamster from control group (13.30-19.30) in LD 14:10.

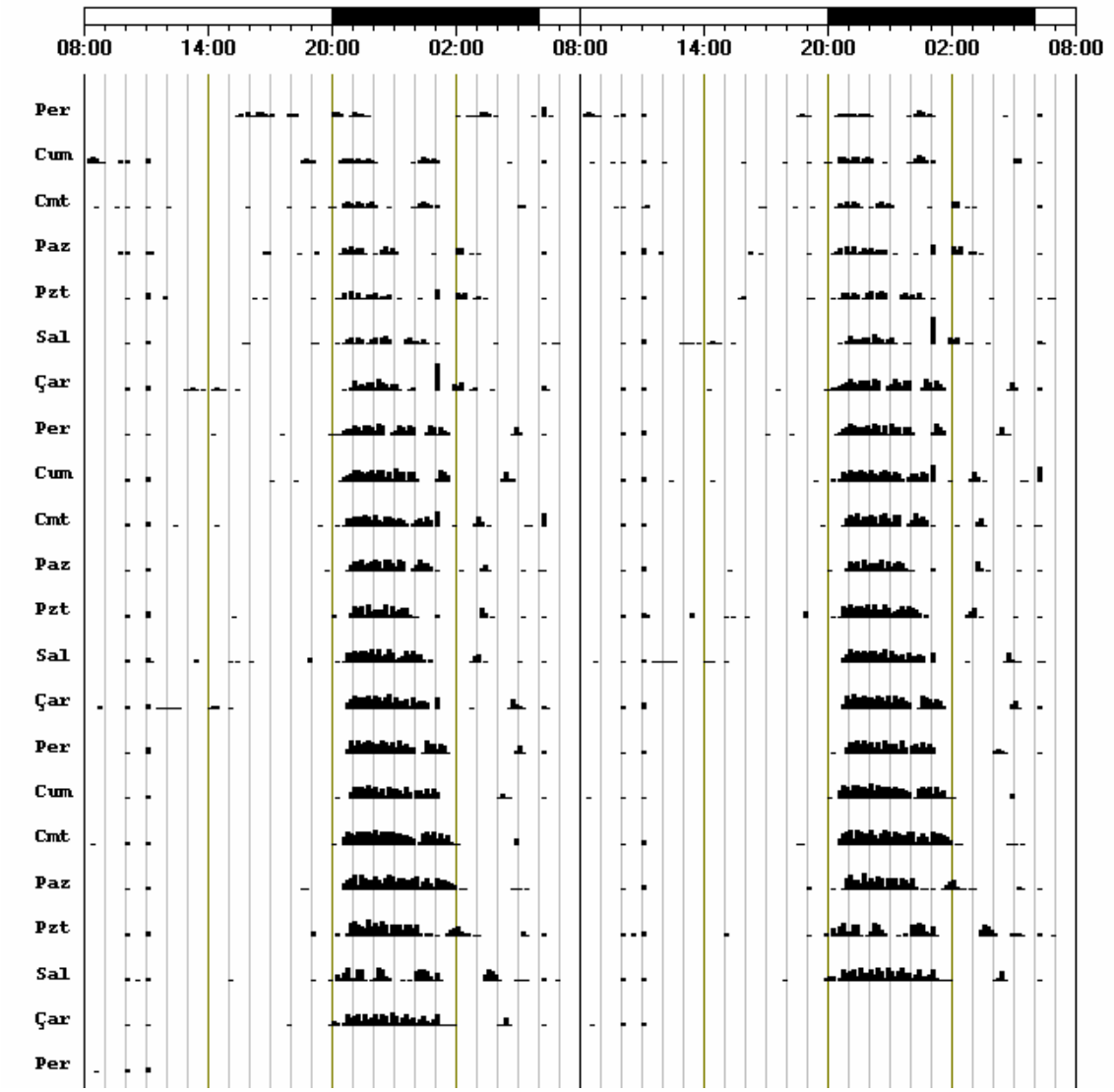

Figure 15: A representative actogram of one juvenile Syrian hamster from pinealectomy group (13.30-19.30) in LD 14:10. Open bars represent the day time and dark bars represent the night time. Y axis indicates the days of the week.

