

**INTERACTION BETWEEN SUPRACHIASMATIC NUCLEUS
(SCN) AND LEPTIN HORMONE AND THEIR EFFECT ON
PUBERTY**

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TO MY PARENTS

INTERACTION BETWEEN SUPRACHIASMATIC NUCLEUS (SCN) AND
LEPTIN HORMONE AND THEIR EFFECT ON PUBERTY

by

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ABSTRACT

INTERACTION BETWEEN SUPRACHIASMATIC NUCLEUS (SCN) AND LEPTIN HORMONE AND THEIR EFFECT ON PUBERTY

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In the first study, hamsters were SCN lesioned, exposed to different feeding regimes, adrenalectomized and implanted with cortisol implants and leptin levels were examined. The results showed that the diurnal rhythm of leptin hormone release is directly regulated by the SCN. In the second study, animals were SCN lesioned, pinealectomized and melatonin infused. Infusions suppressed the leptin levels independently from the duration of the infusion ($p<0,05$). In the third study, five experiments were performed. In the first four experiment, animals were injected, subcutaneously infused, and intracerebroventrically infused with leptin at CT 10. Leptin hormone phase advanced the locomotor activity ($p<0,05$). In the fifth experiment, hamsters

were SCN lesioned and received timed leptin infusions. Infusions restored the arrhythmic locomotor activity of the SCN lesioned animals. In the fourth study, two experiments were performed. In the first, female hamsters were paired and injected with leptin, insulin, melatonin and saline. After weaning, the prepubertal hamsters were examined for their body, testes, adipose, epididymis and seminal vesicle weights. All parameters were heavier in hamsters borned to leptin injected mothers ($p<0,01$). In the second experiment, pregnant hamsters were injected with leptin. After parturition, the newborn pups were decapitated and their brains were examined for their weight, volume and the number of the neurons. Leptin accelerated the development of the brains. In the fifth study, photoperiodic control of leptin rhythm was investigated in hamsters with intact or pinealectomized groups. The leptin levels were the lowest in lipectomy group at the first week however leptin levels were similar to each other in lipectomy and leptin injected groups at the eighth week of the experiment.

Key words: SCN, leptin, rhythm, feeding, adrenalectomy, lipectomy, maternal transfer

ÖZET

SUPRAKİYAZMATİK NUKLEUS İLE LEPTİN HORMONU ARASINDAKİ ETKİLEŞİM VE PUBERTE ÜZERİNE ETKİLERİ

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Birinci çalışmada, hamsterlere SCN lezyonu uygulandı, farklı yeme programları uygulandı, hamsterlerin adrenal bezleri çıkartıldı ve cortisol içeren implantlar yerleştirildi. Leptin seviyeleri incelendi. Sonuçlar gösterdi ki, Suriye hamsterlerinde leptin hormonunun salınım ritmi direkt olarak SCN tarafından düzenlenmektedir. İkinci çalışmada, hamsterlere SCN lezyonu ve pinealektomi uygulandıktan sonra melatonin ile infüze edildiler. Melatonin infüzyonları leptin seviyelerini infüzyon süresinden bağımsız olarak baskıladı ($p<0,05$). Üçüncü çalışmada beş deney yapıldı. İlk dört deneyde, hamsterlere sirkadiyen saat 10'da intraperitoneal enjeksiyon, deri altı infüzyon ve serebrum

içi infüzyon ile leptin uygulandı. Leptin uygulamaları lokomotor aktivite ritminde bir öne kayma yarattı ($p<0,05$). Beşinci deneyde hamsterlere SCN lezyonu ve leptin infüzyonu uygulandı. Leptin infüzyonları aritmik lokomotor aktiviteyi tekrar ritmik duruma geçirdi. Dördüncü çalışmada iki deney yapıldı. Birincisinde dişi Suriye hamsterleri çiftleştirildi ve hamile hamsterlere leptin, melatonin, insülin ve serum fizyolojik enjeksiyonları uygulandı. Sütten kesme sonrası yavru erkek hamsterlerin puberteye ulaşınca kadar vücut, testis, yağ doku, epididimis ve seminal vezikül ağırlıkları ölçüldü. Sonuçlar, ölçülen parametrelerin hepsinin leptin enjeksiyonu yapılan annelerin yavrularında ağır olduğunu göstermekte idi ($p<0,01$). İkinci deneyde dişi hamsterlere leptin enjeksiyonları uygulandı. Yeni doğan yavrular dekapite edildiler, beyinleri çıkartılarak ağırlığı, hacmi ve nöron sayısı ölçüldü. Leptin uygulanan gruplarda ki beyin gelişimi daha hızlı idi. Beşinci çalışmada, intakt ve pinealektomize hamster gruplarında leptin ritminin fotoperiyodik kontrolü araştırıldı. Birinci haftada, leptin seviyeleri lipoektomi grubunda en düşük iken, sekizinci haftada lipoektomi ve leptin enjeksiyonu yapılan grupların leptin seviyeleri birbirine benzer idi.

Anahtar kelimeler: SCN, leptin, ritim, beslenme, adenalektomi, lipoektomi, maternal transfer

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1. INTRODUCTION

1.1 Photoperiod and the Control of Annual Reproductive Cycles

Several species regulate their body weight, reproduction and energy expenditure according to the external environmental factors such as daylength, temperature, rainfall, humidity and food availability (Chapmann, 1970; Reiter, 1974; Stetson et al., 1975). Mammals which utilize information to appraise their internal organs of the season are described as being photoperiodic.

Photoperiod (day length) has been shown to be the most critical environmental cue regulating seasonal reproductive rhythms in mammals of the temperate zone. Mammals living in these ever-changing environments have evolved means to increase the chances of their survival, and more importantly, the survival of their young. The latter is achieved by having the young of the species born during the time of the year that is maximally supportive of their survival. This critical time is the spring of the year.

Reproduction and gonadal maturation in hamsters are very sensitive to photoperiod. Exposure to short photoperiods results in a gonadal regression and loss of sexual behaviour (Pospichal et al., 1991; Schilatt et al., 1993).

Critical photoperiods, often between 12 and 14 hr of light per day, have been characterized for several rodent species. In the Syrian hamster, this photoperiod is 12,5 hours of light per day, in the Siberian hamster it is about 13 hours. In Syrian hamsters daylength shorter than 12,5 hours causes gonadal atrophy whereas the daylength longer than this promote gonadal maintenance (Elliot, 1976; Hoffmann, 1979). In Mongolian gerbil species, there are two

critical photoperiods; the first is between 8-10 hours of light and the second is between 22-24 hours of light (Karakaş and Gündüz, 2002a)

In several species it has been shown that photoperiod may also regulate gonadal development in juveniles (Johnson and Zucker, 1980; Hoffmann, 1978; Hoffmann, 1981). In Montane vole, deer mice, Mongolian gerbil and Siberian hamster, short photoperiod exposure appears to be responsible for the prevention of pubertal development (Horton, 1984; Stetson et al., 1986; Lee and Zucker, 1988; Karakaş and Gündüz, 2002a). In Turkish hamster, the response of intact prepubertal hamsters to photoperiods depends on the lactational period (Gündüz and Stetson, 1998). 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. A short-day exposure, for example, induces a large and reversible reduction in body mass in Siberian hamsters. In contrast, in Syrian hamsters short-day exposure increases body mass and this increase is mostly associated with an increase in fat mass, thus offering the opportunity to study the effects of a photoperiod-induced regulation of adipose tissue metabolism.

1.2. Body Weight Regulation

Body weight responses show a species-specific situation. Some species show a significant decrease in body mass in winter or dry season whereas the other species demonstrate the opposite trend (Hoffmann, 1981; Gower et al., 1994). There are also seasonal breeders that do not change body mass

seasonally (Bartness and Wade, 1984). It is an expected seasonal adaptation for some animals living at higher latitudes to increase their energy stores before winter, like ground squirrel (*Spermophilus beldingi*), Syrian hamster (*Mesocricetus auratus*), and prairie voles (*Microtus ochrogaster*). Conversely, some species like, meadow voles (*Microtus pennsylvanicus*), deer mice (*Peromyscus maniculatus*) and Siberian hamster (*Phodopus sungorus*) decrease their body weight when they exposed to short winter-like days. In three gerbil species, *M. crassus*, *G. dasyurus* and *G. henleyi*, there was a significant reduction in body weight in winter, ranging from %8,9-%20,2 (Vander Wall, 1990). The increase of body weight and lipid mass might seem intuitive before winter but decreases are explained as an adaptive strategy that reduces energy requirements during the harsh conditions (adaptation hypothesis) (Stebbins, 1978) and a response to changes in food-water availability (Abramsky, 1990). The adaptation hypothesis received support from studies on Meadow voles (*Microtus montanus*) and Siberian hamster in which body weight was found to be regulated by photoperiod (Heldmaier and Steinlechner, 1981). Siberian hamster reduces its body weight before winter some %45 for about 45 g to 25 g. Meadow voles also change their energy intake and body weight in response to photoperiod. Reduction in body weight was due to a decrease in total body water and in lean body mass, but brown adipose tissue remained unchanged. However, there was a reduced activity of white adipose tissue lipoprotein lipase. The loss in body weight during the short photophase is in agreement with other studies on winter acclimated voles (Wunder et al., 1977). Also *Cricetus cricetus* born in autumn were unable to increase their body weight in winter

even with excess food under stable conditions (Conguilhem and Marx, 1973). As it seen in different animal examples, photoperiod plays a major role on the regulation of body weight. And pineal gland is the most important component in this photoperiodic regulation.

1.3. Pineal Gland

Pineal gland is a small pine-shaped structure located in the epithalamus of the brain. Most important product of the pineal gland is the hormone melatonin. Melatonin is secreted and released from pineal gland in a rhythmic manner. Pineal gland functions as a chemical neurotransducer which converts the neural stimuli to a hormonal product as melatonin. The mammalian pineal gland is a secretory organ, whereas in fish and amphibians it is directly photoreceptive and in reptiles and birds it has a mixed photoreceptor and secretory function. In some birds and lower vertebrates it serves as a rhythm generating system, or biological clock. In mammals it is intimately concerned with the coordination of rhythm physiology without having the capacity to act as a rhythm generator (Arent, 1995).

Pinelectomy (the remove of the pineal gland) is one of the methods to investigate the effect of melatonin in photoperiodic animals. Pinelectomy eliminates the melatonin hormone from blood circulation. It prevents the animal from responding against the changes in daylength (Hoffmann and Reiter, 1965; Hoffmann, 1974). The effects of pinelectomy in some hamster species have been well documented. Pinelectomy prevents the regressional effect of short photoperiods while gonadal maintenance on long photoperiods is not affected in Syrian hamster (Hoffmann and Reiter, 1965). The effect of

pinealectomy on Siberian hamsters is more complex. Pinealectomy blocks short photoperiod induced gonadal regression of hamsters previously housed on long photoperiod (Hoffmann, 1974). The pineal gland is also involved in transducing long photoperiod effects in these species: Pinealectomy prevents long photoperiod induced gonadal growth of hamsters previously housed on short photoperiod. Thus, the general effect of pinealectomy in adult Siberian hamster is either to prevent the perception of a change in daylength or to maintain the daylength measurement system in preoperative mode, the gonads are maintained in a state appropriate to the previous photoperiod (Gündüz and Stetson, 1994). The responses to pinealectomy in Turkish hamsters are much more different. In this species, pinealectomy causes gonadal atrophy in long photoperiods but in short photoperiods, pinealectomy does not prevent the gonadal regression (Carter et al., 1982). Also there is an effect of pinealectomy on juvenile sexual development in Siberian hamsters. Removal of the pineal gland deletes the effect of long and short photoperiod on gonadal development. The development of pinealectomized juveniles is more rapid than the intact juveniles (Hoffmann, 1973; Brackmann, 1977). Moreover, pinealectomy inhibits the stimulatory effect of long photoperiods (Hoffmann and Kuderling, 1975). Besides the effects on reproductive system, pineal gland may affect the body weight and lipid mass changes. It was reported in Syrian hamsters, Siberian hamsters and prairie voles that pinealectomy may block the photoperiod related changes in body weight (Hoffmann, 1981).