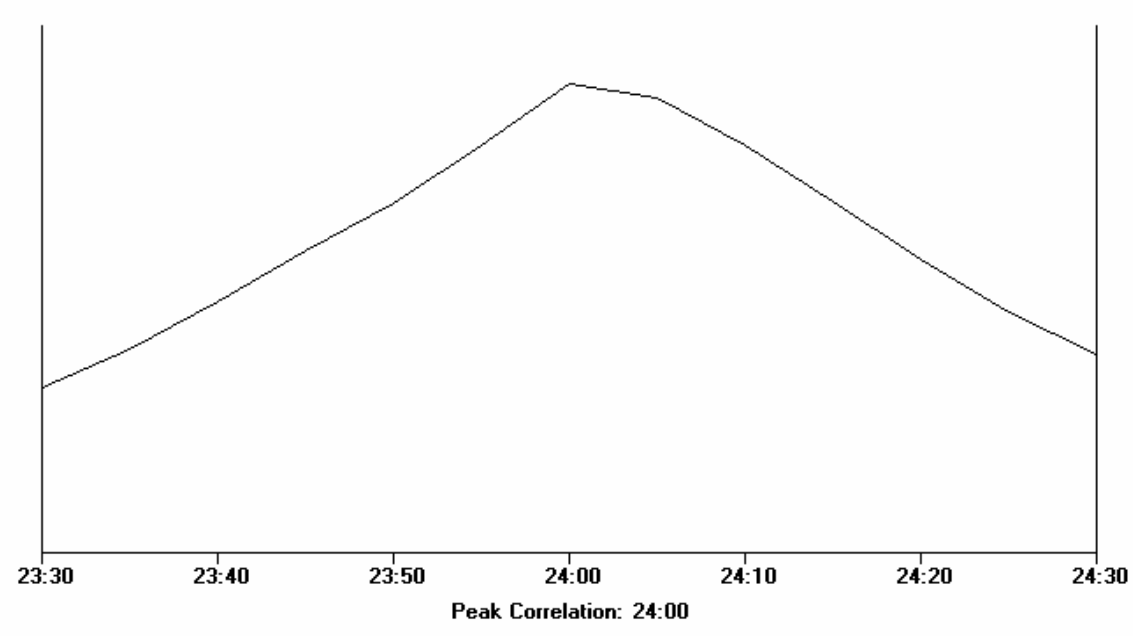

Figure 16: A representative periodogram of one juvenile Syrian hamster from pinealectomy group (13.30-19.30) in LD 14:10.