Light information is first perceived by the retina of the eye. Then this information is transferred to suprachiasmatic nuclei (SCN) by the

retinohypothalamic tract. SCN is a very important brain component because it generates all the internal rhythms in the body and also it conducts the light information to the superior cervical ganglia (SCG). At the end, light information reaches to the pineal gland (Arent, 1995).

1.4. Melatonin

Melatonin is produced and released from the pineal gland into the blood especially at night and is referred to as the chemical experience of darkness (Reiter, 1981; Stetson and Watson-Whitmyre, 1984). The melatonin activity of the pineal gland is determined primarily by the release of norepinephrine (NE) from postganglionic sympathetic nerve endings that terminate in the gland. The release of NE is associated with darkness, when the suprachiasmatic nucleus (SCN) is relieved of the inhibitory signal from the eyes due to the interaction of photons with the retinas. In as much as NE release onto the pinealocytes occurs at night, melatonin synthesis likewise occurs primarily during darkness.

Therefore, the concentration of melatonin in the blood is greater at night than during the day. The rate and the pattern of the nocturnal increase in melatonin production depend on some other factors such as species and tissues. Three different basic pattern of pineal melatonin production are described in mammals (Reiter, 1987). Type A is not very common and demonstrated in Syrian hamsters and Mongolian gerbils. Melatonin peaks late at night. After midnight, melatonin levels increase quickly to its peak values and soon thereafter, before the time of lights on, they decline to daytime values. Type B is the most common one and demonstrated in rat, human and Guinea pig. Melatonin makes peak at midnight. Pineal melatonin levels gradually rise, reaching peaks around

the middle of the night, and then declining slowly during the second half of the night to reach low daytime values near the time lights on. Animals with a type C pattern of the nocturnal melatonin synthesis are also common and they are characterized by a prolonged peak of melatonin levels for virtually the entire night. Thus, peak melatonin production is reached soon after the onset of darkness, these high levels of the hormone for most of the night and decrease before lights onset. This pattern of the melatonin formation is present for example in sheep, cat and the Djungarian hamster (Reiter, 1987).

Melatonin has a half life of nearly 20-40 minutes. It does not remain in the blood very long. Unless the pineal gland continues to produce and secrete melatonin, blood levels of the hormone drop quickly. Melatonin is removed from the blood in at least four ways. 1) It is enzymatically degraded primarily to 6-hydroxy melatonin by the liver. 2) Melatonin is taken up by other cells is non enzymatical degraded when it scavenges hydroxyl radicals. 3) Also, melatonin in the blood rapidly escapes into other body fluids. 4) Finally, melatonin attaches to specific receptors or binding sites located at various locations in the organism (Panke et. al., 1979; Steinlechner, 1996).

The melatonin receptors involved in mediating the effects of melatonin on the reproductive and endocrine systems are presumed to be those located in the pars tuberalis of the anterior pituitary gland (Stankov et al., 1991). These cells are in close proximity to the primary portal plexus and the terminals of the hypothalamic releasing hormone neurosecretory cells in the median eminence. Melatonin theoretically controls the release of substances, e.g., gonadotropins or other factors, that act in a paracrine manner in the nearby median eminence

thereby regulating the release of the hypothalamic releasing hormones, e.g., gonadotropin releasing hormone (GnRH). In this manner melatonin could obviously regulate the functional status of the gonads and control the reproductive capability of an animal on a seasonal basis.

1.5. Photoperiodic time measurement

The nature of the endocrine message communicated via the melatonin rhythm has been the subject of much investigation. Two different possibilities have been proposed to explain how melatonin conveys the photoperiod information to the reproductive axis (Reiter, 1991; 1993). The duration hypothesis, implies that the photoperiod induced modification in the length of the nocturnal melatonin rise, is the message that synchronises biological and reproductive functions to the different seasons of the year (Reiter, 1987; Bartness et al., 1993). Evidence in support of this hypothesis has come from melatonin infusions in pinealectomized Siberian and Syrian hamsters (Carter and Goldman, 1983; Maywood et al., 1990). The internal coincidence hypothesis implies that melatonin exerts an effect only when its circadian secretion is coincident with target tissue sensitivity. This hypothesis supposes that the time of presence of melatonin is important. Support for this hypothesis has come from experiments in intact adult Syrian hamsters (Stetson and Tay, 1983), Turkish hamster (Hong and Stetson, 1987) and Siberian hamsters (Stetson et al., 1986), pinealectomized adult Syrian hamsters (Stetson and Watson-Whitmyre, 1986; Watson-Whitmyre, 1985) and intact and pinealectomized juvenile Siberian hamsters (Gündüz and Stetson; 2001a, b).

1.6. Maternal Transfer of Photoperiodic Information

Pregnant females of several mammalian species transmit information about daylength to their fetuses and this prenatal photoperiodic information programs the postnatal reproductive development and photoperiodic responses of the young. Horton (1984) showed that the reproductive development of Montane voles (*Microtus montanus*) was altered by changing the prenatal photoperiod. Males which were transferred on the day of birth from LD 16:8 to LD14:10 had inhibited reproductive development, while in males gestated in LD12:12 and transferred to LD14:10 testes were stimulated (large). A similar finding has been reported by Stetson et al., (1986) using the Siberian hamster. These findings indicate that a prenatal signal is received by the fetus during the gestational period. Investigation of which factor could influence the pubertal development has shown that melatonin was the potential candidate for this transfer (Gündüz and Stetson, 2003).

1.7. Hypothalamic control of Feeding

The survival of an animal depends on how efficiently it can regulate its supply and use of metabolic fuels and essential nutrients. Food intake regulation depends on the ability of the animal to encode and retain in its memory a variety of information about its experiences with food. The brain controls the feeding. The decision to eat or not to eat, and the selection of food are based on learning to anticipate the post-ingestive consequences of food intake. The hippocampus is involved with either the detection or the utilization of hunger/satiety signals and the specific memories and learning related to food intake (Woods et al.,

1998). Some peptides increase the food intake (orexigenic peptides) and some of them decrease (anorexigenic peptides) it.

1.8. Peptide systems that increase feeding and body weight (Orexigenic Peptides)

Neuropeptide Y (NPY) is expressed in neurons of the arcuate nucleus (ARC) that send their dense projections to the paraventricular nucleus (PVN), ventromedial hypothalamus (VMH), and perifornical area/lateral hypothalamus (PFLH). This peptide is a potent stimulant of feeding. Neurons synthesizing NPY in the ARC are highly responsive to states of energy deficiency and higher metabolic demand. The expression of the peptide is stimulated by food deprivation, as well as during states of increased exercise, cold and pregnancy. The peptide, agouti-related protein (AgRP), is expressed primarily in the ARC. It forms part of the central melanocortin system. It also increases the food intake (Ozata, 2003).

1.9. Peptide systems that reduce feeding and body weight (Anorexigenic Peptides)

Pro-opiomelanocortin (POMC) is synthesized in the ARC. There is evidence relating the melanocortins to circulating leptin and suggesting a role for this peptide system in mediating leptin's effects on energy balance. Leptin stimulates the POMC neurons which express the leptin receptor. Leptin-deficient *ob/ob* mice exhibit a substantial reduction in POMC throughout the ARC, which is restored by leptin replacement. Cocaine- and amphetamine-regulated transcript (CART) is another anorexigenic peptide.

Immunohistochemical evidence shows CART peptides in various nuclei of the

hypothalamus, including the ARC, PVN and PFLH. CART-immunoreactivity is also found in the pituitary and adrenal gland which suggests a role in the hypothalamo–pituitary–adrenal (HPA) axis function. Corticotropin releasing factor (CRF), is widely expressed in the brain, known as another anorexigenic peptide (Ozata, 2003).

1.10. Leptin Hormone

Leptin (167-amino acid polypeptide hormone), the product of the *ob* gene (Zhang et al., 1994) is an adipocyte-secreted protein whose circulating levels in the fed state reflect body fat content in mice and humans and signal the status of energy stores to the brain. Moreover, leptin levels in the blood change in response to fasting and overfeeding independently of changes in adipose tissue stores. In addition to its role in regulating energy balance, leptin mediates the neuroendocrine response to fasting-starvation (Ahima et al., 1996), may play a role in haematopoiesis, immunity (Ozata et al., 1999), antioxidant defense system (Ozata, 2000), functions as the metabolic signal to the reproductive system (Chehab et al., 1996) and appears to signal the onset of puberty in rodents (Ahima et al., 1997; Chehab et al., 1997, Pehlivan and Gündüz, unpublished data) and humans (Ozata, 2003).

Leptin binding to target cells results in the formation of a receptor complex, leading to the cross-phosphorylation and activation of the JAKs. These activated JAKs then rapidly phosphorylate tyrosine residues in the cytosolic domain of the receptor, and such phosphorylated residues provide binding sites for signaling molecules, including members of the STAT family. To date, six splice variants of the leptin receptor, OB-Ra-e (Chen et al., 1996)

and muB219 have been identified. Among these splice variants, only the long form, OB-Rb, is capable of mediating signal transduction through STAT3, while the short form receptors (called common receptors, OB-R) are not (Tartaglia et al., 1995).

The regulation of leptin production has been studied most extensively in rodents. Fasting, cold exposure, β adrenergic receptor agonists and the insulin sensitizing agent triglitazone decrease expression of leptin mRNA expression whereas feeding, dexamethasone and insulin increase expression. Serum leptin concentrations are positively correlated with the white adipose tissue (Wade et al., 1996). Leptin expression in adipose tissue and circulating leptin depends upon fat/ energy stores, which are affected by sex and dietary patterns. The activity of reproductive axis is sensitive to the sufficient quantity of food and the store of the metabolic sources. It functions as a metabolic gate to reproductive system (Wade et. al., 1996).

There is an interaction between the hormones leptin and the insulin. Insulin can regulate leptin expression. It has been shown that *in vitro* insulin stimulates the mRNA expression and secretion of the leptin (Boden et al., 1997). There are also some *in vivo* studies which support this data. Rodent studies have shown that insulin induces an increase in ob mRNA in adipose tissue and increases plasma leptin levels. On the other hand leptin can modulate insulin secretion and action. In ob/ob mice, leptin administration decreased glucose and insulin levels and corrected insulin resistance. Leptin receptors have been shown on insulin-secreting pancreatic β -cells.

Glucocorticoids modulate food intake and body weight (Dagogo-Jack et al., 1997; Bradley and Cheatham, 1999). They have a central effect on the central nervous system. Leptin suppresses the hypothalamic-pituitary-adrenal axis. In fasted rodents, leptin levels reduce however plasma corticosterone and adrenocorticotrophic hormone levels increase (Licinio et al., 1997). Leptin receptors are present in adrenal tissue, cortex and medulla (Guan et al., 1997).

Genetically obese ob/ob mice (lacking endogenous leptin) are infertile due to the absence of the leptin hormone. Leptin treatment to these mice restores the fertility (Chehab et. al., 1996). Food restricted animals have reduced circulating levels of leptin, which are associated with markedly reduced secretion of gonadotropins (Matthew et. al., 1999). Refeeding restores the normal leptin levels. In Siberian hamsters, exposure to short photoperiod decrease leptin gene expression and hormone release related to decrease in adipose tissue (Klingenspor et.al., 1996; Klingenspor et.al., 2000). Leptin might be affected independently upon to food deprivation in seasonal behaviors of animals mediated with photoperiod.

During pregnancy, maternal serum leptin concentrations are greater than those in nonpregnant women (Butte et al., 1997). Substantial increases in early pregnancy, before the occurrence of any notable increase in body weight due to progressive gestation, imply that factors other than increased adiposity mediate maternal leptin levels (Highman et al., 1998). Numerous studies have demonstrated that maternal peripheral leptin levels are enhanced during pregnancy (Sivan et al., 1998) and collectively suggest that leptin concentrations peak in the second trimester and remain elevated until

parturition. In the pregnant mouse, serum leptin levels peak on Day 17 and are many fold higher than nonpregnant levels. Leptin is indeed produced during pregnancy, expressed by the placenta but the importance of leptin is not clear. In the pregnant rat, serum leptin increases during pregnancy (Garcia et al., 2000), perhaps declining before parturition (Chien et al., 1997).

During pregnancy and lactation, major adaptations in maternal energy metabolism must occur to meet the requirements of fetal growth and milk production. Although these energetic adaptations have been studied extensively during pregnancy, the extracellular factors responsible for their onset have not been well-defined. Leptin, because of its role in the regulation of energy metabolism, has been implicated in the coordination of maternal adaptations during pregnancy and lactation. Maternal plasma leptin concentration is elevated during pregnancy in rats, mice and humans and falls during the transition to lactation (Masuzaki et al., 1997; Butte et al., 1997). Leptin is regulated by adiposity and energy balance and it is unclear whether factors specifically associated with pregnancy or lactation can alter plasma leptin concentration independently of changes in energy balance or adiposity.

In a recent study, day and night serum leptin levels were determined in adult female Syrian hamsters during gestation and lactation periods in long and short photoperiods. It was also investigated that if pineal gland had an effect on leptin levels. According to the data, day and night leptin levels in long photoperiod exhibited a decline. At the last phase of the gestation, day and night leptin levels of control and pinealectomized animals were similar to each other. When we looked at the short photoperiod, serum leptin levels were high

at the first phase of the gestation while they were low at the last phase of the gestation. However, no significant difference was determined between day and night leptin levels in control and pinealectomized animals except the second phase of the gestation in short photoperiod (Pehlivan and Gündüz, unpublished data).

It has been shown that leptin concentration changed with photoperiod in adult Syrian hamsters and leptin level exhibited circadian rhythm (Gündüz, 2002). There is a reverse relationship between plasma melatonin and leptin levels connected with photoperiod and/or pineal gland; leptin levels of hamsters in light phase high but melatonin levels are low (Gündüz, 2002).

Daily rhythm of leptin changes in rodents (Ahima et. al., 1996) and humans (Sinha et. al., 1996). It was demonstrated in rats that serum leptin concentrations and mRNA content of leptin in adipose tissue decrease in light phase and increase in dark phase (Ahima et. al., 1998; Pickavance et. al., 1998; Shimokawa and Higami, 1999).

There is considerable debate concerning the potential role of leptin as a physiological trigger for initiating the onset of puberty. Because leptin is a metabolic signal that indicates the level of somatic development, it may signal the brain that the body is capable of supporting pubertal development and subsequent reproductive function. As such, leptin could potentially serve as a trigger for the initiation of sexual maturation.

Leptin-treated prepubertal female mice reproduced at an earlier age than did nontreated controls, indicating that leptin may help to trigger puberty (Chehab et al., 1997; Pehlivan and Gündüz, unpublished data). Administration

of leptin is associated with elevated LH concentrations and dramatic changes in ovarian and uterine weights and histology. Leptin may also serve as a permissive regulator of human reproductive maturity as increases in peripheral levels are associated with the onset of menarche. Women of reproductive age typically exhibit higher levels than men of comparable age or adiposity.

1.11. The relationship between leptin and melatonin

The recent studies have shown that there is a direct or indirect relationship between leptin and melatonin hormones. Melatonin application in rats causes a significant reduction in serum leptin concentrations (Mastronardi et al., 2000). In contrast, leptin levels increase after pinealectomy (Wolden-Hanson et al., 2000). According to this data melatonin declines the secretion of leptin hormone. On the other hand, leptin and melatonin hormones exhibits an antagonistic influence in some physiological processes such as the onset of puberty, pain transmission, food intake and reproduction (Chehab, 1997).

Puberty onset is accelerated by leptin whereas it is delayed by melatonin. Serum melatonin levels decrease before puberty and this led the increase in serum leptin concentrations. Another antagonistic influence is seen in the regulation of pain transmission. When melatonin was applied, the pain threshold is increased and by this way decreases the sensitivity to pain. Although the studies about analgesic effects of leptin are unclear, it has been shown in mice that leptin application decreases the threshold of pain and increases the sensitivity to pain. Appetite suppressive effects of leptin is well known. Apart from that, leptin also increases the energy consumption. On the contrary, melatonin increases the appetite. The decrease in food intake in

pinealectomized mice is returned to normal amounts with melatonin application. Another antagonistic interaction between leptin and melatonin hormones is seen in oxidative stress. It has been shown that, melatonin decreases the oxidative stress by rendering free radicals and increasing the activity of antioxidative enzymes (Reiter, 1997). Conversely, leptin increases the oxidative stress in endothelial cells. Reproductive physiology is also affected from leptin and melatonin hormones in an antagonistic character. Melatonin inhibits the reproduction of photoperiodic animals. Whereas leptin stimulates (Matthew et al., 1999).

1.12. Obesity

Obesity is a major health problem in developed countries, and is rapidly becoming an important health problem in developing countries. Obesity is strongly associated with many chronic diseases : hyperlipidemia, high blood pressure, carbohydrate intolerance and diabetes, coronary atherosclerotic heart disease, gout, restrictive lung disease, gall bladder disease, cancer, degenerative arthritis, and infertility (Ozata, 2003). Because of the strong association between obesity and several risk factors for morbidity and mortality, reversing the obesity epidemic is an urgent priority.

Obesity is a disorder that results from excess white adipose tissue. Normal values of fat mass are 15–18% of body weight in males and 20–25% in females but it may consist of 60–70% of body weight in massively obese individuals (Ozata, 2003). In addition to storing excess energy as fat, adipose tissue has much more complex and dynamic functions and acts as an endocrine organ secreting various factors into the blood, termed adipokines (Harvey and

Ashford, 2003). These factors include leptin, adiponectin, resistin, acylation-stimulating protein, adipsin, angiotensin II, prostoglandins, plasminogen activator inhibitor-1, tumor necrosis factor- α , macrophage migration inhibitory factor etc., and the list of these factors is growing rapidly. Most of these factors have important roles in energy homeostasis, insulin sensitivity, immune function, inflammation, and even in tumor progression in the local oncogenic microenvironments. The impact of these secretory and non-secretory components of the adipose tissue on obesity related complications indicate that adipose tissue is an active endocrine organ (Augustine and Rossi, 1999).

1.13. Biological Rhythms

Biological rhythms are recurrent events within a biological system. Recurrent events mean biological processes that take place more than once usually many times throughout the system's life. The frequency of recurrence of the events may be very high (once every fraction of a second) or very low (once each year or once every several years). Biological processes that repeat themselves every 24 hours are called daily or circadian rhythms. Circadian rhythms are so pervasive in the body that affect the operation of other rhythms. Biological rhythms with frequencies higher than that of circadian rhythms (such as electrocardiogram) are called ultradian rhythms. Rhythms with frequencies lower than that of circadian rhythms (such as the menstrual cycle) are called infradian rhythms (Aschoff, 1981).

In animals that live close to the ocean and graze in intertidal areas, tidal rhythms (12,4 hours) and lunar rhythms (24,8 hours) have been observed. Especially well studied have been the biological rhythms associated with

reproduction. Seasonal cycles affect reproduction in many species. For instance, in hamsters and voles, gonads undergo seasonal regression during the winter, so that the animals are physically unable to reproduce. Many physiological functions in addition to reproduction undergo seasonal changes. These include body weight gain or loss, pelage molt, alterations in the level of locomotor activity, and, of course, drastic changes in body temperature in hibernators (Refinetti and Menaker, 1992; Refinetti, 1996).

When the majority of the activity of an animal occurs at night it is said to have a nocturnal activity pattern. Animals that show a diurnal pattern of activity exhibit the majority of their locomotor activity during the day or light portion of the LD cycle. Crepuscular activity rhythms are characterized by a bimodal distribution of activities occurring around the light/dark transition periods-that is, at dawn and dusk (Daan and Pittendrigh, 1976).

1.14. Entrainment of the Rhythms by Light and Other Signals

One of the most important properties of circadian clocks is their ability to become synchronized with the 24-h LD cycle. The synchronization process is called entrainment and is the result of the ability of the clock to be reset by light. Several authors have studied the influence of the most potent known synchronizer: the alternance of day and night. For living organisms, the L:D cycle is transposed in biological representations. Circadian rhythms are known to be entrained under natural conditions of the day-night alternance. Studies on the circadian rhythms in animals maintained under constant conditions lead to address several hypothesis on the functional organization of the biological clock. In an environment without time cues, the circadian rhythms are persist

with a period close but different from exactly 24-h: these are called free running rhythms. Species differences are observed in the free running periods but most nocturnal rodents exhibit a free running circadian period of approximately 25-h when they are housed in constant darkness. The circadian rhythms of temperature and locomotor activity were abolished when the animals were transferred from LD 12:12 to prolonged continuous light (Depres-Brummer et al., 1995). It is well established that pinealectomy has no effect on the expression of activity circadian rhythms in animals both placed in constant darkness and submitted to a modification of photoperiod (Cheung and McCormack, 1982).

The first evidence that scheduled food availability of food influences circadian control came from studies showing that locomotor activity was increased markedly before the time that food was presented each day in rats (Richter, 1922). There is now clear evidence that restricted food availability is capable of entraining a circadian clock; that is, limited food access can serve as a zeitgeber and it has many of the same effects on clocks as other entraining stimuli (Rusak, 1990). The majority of research on the entraining effects of food availability have been conducted in laboratory rats and have found that the scheduling of food can influence the circadian control of a number of circadian rhythms including wheel running and drinking. It is not feasible to conduct similar experiments in Syrian hamsters because they do not survive under conditions where food availability is scheduled only for short intervals (Silverman and Zucker, 1976). If freely-feeding Syrian hamsters are given

restricted opportunities to hoard food, however, then this time cue capable of serving as an entraining stimulus (Rusak et al., 1988).

1.15. Organization of the Mammalian Circadian System

From the simplest algae to mammals, nearly all organisms use sunlight to adjust their period of activity to optimize survival. Consequently, organisms have adapted their life style in such a way that they organize their activities in 24-h cycles determined by sunrise and sunset. There are two dominant environmental oscillators shaping the living conditions of our world: The day-night cycle and the succession of the season. Organisms have adapted to these by evolving internal clocks slaved to the master pacemaker of the suprachiasmatic nuclei (SCN) synchronizes the body to the daily 24 hour cycle. However, this clock's work closely interacts with the seasonal time-teller. Recent experiments show that photoperiod, the dominant zeitgeber of the circannual clock might be deciphered by the organism using the tools of the circadian clock itself. Destruction of the SCN in the Syrian hamster by lesions, abolishes three well documented circadian rhythms, locomotor activity, estrous cyclicity and photoperiodic photosensitivity. Entrainment of these rhythms by light cycles fails in lesioned hamsters; females become persistently estrous. Locomotor activity in both sexes is abolished and is totally arrhythmic when lesioned animals are exposed to continuous darkness. The photoperiodic gonadal response (gonadal regression induced by short daylengths) is abolished, lesioned animals remain reproductively mature irrespective of photoperiodic treatment (Ellison et al., 1992).