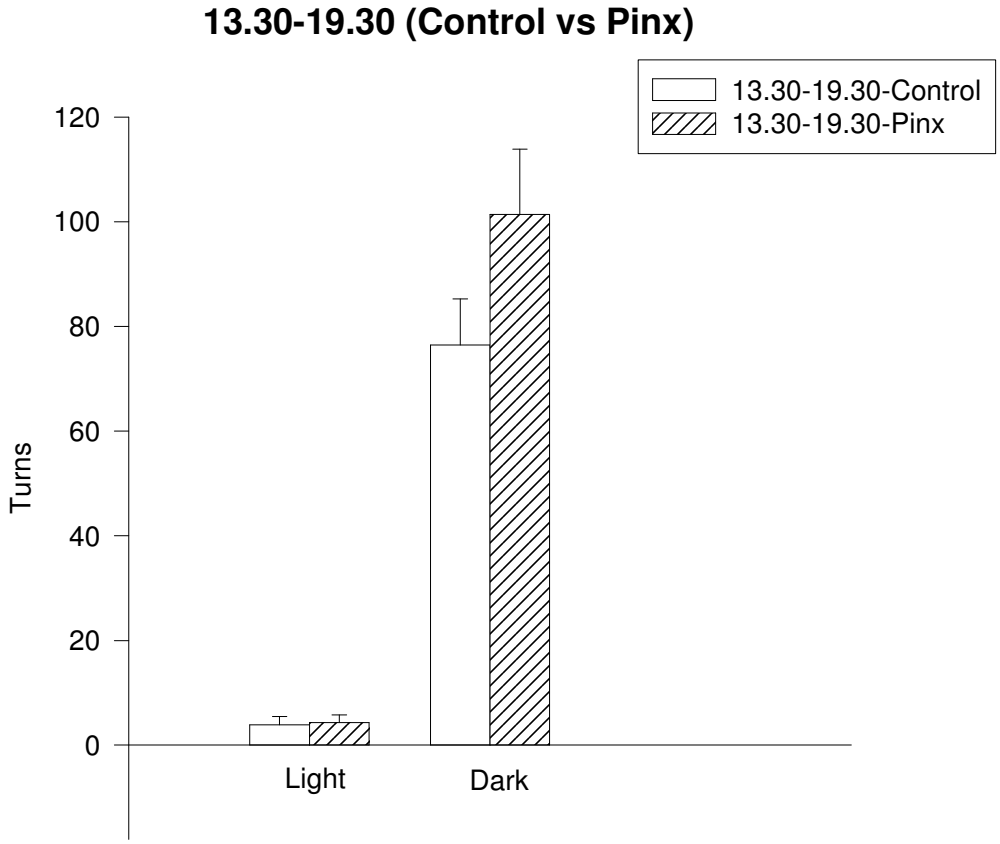

Figure 17: Comparison of running wheel turns of the juvenile Syrian hamsters between control and pinealectomy groups in light and dark period. Data are given as mean ± S.E.M. (Pinx: pinealectomy; 13.30-19.30 indicates the leptin infusion times).

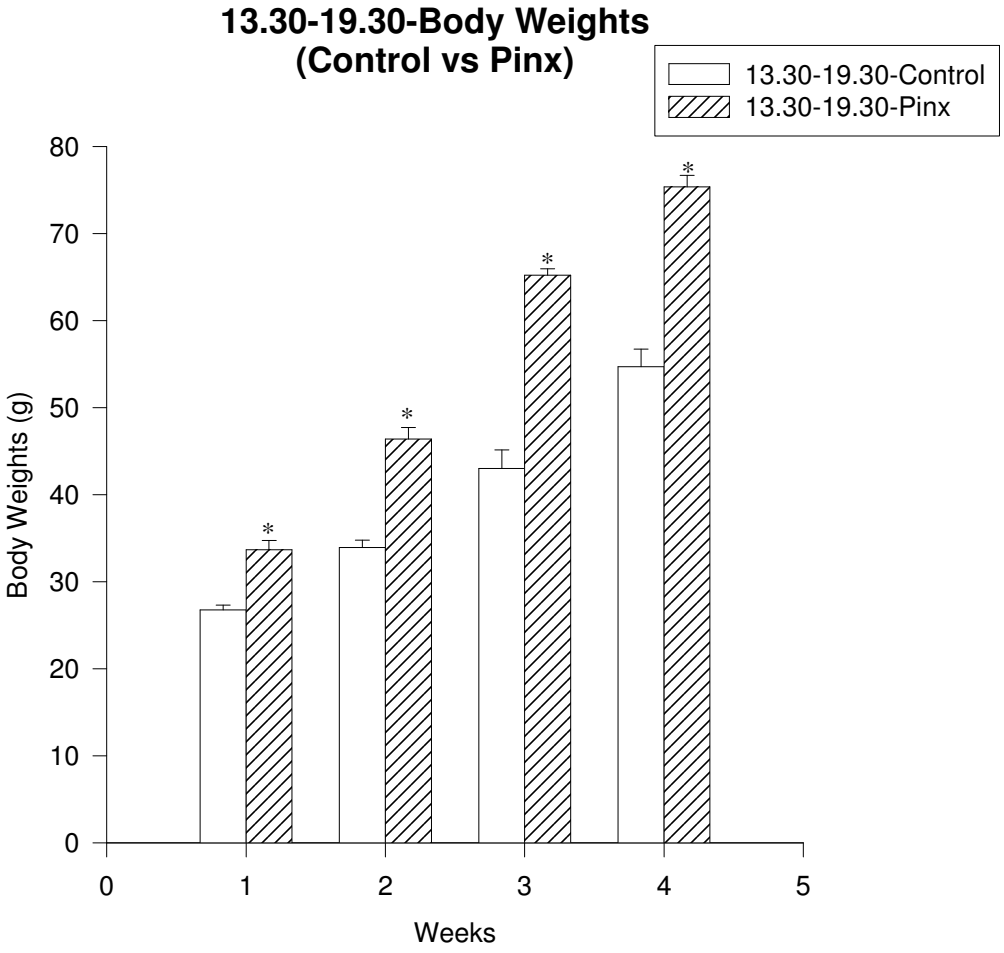

Figure 18: Comparison of body weights of the juvenile Syrian hamsters between control and pinealectomy groups. * indicates statistically significant difference ($p<0.001$). Data are given as mean ± S.E.M. (Pinx: pinealectomy; 13.30-19.30 indicates the leptin infusion times).

The results show that in control group, wheel running activity of dark period was significantly higher ($p<0.05$) in group 2 (00.00-01.00 h) (Fig 19). But this statistically significant difference of wheel running activity could not be observed in pinealectomy group ($p>0.05$) (Fig 20). In pinealectomy group, there is a tendency of higher wheel running activity for group 2 (00.00-01.00 h) in dark period. But this tendency is not statistically significant ($p>0.05$). If wheel running activity of light period is compared, as it was expected, there were not statistically significant difference ($p>0.05$) observed between different time of leptin infusion groups in both control and pinealectomy groups (Fig 19-20, Table 1-2).

Body weights of juvenile Syrian hamsters of both control and pinealectomy groups from different leptin infusion times are summarized in tables (Table 3-4).

Light Condition	Turns (Control) 12.00-13.00 Infusion Mean ± S.E.M.	Turns (Control) 00.00-01.00 Infusion Mean ± S.E.M.	Turns (Control) 13.30-19.30 Infusion Mean ± S.E.M.
Light	8.857 ± 4.230	8.143 ± 3.640	3.869 ± 1.631
Dark	69.28 ± 9.627	116.8 ± 9.755	76.45 ± 8.783

Table 1: Light and dark running wheel turns of control group of juvenile Syrian hamsters in 14L. Data are given as mean ± S.E.M. (12.00-13.00, 00.00-01.00, 13.30-19.30 indicates the leptin infusion times).

Light Condition	Turns (Pinx) 12.00-13.00 Infusion Mean ± S.E.M.	Turns (Pinx) 00.00-01.00 Infusion Mean ± S.E.M.	Turns (Pinx) 13.30-19.30 Infusion Mean ± S.E.M.
Light	8.155 ± 3.494	12.38 ± 4.153	4.333 ± 1.463
Dark	90.93 ± 13.95	116.7 ± 14.46	101.4 ± 12.46

Table 2: Light and dark running wheel turns of pinealectomy group of juvenile Syrian hamsters in 14L. Data are given as mean ± S.E.M. (Pinx: Pinealectomy; 12.00-13.00, 00.00-01.00, 13.30-19.30 indicates the leptin infusion times).