In contrast, other parts of the brain that normally show diurnal variation in activity do not show a circadian rhythm after surgical isolation of the SCN. In vitro, SCN neurons continue to show a 24 hour pattern of electrical activity. Further evidence indicating the SCN as primary circadian oscillator comes from work showing that electrical stimulation of the nucleus causes a phase shift of circadian rhythms (Rusak and Gross, 1982). Transplantation of the embryonic SCN can restore circadian rhythmicity in SCN lesioned rodents (Lehman et al., 1987) and when short period mutants are used as SCN tissue donors, the host takes on the period characteristic of the donor (Ralph et al., 1990). Taken together, these studies indicate that the SCN is involved in the generation of the circadian rhythms and that, even in the absence of the environmental cues or input from other parts of the central nervous system, the neurons of the SCN are capable of sustaining a circadian rhythm.

1.16. Anatomy and the Neurochemistry of the SCN

The suprachiasmatic nucleus is a nucleus in the hypothalamus and is so named because it resides immediately above the optic chiasm. It consists of two nuclei each of which lies on either side of the hemisphere separated by the third ventricle. SCN has been divided into dorsomedial and ventrolateral subdivisions (Moore and Card, 1985). Neurons within the two subdivisions produce different neurotransmitters and receive different patterns of afferent input. The ventrolateral subdivision is the site of termination for three of major afferent projections to the SCN. The retinohypothalamic tract (RHT), which is the direct projection from the retina to the SCN, appears to be necessary for the entrainment of circadian rhythms to light (Johnson et al., 1988). The two other

pathways to the SCN are the geniculohypothalamic tract (GHT), that originates in the intergeniculate leaflet of the thalamus (Ribak and Peters, 1975) and a projection from the raphe nuclei (Fuxe, 1965). There is evidence that these pathways may influence the response of the SCN to light/dark information (Albers et al., 1991), although neither pathway appears to be necessary for entrainment to light (Pickard et al., 1987). It also has been hypothesized that these pathways are involved in mediating at least some of the phase shifting effects of non-photic cues (Edgar et al., 1993).

The primary neurotransmitter in the projection from the retina appears to be glutamate (GLU), the projection from the IGL contains both neuropeptide Y (NPY) and gamma-aminobutyric acid (GABA), and serotonin (5-HT) is found in the projection from the raphe. Neurons within the ventrolateral subdivision produce a large number of neurochemical signals including vasoactive intestinal peptide (VIP), peptide histidine isoleucine (PHI), gastrin releasing peptide (GRP) and GABA. Neurochemicals produced within the dorsomedial subdivision include vasopressin (AVP), somatostatin (SS) and GABA (Albers et al., 1992).

1.17. Targets of the SCN

Several studies have shown that the SCN specifically targets the following: 1) gonadotropin releasing hormone (GnRH) neurons in the rostral forebrain (Van der Beek et al., 1997) and neurons that contain estrogen nuclear receptors (Delaiglesia et al., 1995), providing the anatomical basis for the influence of the SCN on the reproductive cycle in females and on the daily testosterone surges in males; 2) neurons that are activated during stress mainly

around the paraventricular nucleus of the hypothalamus (PVN) (Buijs et al., 1993), providing the anatomical basis that explains the role of the SCN in stress controlled corticosteroid regulation (Kalsbeek et al., 1996, Choi et al., 1998); 3) dopamine containing neurons probably implicated in the regulation of prolactin secretion by dopamine release in the tubular infundibular region (Horvath, 1997); 4) neurons projecting to the autonomic nervous system, providing the anatomical basis for the circadian control of the pineal, adrenal, pancreas, liver, ovaries and other organs (Gerendai et al., 2000; Buijs et al., 2001); 5) neurons below the PVN and within the dorsomedial nucleus of the hypothalamus (DMH) seem essential for the transmission of sleep/wake information (Aston-Jones et al., 2001); 6) magnocellular neurons, having a putative role in the secretion of neurohypophysial hormones (Hermes et al., 1996, 2000)

1.18. Circadian Control of Hormone Secretion

It is proposed that the SCN affects hormone secretion and affects the sensitivity of the target organs of these hormones by neuronal mechanisms. Anatomical and physiological evidence shows that the level of insulin secretion from the pancreas, melatonin secretion from pineal and blood corticosterone levels are controlled by the SCN (Nagai et al., 1996). When SCN stimulates glucose secretion from the liver, at the same time it stimulates uptake of glucose by other tissues (La Fleur et al., 2001). This action of the SCN on glucose metabolism prepares our body just before waking for the coming period of activity. Injection of transneuronal tracers into various organs and tissues ranging from white to brown adipose tissue results in the labeling of neurons in the SCN via the sympathetic and parasympathetic branches of the autonomic

nervous system (Gerendai et al.,2000). These data demonstrate that the SCN may transmit its circadian message influencing the activity/rest cycle of peripheral organs not only by means of the secretion of hormones that may freely pass all kinds of tissue barriers such as corticosterone and melatonin, gonadal hormones and thyroid hormones but also by a direct nervous control of these organs.

1.19. Syrian hamster

Syrian hamsters (*Mesocricetus auratus*: Cricetidae) (Waterhouse, 1839) have been preferred as a laboratory model species because of their strong photoperiodic characteristics that illuminate effect various mechanisms of the day length. However, the body weights and gonadal development of prepubertal individuals of this species are independent from ambient photoperiod (Gaston and Menaker, 1967).

Syrian hamsters display a 4-day estrous cycles that are exceptionally predictable and easy to monitor compared to the estrous cycles of other laboratory animals. On day 4 of the estrous cycle, adult females show stereotypical estrous behaviour (lordosis) promptly upon being introduced to a sexually experienced male. Ovulation occurs during the night of day four. The next day, is characterized by the appearance of a conspicuous vaginal discharge (Orsini, 1962).

The hamster has the shortest gestation period in eutherian mammals. Gestation length depends on the age of the mated hamsters. It is about 373 hours for one month-old mated hamster while 402 hour for 14 months of age. There is a sharp drop in mating and deliveries after 14 months of age.

Sexual maturation and the onset of puberty are aspects of mammalia reproduction which have received considerable attention. Rather than being under strict genetic control, the timing of the prepubertal events can be modulated for environmental conditions such as nutrition, temperature and noise. Photoperiod which is an important regulator of annual breeding cycles in many adult mammals, also has been thought to play a role in the sexual maturation process. Although the reproductive system of adult syrian hamster is exquisitely sensitive to photoperiod being inactivated when daylength is less than 12,5 h. It has not been clear from early work whether the prepubertal sexual maturation process is under photoperiod control. Syrian hamsters born in the spring and summer have been reported to reach puberty more rapidly than those born in the fall under conditions of decreased day length (Czyba, 1968). Yet male hamsters blinded at birth demonstrated gonadal weights at 6 weeks of age similar to those of sighted controls suggesting a lack of photoperiodic involvement in early sexual development.

Body weights and fat content of this species is preciaely controlled by photoperiodic changes. They reduce body weight in long photoperiods but increase in short photoperiods; opposite to other counterparts (e.g. Siberian hamsters). This mechanism in altering body fat and weight is not well known and is suggested that leptin may adjust these changes (Karakaş and Gündüz, 2002).

2. MATERIALS AND METHODS

2.1. Animal care

Syrian hamsters were obtained from our laboratory colony maintained at the Abant Izzet Baysal University. Hamsters exposed from birth to 14L (14 hour of light, 10 hour of darkness, lights off at 2000 hr). Animals were maintained in plastic cages (16x31x42 cm) with pine shavings used as bedding. Food pellets (Purina rodent chow) and tap water were accessible ad libitum. The procedures used in these studies were carried out in accordance with the Animal Scientific Procedure, Act of 1986 and approved by the Institutional Animal Care and Use Committee. All lighting was provided by 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.

2.2. Anesthesia

Before surgery, hamsters were anesthetized subcutaneously with Ketamine (20 mg/kg BW, Sigma Chemical Company, MO, USA) and intraperitoneally with pentobarbitol (32.5 mg/kg BW). Depth of anesthesia was monitored by frequent testing for the presence of leg flexion reflexes and active muscle tonus.

2.3. Surgical Procedures

2.3.1. Pinealectomy

Pinealectomy of hamsters was performed according to the method of Hoffmann and Reiter (1965); aspiration was used to control the hemorrhaging.

Anesthetized hamsters were placed in a stereotaxic apparatus to stabilize the head during surgery. After the head was shaved the surgical area was sterilized with 70% ethanol, an incision was made in the scalp. Muscle attachments were removed from the dorsal skull. After drying the skull, an incomplete circular cut was made with a dental drill burr at the λ (lambda) suture and a piece of cranium covering the pineal gland was folded forward anteriorly. Fine-tipped forceps were 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. The scalp was closed with a stainless steel surgical clips. After surgery, the incision was treated with Newskin adhesive to prevent any contamination.

2.3.2. Lipectomy

Under general anesthesia the abdominal midline was cut open and as much as possible of epididymal, perirenal, abdominal, and ventral subcutaneous fat was removed and weighed. To limit the surgical trauma, no great effort was made to remove all the visible fat. The hamsters of the control group were subjected to a similar intervention, including manipulation of the intestines, but were not lipectomized. The fascia and the skin was closed with clamps. There were no wound complications.

2.3.3. Adrenalectomy:

All adrenalectomies were done bilaterally through two dorsolateral midflank skin and muscular incisions. Sham surgeries were identical to

adrenalectomies except that the adrenals were not removed. All incisions were sutured. All hamsters were anesthetized during surgery. All hamsters that underwent adrenalectomies were provided with 0.5% saline in their drinking water.

2.3.4. Cortisol replacement:

Cortisol (sigma) was dissolved in melted beeswax (1 mg cortisol/10 mg beeswax). Then the mixture was aspirated into 15 cm lengths of PE 320 tubing (2,69 mm i.d. x 3,5 mm o.d.; intramedic, clay Adams, Parsippany, NJ). When the beeswax had cooled to room temperature and hardened, the tubing was cut into 10 mm capsules. Control capsules were prepared in a similar way but filled with beeswax only. Implants were inserted s.c. in animals under ketamine and pentobarbital anesthesia through a small dorsal skin incision at a shaved area on the back. The wound was closed by a steel wound clip.

2.3.5. Cannulation

The methods of cannulation and infusion was based on Carter and Goldman (1983). Briefly, after the skin at the back of the neck is shaved, the area was sterilized with 70% ethanol and a small incision was made to allow insertion of a subcutaneous for leptin or vehicle infusion. The cannula will consist of a 60 cm length of polyethylene tubing (PE 20). Only one cm of the tubing remained beneath the skin of the animal; the remainder was for transport purpose only. At the distal end of the cannula, 5 cm length of stainless steel tubing was fixed to prevent the cannula from being chewed. The tubing was attached to a disk of nylon mesh, saturated with Nitrofurazone that prevented the nylon mesh sticking to the animal's skin. The cannula was then secured by

a purse-string suture. Newskin adhesive was applied to the incision area to prevent any contamination. The entire cannula assembly was sterilized with 70% ethanol before use.

2.3.6. Intracerebroventricular (Icv) cannulation:

For the study involving the i.c.v. administration, cannula was implanted into the SCN. Hamsters were anaesthetized with pentobarbital sodium (50 mg/kg, i.p.) and fixed in a stereotaxic frame (Stoelting Co., IL, USA). A 22-gauge stainless steel guide cannula 313-G/Spc (Plastics One Inc., VA, USA) was implanted aseptically into SCN region (coordinates anterior +0.6 mm; lateral from midline +0,1 mm and ventral -7,8 mm; relative to bregma). The guide cannula was secured in place by dental cement (Dental Products of Turkey, Istanbul) affixed to two mounting screws. A stainless steel dummy cannula was used to occlude the guide cannula when not in use. Each cannulated hamster was then kept individually for a week to recover from surgery.