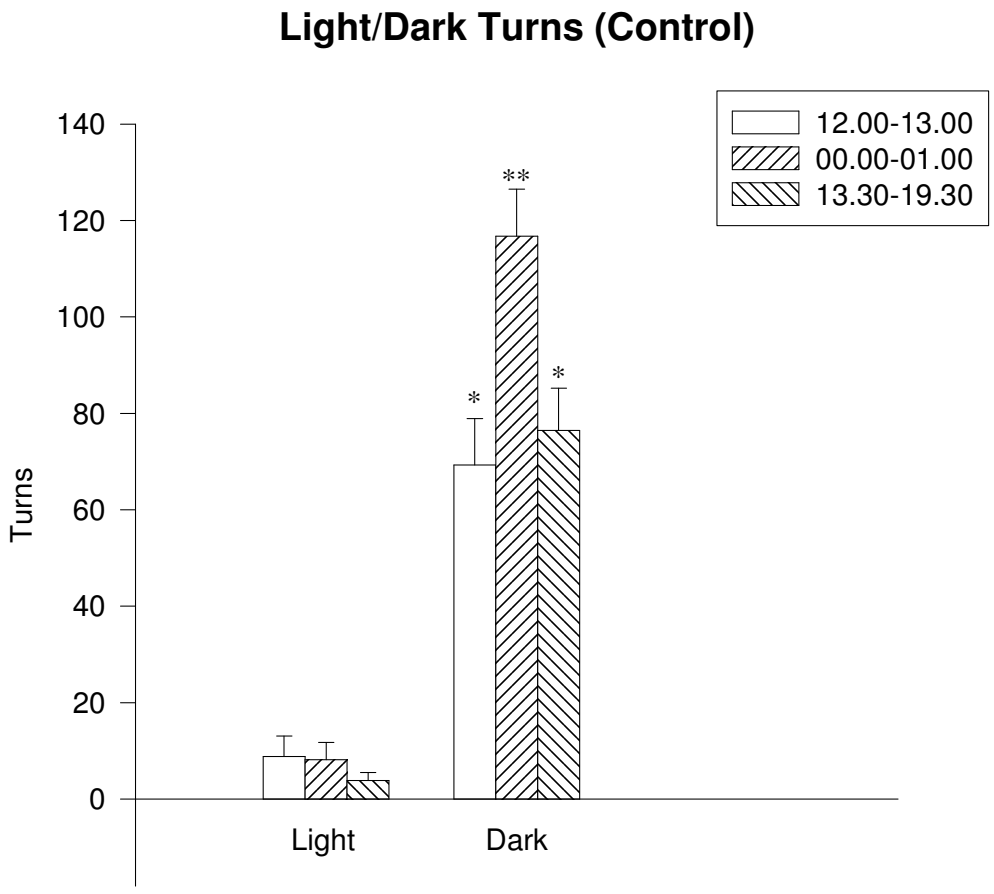

Figure 19: Running wheel turns of the juvenile Syrian hamsters in control groups. * indicates statistically significant difference ($p<0.05$). Data are given as mean ± S.E.M. (12.00-13.00, 00.00-01.00, 13.30-19.30 indicates the leptin infusion times).