2.3.7. SCN Lesions

The running wheel rhythmicity of individually housed adult hamsters was recorded at least one week on a 24 h light-dark cycle to demonstrate entrainment. The rhythmic animals were anesthetized with ketamine and pentobarbital and placed in a stereotaxic instrument. After drilling a hole on the skull, an electrode was inserted into the SCN using stereotaxic coordinates (anterior +0.6 mm; lateral from midline +0,1 mm and ventral -7,8 mm; relative to bregma). Lesions were made by current injection (4 mA for 10 sec) through a stainless steel wire electrode insulated except for 0,3 mm at the tip. After lesioning the SCN, the hamster was returned to the running wheel cage for one

week and then were used in experiment. Locomotor activity was recorded.

Only the SCN lesioned (arrhythmic) animals were used in experimental studies.

2.4. Wheel Running Activity

Wheel running activity was recorded using the MiniMitter data acquisition system. Hamsters were housed individually in polypropylene cages, each equipped with a running wheel that closed a microswitch mounted on the outside of the cage with each revolution. Counts of wheel revolutions were stored in ten minute bins. The graphic display and analysis of activity records were done using Vital View Software. Estimates of free running period with a resolution of ± 5 min were made using the chi-square periodogram.

2.5. Testes Measurements

The length and the width were measured with calipers to the nearest 0.1 mm by external palpation of the left testis in the scrotum. In general, only one of the testes was measured, alternating sites on successive animals. However, if the testes were suspected of being disparate in size (as assessed by gentle palpation) both were measured. Paired testicular volume was calculated from this measurement using the formula for a prolate spheroid (Watson-Whitmyre and Stetson, 1985);

$$\text{Volume} = 0.5236 (\text{length}) (\text{width})^2$$

This single testis volume (STV) was converted to paired testes weight (PTW) using a pre-determined linear regression formula;

$$\text{PTW} = 1.846 (\text{STV}) - 0.015$$

All data from in situ testicular measurements were reported in the form of paired testes weights derived by this method.

2.6. Injections

Leptin (4µg/kg) and melatonin (10 mg/kg) hormones were injected intraperitoneally to the animals.

2.7. Infusions

Animals were housed singly in plastic cages. A plastic syringe guard was secured above the cage. A small hole was drilled in syringe guard through which the cannula was passed and loosely knotted above to prevent the distal cannula from becoming too slack. The proximal end of the cannula was attached to a 1-ml syringe filled with vehicle, melatonin or leptin solutions. Melatonin solutions were made in absolute ethanol and diluting this mixture in saline (0,9% NaCl) to the desired concentrations. Leptin solutions were made in saline (0,9% NaCl). Stock solutions were kept at 4 C⁰ prior to use. Fresh working solutions were made by diluting stock with sterile saline to the desired concentration. Vehicle solution was made in the ratio of one part absolute ethanol to 1000 parts saline. To aid refilling of the syringes during the experiment, a three-way stopcock was inserted between each syringe and 25-gauge needle. The syringes were mounted onto an infusion pump modified to deliver fluid to ten animals simultaneously through 10 separate 1 ml syringes. This pump was controlled by an electronic timer so infusions of controlled duration can be administered at specific times of the day. The infusion apparatus, including the syringe pumps and the polyethylene tubing up to the flow-through swivels were covered with aluminum foil as a precautionary measure against light exposure and subsequent degradation of melatonin.

2.8. Stereological analyses of the cerebral cortex

Stereological analyses were performed to measure total volume of cerebral cortex, the numerical density and total number of cortical neurons. The cerebral neocortex was defined as all six layered regions of the cerebral cortex according to established boundary criteria. The total volume of the neocortex was measured using Cavalieri's direct estimator. Sections were examined with an Olympus compound microscope and visualized on a monitor. The outline of the cerebral cortex was traced and its area was measured in μm^2 . The total volume of the cerebral cortex was calculated from $V = \sum A \times T \times 4$, where $\sum A$ is the sum of the area measurements, T is the section thickness, and 4 is the periodicity of the section sample.

The numerical density of neurons in the cerebral cortex was determined by directly counting. Seven sections that were equally spaced along caudal-to-rostral extend of the cerebral hemispheres were selected from the serial sections for analysis. A random set of sampling points were generated over the neocortex on each section using a equally divided transperant page.

Approximately 30 points were sampled on each section. Sections were examined at each sampling point using a 100x oil-immersion objective. For a given sampling point, a square counting frame measuring 30 μm on a side was positioned approximately 3 μm from the section surface and moved through 10 μm of section thickness in the Z axis plane. Nuclei of neurons were counted that came in the focus entirely within the counting frame or were intersected by its inclusion edges. Approximately 400-500 neurons were counted per animal. The numerical density of neurons was calculated using the equation $N_v = \sum Q-$

$\sum V_{Dis}$, where $\sum Q$ - is the sum of the neurons counted and $\sum V_{Dis}$ is the sum of the dissector volumes. Estimates of neuronal numerical density were used to calculate the total number of neurons in the cerebral cortex of each animal.

2.9. Hormone Measurement

Blood samples from each group were obtained for leptin measurements. Blood (approx. 0,5-1 ml) was taken from the orbital sinus of each animal under light ether anesthesia. Samples during dark phase were taken under light ether anesthesia using dim red light. To prevent the loss of circulating plasma volume, 0,9 % NaCl was injected immediately after each blood collection in the same volume as that drawn. NaCl replacement solution was sterilized and warmed to body temperature prior to replacement. Blood samples were centrifuged at 4 °C for 30 minutes at 4000 rpm. Serum aliquots were aspirated and frozen at -20°C. Hormones were measured by commercial ELISA kits according to the manufacturer's instructions (ICN, CA, USA). Optical densities were determined at 450 nm in an automatic microplate reader. Serum concentrations of hamster leptin were measured in duplicate, with a lower detection limit of 0.5 ng/ml. The intra and inter-assay coefficient of variation (CV) were less than 10%.

2.10. Statistics

Data were analyzed using SPSS (SPSS Statistical Software, SPSS Inc., Los Angeles, CA, USA, Ver. 11.0). Testes and body weights were analyzed using a repeated measures two-way analysis of variance (ANOVA). Hormone levels were analyzed by one way analysis of variance followed by Duncan's

multiple range test. Differences between means within or between groups were determined by t-tests. Values were considered statistically significant at $p < 0,05$. Data were presented as mean $\pm$ SEM.

2.11. STUDIES

2.11. 1. STUDY - 1

In mammals the biological clock is located at the base of the anterior hypothalamus in the suprachiasmatic nuclei. The SCN plays a key role in the induction, synchronization and entrainment to the environmental light/dark cycle of circadian rhythms in behavioral, metabolic and endocrine functions. Disappearance, however of the diurnal leptin rhythm after ablation of the SCN does not prove a direct clock control of leptin release, because in these SCN ablated animals other rhythmic signals like feeding have also become arrhythmic. Therefore, to investigate separately the respective roles of the SCN, feeding rhythm in the control of rhythmicity, we planned to determine daily plasma leptin rhythms in three experiments.

Experiment 1: This experiment tested whether the rhythmic release of leptin hormone is controlled by suprachiasmatic nucleus and investigated the possible effects of photoperiod on this control. Therefore, possible changes in leptin rhythm after SCN lesion was investigated. Before the experimental start, male hamsters were checked for rhythmicity and only the rhythmic animals were used in experiment. Two groups were established. Animals were exposed to long (LD 14:10) and short photoperiod (LD10:14). Pre-lesioned blood samples were collected at four different time points. Then, experimental group (n=20)

was lesioned and control group (n=10) was sham-lesioned. The success of lesion was determined by locomotor activity system. Blood samples were collected at four different times (06:00, 12:00, 18:00 or 24:00 hr) from lesioned-animals. Serum leptin profiles were determined. In order to evaluate the success of the lesions, brain (SCN) histology sections were prepared and examined.

Experiment 2: This experiment investigated the relationship between SCN and day-night feeding activity. By controlled feeding, we could check whether leptin hormone is directly controlled by suprachiasmatic nucleus or by the feeding time. In fact, it is not even clear whether the diurnal plasma leptin rhythm is under direct control of the biological clock or, conversely, whether it is a mere consequence of circadian rhythms in hormone release and/or behaviour. Meal shifts entrain the diurnal rhythm of leptin secretion, suggesting that daytime feeding contributes to the nocturnal increase in plasma leptin levels. This feeding effect would be in accordance with the acute effect of fasting and insulin on leptin secretion. On the other hand, diurnal leptin release was maintained during prolonged hyperinsulinemia in normal human subjects, indicating that factors other than feeding behaviour must also be involved.

Male adult Syrian hamsters in 14L were used in the experiment. Before the experimental start, hamsters were checked for rhythmicity and only the rhythmic animals were used in experiment. There were three groups;

Group 1: Intact male Syrian hamsters (n=20) were exposed to 14L photoperiod.

i) Animals were fed ad libitum ONLY at day time (food was given at 0600 and taken back at 2000) for one month. Weekly blood samples were collected at day (12:00) and night (00:00) and serum leptin profiles were determined.

ii) The animals were fed ad libitum ONLY at night (food was given at 2000 and taken back at 0600) for one month. Weekly blood samples were collected at day (12:00) and night (00:00) and serum leptin profiles were determined.

Group 2: Same animals in group 1 were SCN lesioned.

i) SCN-lesioned animals were fed ad libitum ONLY at day time for one month. Weekly blood samples were collected at day (12:00) and night (00:00) and serum leptin profiles were determined.

ii) SCN-lesioned animals were fed ad libitum ONLY at night for one month. Weekly blood samples were collected at day (12:00) and night (00:00) and serum leptin profiles were determined.

Group 3: In this group, feeding time were shifted between groups therefore animals which were fed at day were fed at night and animals which were fed at night were fed at day time for one month. Weekly blood samples were collected at day (12:00) and night (00:00) and leptin profile were determined.

Experiment 3: This experiment investigated the relationship between SCN and the adrenal cortex. By constant glucocorticoid release, we could check whether leptin hormone is directly controlled by suprachiasmatic nucleus or by the glucocorticoid release. For this aim, male Syrian hamsters were first adrenalectomized and then glucocorticoid implants were placed to these animals for a constant release. Control animals were sham adrenalectomized and they were implanted with empty tubes. Blood samples from experiment and control

hamsters were collected before and after the surgical operations and they were evaluated for their leptin levels.

STUDY - 2

In this study, the role of pineal gland and its hormone melatonin were investigated on the rhythmic release of leptin hormone in 14L photoperiod. The inverse relationship between leptin and melatonin hormones has already been shown. We suggested that timed melatonin infusion may suppress the leptin hormone release. On the other hand, it is not known whether the time of melatonin action on leptin profile is important. In this study, adult male Syrian hamsters (n=10) were SCN lesioned to lift leptin rhythmicity and were pinealectomized to eliminate melatonin hormone circulation in the blood. Therefore the only source for melatonin hormone was the infused melatonin. This gave an opportunity to apply melatonin hormone at the time that we suspect hormone's suppressive role on leptin hormone. Rhythmic activity of animals was measured before the start of experiment. Only the rhythmic animals were used. At the beginning of the experiment, rhythmic hamsters were SCN lesioned. After 1 week of acclimatization arrhythmic animals were pinealectomized and cannulated for melatonin infusion. One group of hamster (n:5) was infused with 50 ng/h at 20:00-21:00 h (one hour) and another group of hamsters (n:5) were infused with 50 ng/h melatonin at 20:00-06:00 (ten hours) for 15 days. Blood samples were taken from those animals at day time (1200) and night time (2400). Serum leptin profiles were determined.