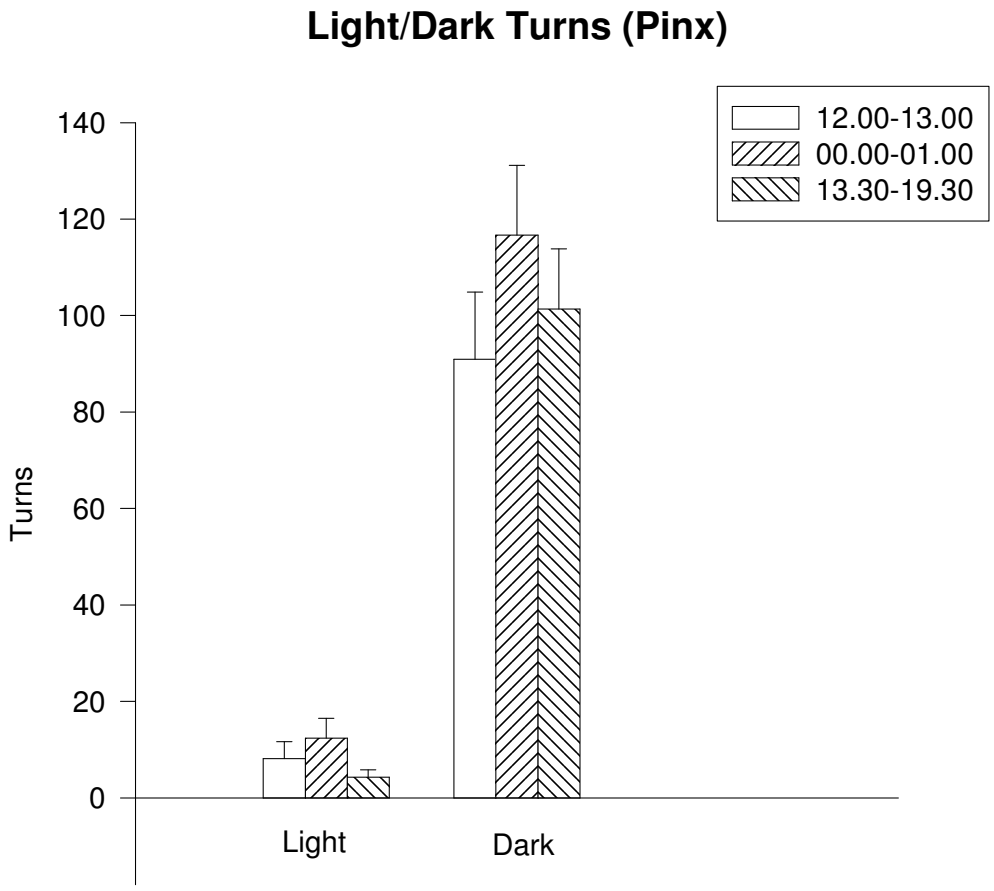

Figure 20: Running wheel turns of the juvenile Syrian hamsters in pinealectomy groups. Data are given as mean ± S.E.M. (Pinx: pinealectomy; 12.00-13.00, 00.00-01.00, 13.30-19.30 indicates the leptin infusion times).

Weeks	Body Weights (g) (Control) 12.00-13.00 Infusion Mean±S.E.M.	Body Weights (g) (Control) 00.00-01.00 Infusion Mean±S.E.M.	Body Weights (g) (Control) 13.30-19.30 Infusion Mean±S.E.M.
1-	20.033 ± 0.567	32.933 ± 0.993	26.778 ± 0.536
2-	36.367 ± 0.418	43.294 ± 0.871	33.922 ± 0.860
3-	57.833 ± 0.689	56.747 ± 1.367	43.000 ± 2.124
4-	70.433 ± 1.120	71.518 ± 1.470	54.686 ± 2.033

Table 3: Body weights of control group of juvenile Syrian hamsters in 14L for 4 weeks. Data are given as mean ± S.E.M. (12.00-13.00, 00.00-01.00, 13.30-19.30 indicates the leptin infusion times).

Weeks	Body Weights (g) (Pinx) 12.00-13.00 Infusion Mean±S.E.M.	Body Weights (g) (Pinx) 00.00-01.00 Infusion Mean±S.E.M.	Body Weights (g) (Pinx) 13.30-19.30 Infusion Mean±S.E.M.
1-	25.000 ± 2.000	38.400 ± 1.217	33.671 ± 1.068
2-	39.000 ± 1.400	51.807 ± 1.505	46.414 ± 1.286
3-	57.350 ± 1.050	72.840 ± 1.323	65.186 ± 0.771
4-	69.150 ± 1.750	79.020 ± 1.484	75.329 ± 1.350

Table 4: Body weights of pinealectomy group of juvenile Syrian hamsters in 14L for 4 weeks. Data are given as mean ± S.E.M. (Pinx: Pinealectomy; 12.00-13.00, 00.00-01.00, 13.30-19.30 indicates the leptin infusion times).

4. DISCUSSION

The aim of this thesis was to investigate the regulatory effects of leptin on fetal SCN through maternal system. It was concluded that the locomotor activities and body weights of juvenile Syrian hamsters are controlled by maternal leptin transfer.

In rodents, circadian rhythms are synchronized to the outside environment through the maternal system which is in synchrony with those of their mothers during late embryonic and early neonatal development. The effects of maternal entrainment has been studied by rearing rat pups from their mother and exposing them to constant lighting from birth. Measuring in a population of 10-day-old rats circadian phase of pineal serotonin *N*-acetyltransferase (NAT) activity showed that the pup's NAT activity is close to that of the biological mother even though the photoperiod during gestation was different from that of the mother (Deguchi, 1975; Reppert et al., 1984).

Since clock function can be modulated by pineal melatonin through direct action on hormone receptors in the SCN (Vanecek et al., 1987), melatonin has been proposed as one of the humoral factors for the synchronization of fetus through the maternal system. Maternal entrainment under circadian control may arise from a more complex mechanism than a single hormonal output. This implies that central and peripheral oscillators should all interact in defining the behavior of the organism. Several maternal hormones were removed by the ablation of any maternal endocrine glands (pineal, pituitary, thyroid, adrenal, ovaries) early in the gestation to test the role of them in mediating prenatal entrainment which would have similar effects to

maternal SCN lesions (Reppert and Schwartz, 1986b) suggested that the maternal signal might be redundant, with several maternal rhythms acting in concert to entrain the fetal and/or neonatal circadian system and eliminating any one of these hormonal signals would not be enough to disrupt maternal entrainment (Reppert, 1995).

In our study it was shown that wheel running turns of control juveniles with group 2 (00.00-01.00 h) was significantly higher. This implies the role of leptin in controlling locomotor activity rhythms through maternal system. It has been known that leptin receptors are found in the SCN of the rat (Guan et al., 1997). In our study there was no statistically significant difference of wheel running turns in group 2 (00.00-01.00 h) of both control and pinealectomy groups. Leptin may excite another mechanism within the SCN other than the pineal pathway. Since wheel running turns are similar in group 2 of control and pinealectomy groups and leptin exerted similar effects even in the presence of pineal gland and its hormone melatonin, leptin might effect SCN stronger than that of the melatonin. Study of Gündüz (2002) showed that serum leptin concentrations decrease in the dark phase and increase in the light phase. High wheel running turns in group 2 is also in coincidence with the melatonin peak time. Hormone release begins after lights-off (lights off at 20.00 h), reaches its peak levels at around midnight (00.00 h) and begins to fall after 02.00 h and reaches daytime levels after 06.00 h (lights on at 06.00 h). This suggests that the high amounts of leptin at night may coincide with the sensitivity to melatonin and hence increases wheel turns in juvenile Syrian hamsters. However, there were not any statistically significant difference found between control and pinealectomy groups in both group1 (12.00-13.00 h) and group 3 (13.30-19.30 h). There was a tendency of high wheel running turns in pinealectomy groups. This may also be explained by the sensitivity of the SCN. In the study of Prosser and Bergeron

(2003), leptin induced significantly phase advances of 1-3 h in the SCN of rat *in vitro*, when applied at all times except for ZT 22 (lights-on at ZT 0), when it had no effect. It is important to note that serum leptin concentrations and mRNA content of leptin in adipose tissue of mice and rats decrease in the light phase and increase in the dark phase (Ahima et al., 1998; Pickavance et al., 1998; Shimokawa and Higami, 1999). In Syrian hamsters, however, serum leptin concentrations decrease in the dark phase and increase in the light phase (Gündüz, 2002). Moreover Prosser and Bergeron (2003), stated that the time when brain leptin levels peak may coincide with the time when the SCN clock becomes insensitive to phase-resetting by leptin. Thus, circadian clock sensitivity to leptin may be down-regulated at the time when leptin levels normally peak. In our study, the peak time of leptin in Syrain hamsters coincided with the time of leptin infusion at light periods, and may be insensitivity of the SCN down-regulated the activity of leptin in light periods. It is important to take into account that the leptin transport into the brain appears to be rate-limited (Burguera et al., 2000). Therefore there might be a delay between when leptin levels increase in the plasma and when they increase in the brain. This information may help to explain the long lasting insensitivity of the SCN to leptin in group 3. In addition to the study of Prosser and Bergeron (2003), Karakaş et al. (2005) provides *in vivo* evidence for the phase advance effect of leptin on locomotor activity rhythms in Syrian hamsters. In that study treatment of Syrian hamsters by intraperitoneal injections, subcutaneous and intracerebroventricular (icv) infusions of leptin phase advances the activity rhythm. Those studies provide evidences for the regulatory effects of leptin on the SCN both *in vivo* and *in vitro* and it is possible that through maternal pathway and maternal SCN, leptin could regulate and programme the SCN of pups during pregnancy.