STUDY - 3

Leptin secretion is regulated in part through the daily feeding rhythm and it exerts prominent effects in various parts in the hypothalamus, including the arcuate nucleus, paraventricular nucleus and ventromedial hypothalamus. In these regions, leptin acts on glucose-sensitive neurons possibly through modulating potassium ATP channels. Interestingly, leptin receptors have also been reported in the SCN. The SCN contains glucose sensitive neurons whose activity may be modulated by potassium ATP channels, and the SCN circadian clock can be entrained by feeding a hypocaloric diet or by injections of 2-deoxyglucose, which decrease glucose availability. It became clear that the lipostatic, behavioral, neuroendocrine and the reproductive effects of leptin are mediated by the brain through binding of leptin to its hypothalamic receptors. Disappearance of the diurnal rhythmicity of circulating plasma leptin levels after ablation of the SCN proves a direct control of leptin release. The SCN circadian clock, when isolated in vitro, can be phase advanced by leptin (Prosser and Bergeron, 2003).

Taken together, these data suggest that leptin might be able to modulate SCN circadian clock activity by itself. Therefore, in this study it was aimed to investigate whether the phase of biological clock can be changed in response to leptin administration.

14-L born and kept adult male Syrian hamsters were maintained at 22 ± 2 °C and received food and tap water ad libitum. Changes in locomotor activity in response to different leptin doses were examined in LL (constant light) and DD (constant darkness) photoperiods. Three experiments were performed;

Experiment 1: Control (n=10) and leptin treated (n=20) groups were moved into LL room and allowed to entrain to this photoperiod for 1 week. Leptin groups were injected intraperitoneally with rat recombinant leptin at the doses of 2µg/hamster and 4 µg/hamster at 08:00 h whereas controls received saline (0,9% NaCl) in order to eliminate stress factors.

Experiment 2: Same experiment were performed as in experiment 1 but this time the photoperiod was constant darkness (DD). Total 30 animals (10 for control and 20 for leptin) were used.

The data were depicted as double plots (10 min intervals) and the onset of activity was determined by visual analysis on screen. The period lengths were determined by fitting linear regression lines to the activity onsets before and after injections.

Experiment 3: Control (n=10) and leptin treated (n=20) groups were moved into DD room and allowed to entrain to this photoperiod for 1 week. Leptin groups were infused subcutaneously with rat recombinant leptin at the dose of 4 µg/hamster at 08:00 h whereas controls received saline (0,9% NaCl) in order to eliminate stress factors.

Experiment 4: Control (n=10) and leptin treated (n=20) groups were moved into DD room and allowed to entrain to this photoperiod for 1 week. Leptin groups were infused intracerebroventricully with rat recombinant leptin at the dose of 0,4 µg/hamster at 08:00 h whereas controls received saline (0,9% NaCl) in order to eliminate stress factors.

Experiment 5: Animals (n:10) were SCN lesioned and moved into DD room. Leptin were infused subcutaneously at the dose of 4 µg/hamster at 17:00 h

whereas controls received saline (0,9% NaCl) in order to eliminate stress factors. The locomotor activity rhythm was reentrained by the timed one hour leptin infusions.

STUDY - 4

In rodents, circadian rhythms in behaviour and neuroendocrine function are regulated by circadian oscillations generated within the SCN of the hypothalamus. SCN can be transplanted to the brain of an adult host that has had its own SCN ablated. The transplanted SCN not only restores previously disrupted rhythmicity, but also transfers timing properties characteristic of the donor circadian pacemaker. This demonstrates that a circadian pacemaker develops within the transplanted tissue. Research on the development of circadian rhythms indicates that circadian oscillations are already generated at the time fetal tissue is transplanted. For example, postnatal behavioral rhythms in hamsters can be entrained by daily injections of hormone melatonin, given to the mother prenatally. Oscillations generated within the fetal SCN appear to be entrained by rhythmicity of the mother. The mechanisms underlying maternal entrainment during development are not understood.

In this study, maternal transfer of photoperiodic information and the effect of leptin injections on the onset of puberty were examined. This study tested whether leptin has any affect during gestation and whether leptin functions on reproductive system without SCN in the system. Two experiments were performed.

Experiment 1: Female hamsters (n:25) were paired and injected with leptin (2 µg/kg), insulin (10 µg/kg), melatonin (25 mg/kg) and saline (0,9% NaCl)

between the gestational days 8 and 15. After the weaning, the prepubertal male hamsters (n:50) were examined for their body, testes, adipose, epididymis and seminal vesicle weights. Testis histology of the hamsters will be examined.

Experiment 2 : Pregnant hamsters were injected with leptin (2 µg/hamster) between the gestational days 8 and 15. After parturition, the newborn pups (n:20, 10 for controls and 10 for leptin group) were decapitated and their brains were examined through their weight, volume and the number of the neurons.

STUDY - 5

There is a relationship among adipose tissue, pineal gland and day length. Melatonin suppresses the leptin levels. Moreover, photoperiod may regulate the the levels and the rhythms of the hormones leptin and melatonin. For examining the relationship among these three parameter, leptin hormone levels were investigated in the adult male Syrian hamsters which were pinealectomized, lipectomized and received leptin injections. Under long and short photoperiod, pinealectomy and control groups and control (n:8), leptin injection (n:8) and lipectomy (n:8) subgroups were performed. Injection groups were injected with leptin throughout two months. Weekly blood samples were collected throughout 8 weeks and they were examined for leptin by the ELISA at the end of the experiment.

3. RESULTS

3.1 STUDY 1

3.1.1 Experiment 1:

Leptin results show circadian rhythm in 14L (Fig. 1) and 10L (Fig. 2) photoperiods. SCN-intact hamsters show that leptin levels decreased in 10L when compared with the 14L photoperiod groups ($p < 0,05$). However, the rhythm of leptin hormone did not change in both photoperiods. Leptin levels were the highest at noon (1200 h) whereas the lowest at midnight (2400 h) (Table 1, 2). After SCN lesions, hamsters were placed in running wheels in both photoperiods to observe the success of the lesions. Fig. 3 shows the rhythmic locomotor activity of the SCN intact hamsters. Fig. 4 shows the arrhythmic locomotor activity in SCN lesioned hamster. In SCN lesioned hamsters, leptin rhythm was abolished. In 14L photoperiod (Fig. 5), all leptin values were similar to each other (app. 13 ng/ml). In 10L photoperiod (Fig. 6), the leptin levels were similar and they were app. 10 ng/ml.

At the end of the experiment 1, to confirm the presence of the lesions hamsters were decapitated and their brains were examined for the success of the lesions. Fig. 7 shows the intact hamster brain. Fig. 8 shows SCN lesioned hamster brain.

Table 1: Table represents the serum leptin levels in Sham SCN lesioned and SCN lesioned Syrian hamsters in 14L photoperiod.

14 L	Sham SCN_x	SCN_x
12:00	15,00 ± 0,40	13,10 ± 0,40
18:00	12,90 ± 0,40	13,30 ± 0,40
24:00	10,20 ± 0,30	12,80 ± 0,25
06:00	13,80 ± 0,40	13,15 ± 0,25

Table 2: Table represents the serum leptin levels (ng/ml) in Sham SCN lesioned and SCN lesioned Syrian hamsters in 10L photoperiod.

10 L	Sham SCN_x	SCN_x
12:00	12,10 ± 0,40	10,10 ± 0,20
18:00	10,20 ± 0,40	9,80 ± 0,20
24:00	6,50 ± 0,30	10,00 ± 0,18
06:00	10,40 ± 0,40	9,90 ± 0,18

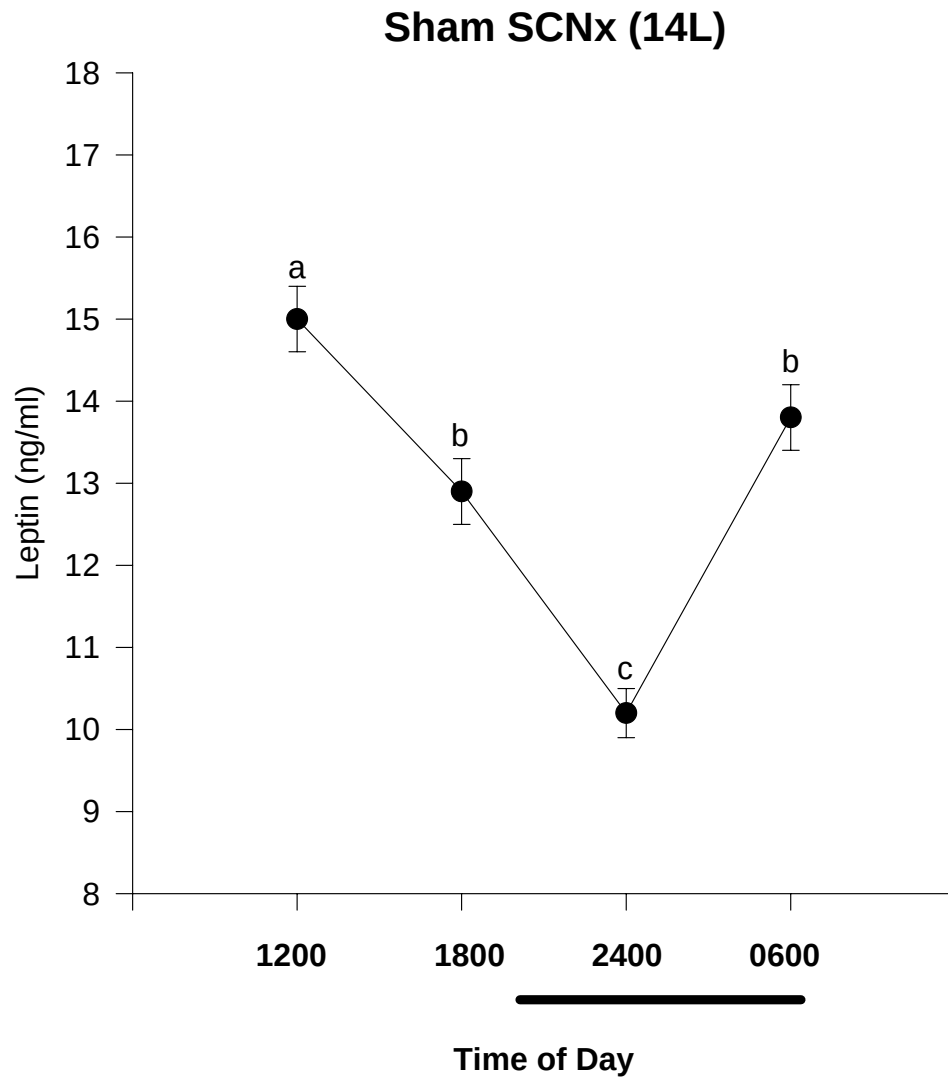


Fig. 1: Serum leptin level in sham SCN lesioned hamsters in 14L photoperiod. Blood samples were collected every 6 h from the same animal (n=10) throughout the day. Letters indicate the statistical difference. The black bar indicates the period of darkness.