In rodents and monkeys, the biological clock in the SCN is functional and entarined prior to birth (Reppert and Weaver, 1991) and it is entrained during development prior to development of the retinohypothalamic pathway. The potency of the mother as an entraining signal decreases during the first week after birth (Reppert et al., 1984; Reppert and Schwartz, 1986a), coincident with the appearance of retina-mediated photic entrainment (Duncan et al., 1985). In our study the entraining effect of leptin which started at the 10^{th} day of pregnancy and ended with the parturition, may be modulated by the development of the pup's retinohypothalamic pathway after the first week of birth.

It has been reported that melatonin may have a direct effect on adipocytes (Blask et al., 1999). In that study fatty acid transport is blocked by melatonin via a melatonin receptor-mediated mechanism. In the study of Le Gouic et al. (1997), melatonin binding sites were reported in brown adipocytes in Siberian hamsters which are photoperiodic-sensitive species. In our study there was statistically significant differences of body weights observed in both group 2 (00.00-01.00 h) and group 3 (13.30-19.30 h) of control and pinealectomy groups. Body weights of control and pinealectomy groups in first week of group 1 (12.00-13.00 h) was statistically different. It is known that brown adipose tissue is a thermogenic organ which present in almost all mammals. It would be logical to say that the maternal pinealectomy can cause the differences of body weights. But it is known that brown adipocyte is mainly active in infancy and over the long term, white fat mass reflects the net balance between energy expenditure and energy intake. It has also been known that pineal melatonin production and rhythmicity is estabilished at the first week after birth with the formation of retina-mediated photic entrainment. On the basis of these informations it is difficult to say that the body weight differences are

caused by maternal pinealectomy. On the other hand body weight differences on the first week might be explained by the above mechanism, which is appearent especially in group 1 (12.00-13.00 h). In group 1, body weights of other weeks were not statistically different between control and pinealectomy groups. This can be explained by the sensitivity to leptin. Because it is known that in Syrian hamsters leptin levels reach its peak value at mid-day and the infusion of leptin at this time (i.e. leptin infusion between 12.00-13.00 h) coincides with the peak time of endogenous leptin. It means that may be the infusion of leptin at this time is insensitive. Infusion of leptin at other times (i.e. between 00.00-01.00 h and between 13.30-19.30 h), on the other hand, coincides with the sensitive phase to leptin. It is known that leptin informs the brain about body energy stores and it has been reported that pinealectomy increases leptin secretion, therefore those findings provide further evidence that melatonin may exert an inhibitory effect on leptin release (Canpolat et al., 2001; Gündüz, 2002). It means that after pinealectomy there is an increase of endogenous leptin in addition to the exogenous leptin. The increased amount of maternal leptin at sensitive phase may inform and programme the brain of fetus to gain weight. It has been known that arcuate nucleus is the brain site for the adipostat control of body fats (Cowley et al., 1999) which contain leptin receptors as an input signal involved in the conveying of the information on the size of body fat stores, and it has SNS outflow to WAT and BAT as an output component (Bamshad et al., 1998, 1999). This reciprocal connection has also been determined by neuroanatomical tracing studies between the hypothalamus arcuate nucleus (ARC), and the SCN (Bouret et al., 2004; Buijs et al., 2006; Dawson et al., 1997; Yi et al., 2006). This suggest the role of SCN for the regulation of lipostatic activity of leptin. It is possible that exogenous leptin during pregnacy is interpreted by the maternal

SCN and may be ARC is involved in this interpretation by its connection with SCN to programme the body weight regulation of fetus through maternal system.

In conclusion, the present thesis shows that the maternal leptin can control the locomotor activity of juveniles possibly through the receptors on the SCN. It is also possible that the body weights of juveniles are controlled by maternal leptin but the precise mechanism of body weight regulation of juveniles should need to be further investigated.

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