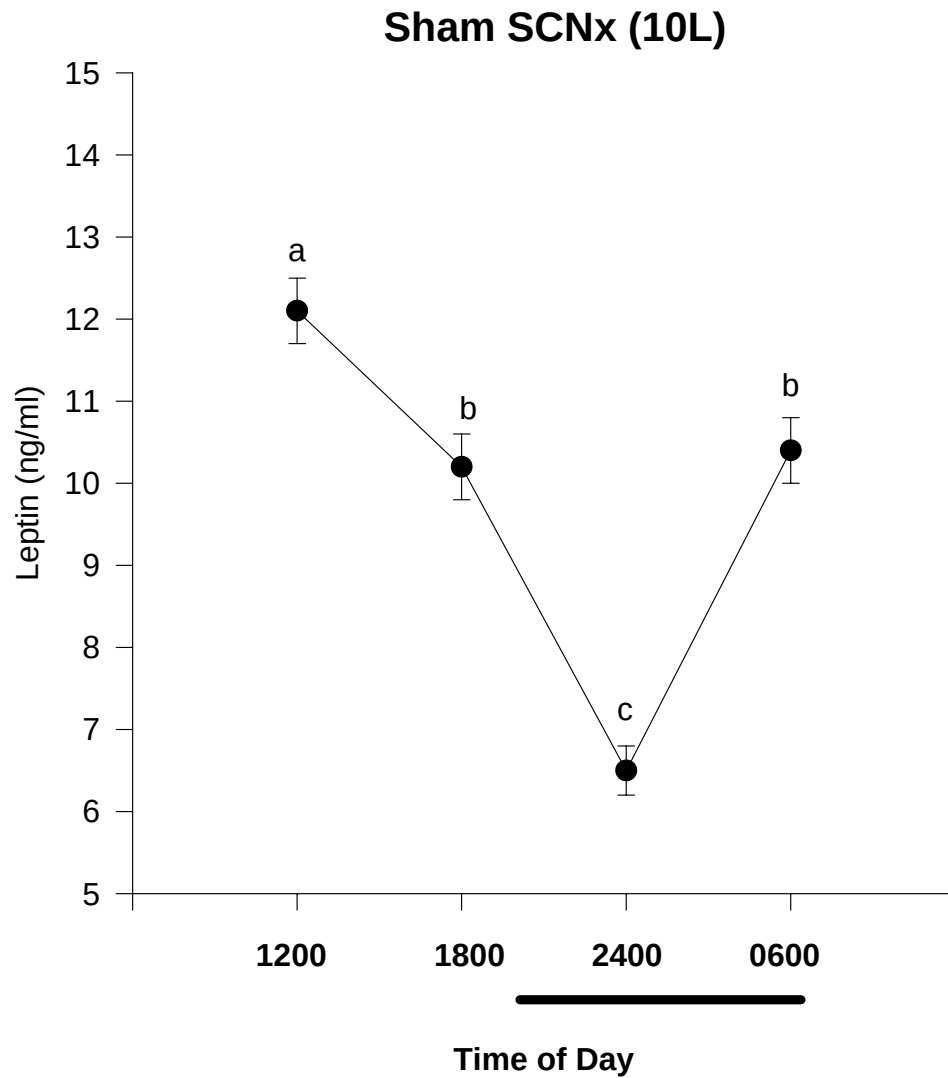


Fig. 2: Serum leptin level in sham SCN lesioned hamsters in 10L photoperiod. Blood samples were collected every 6 h from the same animal (n=10) throughout the day. Letters indicate the statistical difference. The black bar indicates the period of darkness.

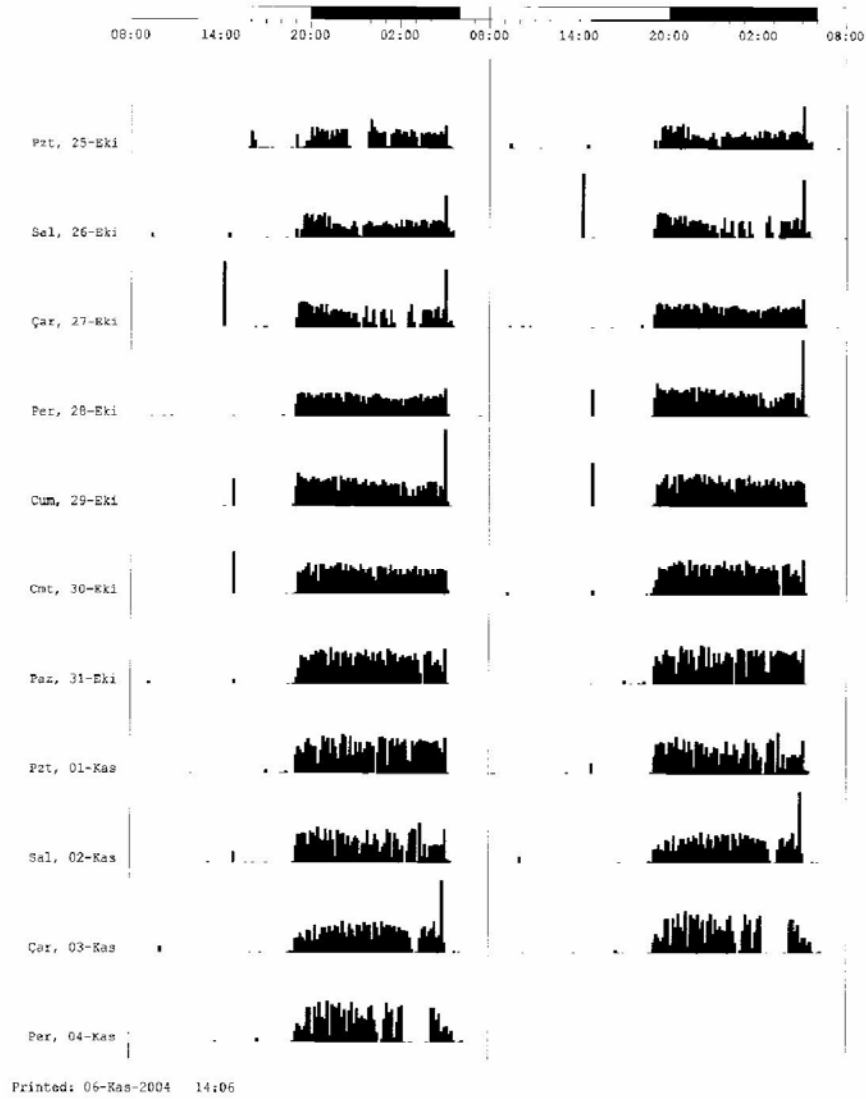


Fig. 3: The actogram of the SCN intact hamster. Note rhythmic locomotor activity.

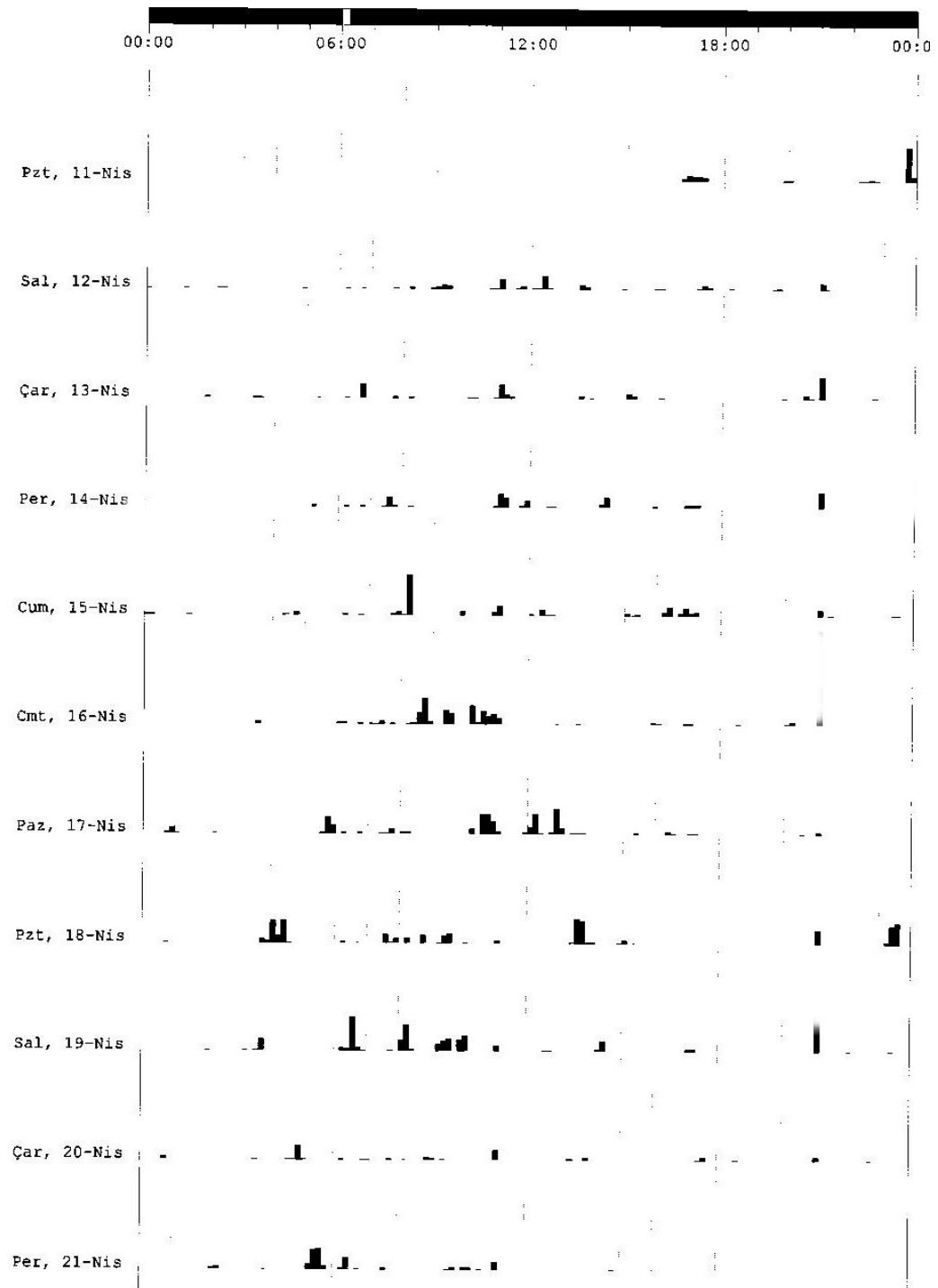


Fig. 4: The actogram of the SCN lesioned hamster. Note arrhythmic locomotor activity.

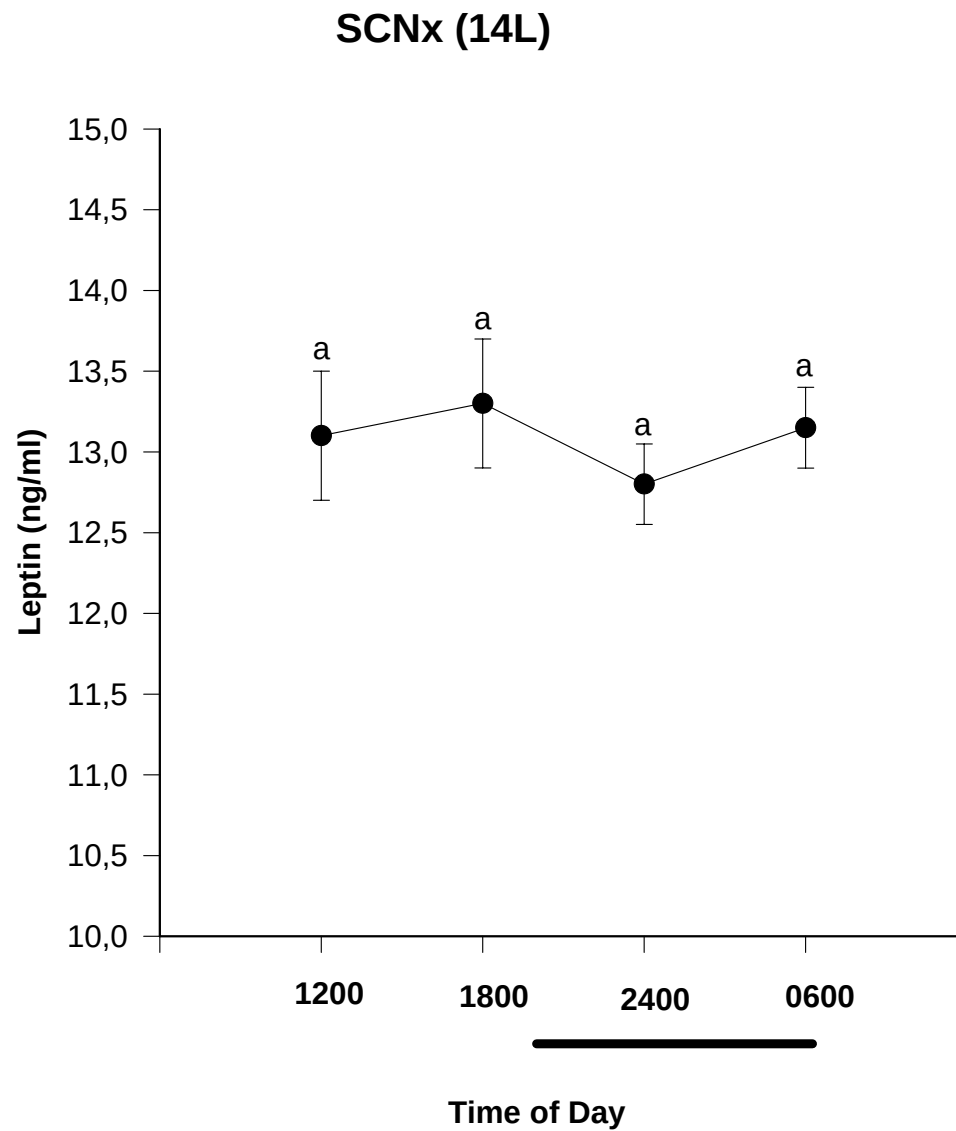


Fig. 5: Serum leptin level in SCN lesioned hamsters in 14L photoperiod. Blood samples were collected every 6 h from the same animal (n=10) throughout the day. Letters indicate the statistical difference. The black bar indicates the period of darkness.

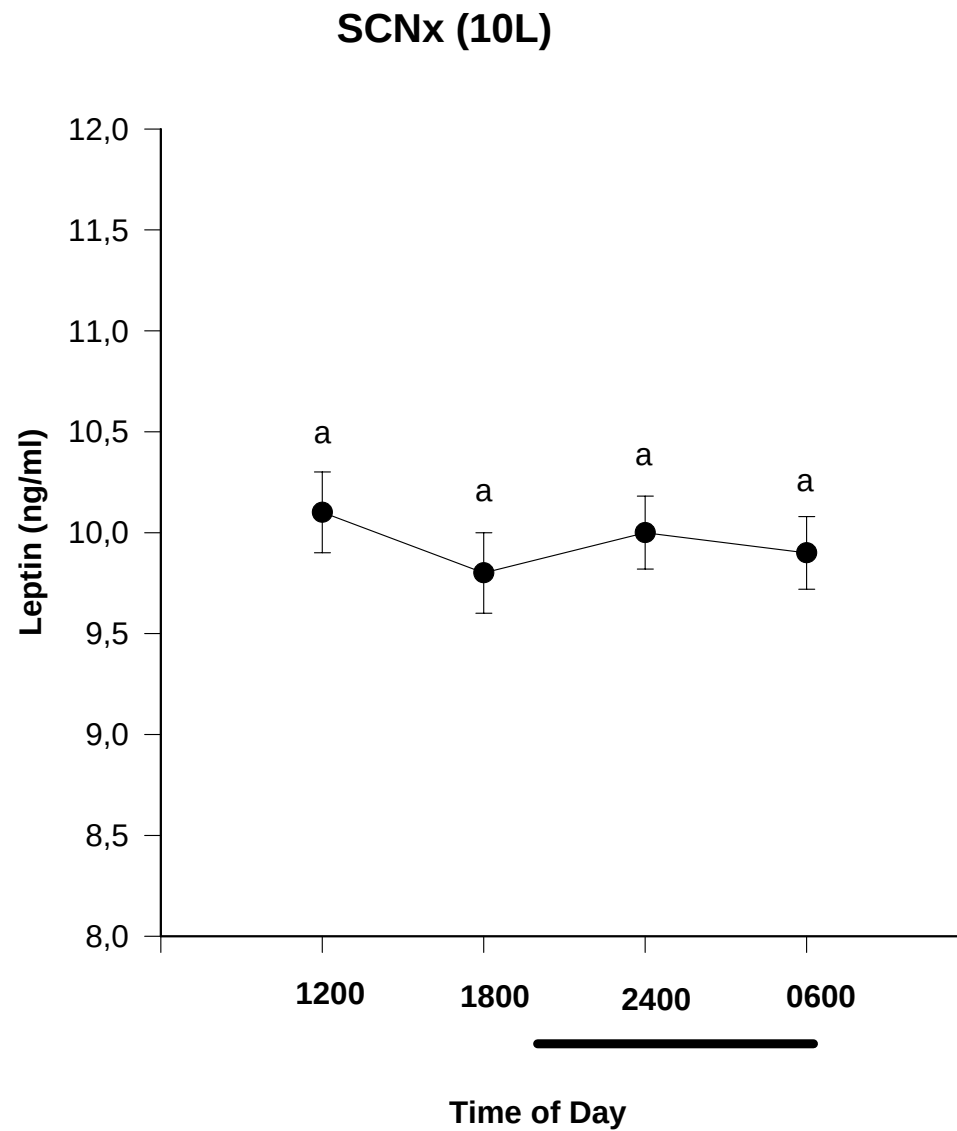


Fig. 6: Serum leptin level in SCN lesioned hamsters in 10L photoperiod. Blood samples were collected every 6 h from the same animal (n=10) throughout the day. Letters indicate the statistical difference. The black bar indicates the period of darkness.

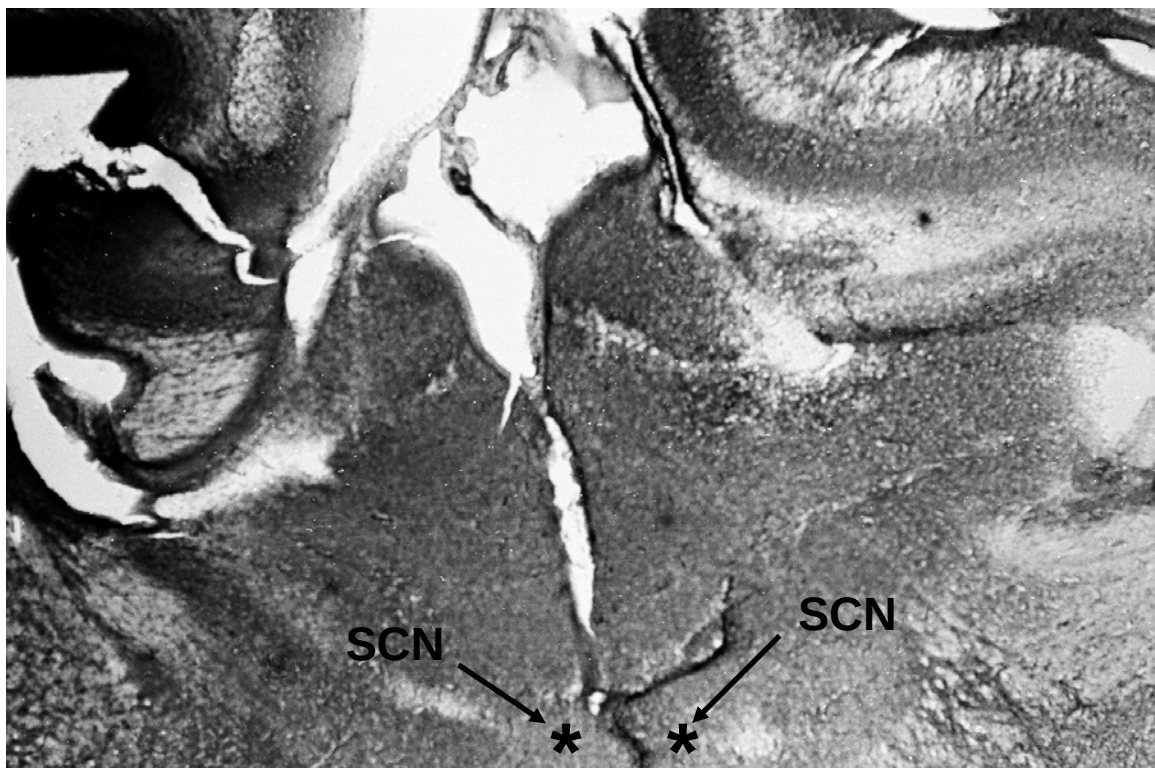


Fig. 7: The coronal section of the intact hamster brain. Note presence of the SCN regions. (X 400)

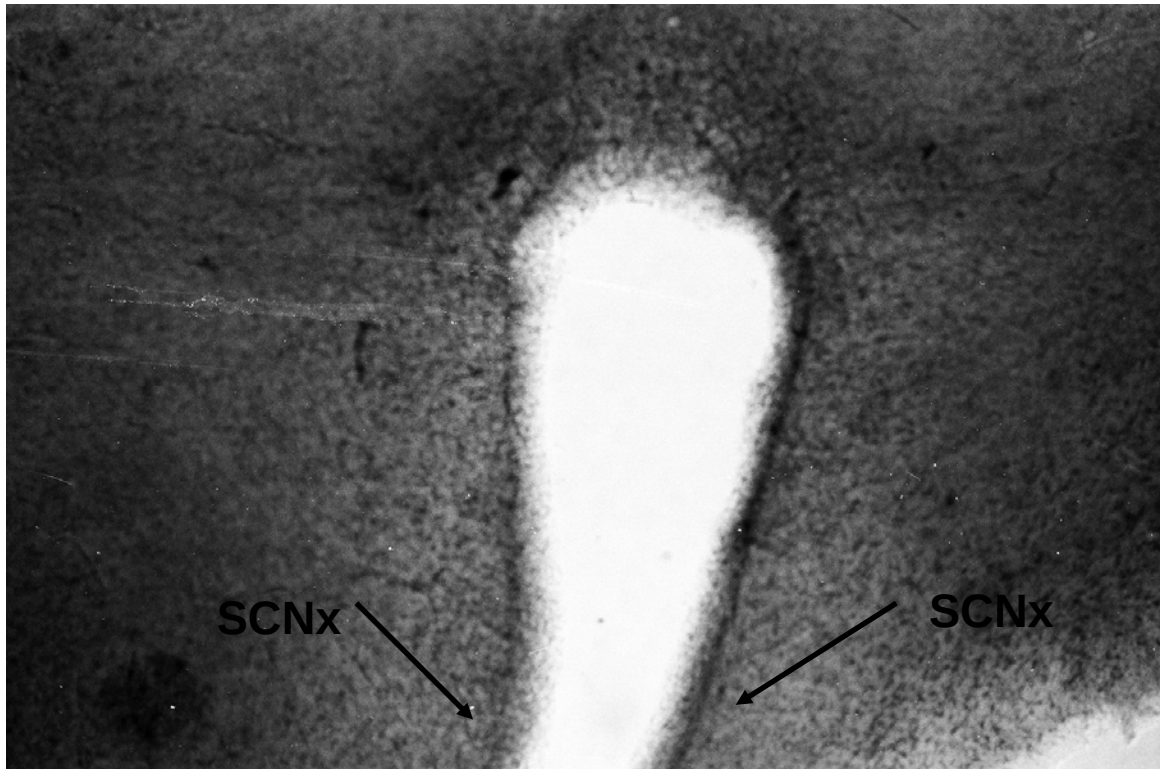


Fig. 8: The coronal section of the partially SCN lesioned hamster brain. Note absence of the SCN regions. (X 600)

3.1.2 Experiment 2:

Grup 1:

i) Table 3 shows leptin values in day-time fed animals. When animals were fed only at the day time, leptin levels at 1200h and 2400 h did not change during the course of the experiment ($p>0,05$). On the other hand, the day time (1200 h) and the night time 2400 h leptin levels were different from each other ($p<0,05$). Leptin levels were higher at day time than that of night time levels (Fig. 9).

ii) Table 4 shows leptin values in night-time fed animals. When animals were fed only night time, leptin levels did not change during the course of the experiment ($p>0,05$). On the other hand, the day time and the night time leptin levels were different from each other ($p<0,05$). Leptin levels were higher at day time than that of night time levels (Fig 10).

Feeding regimes did not change the rhythm of the leptin release.

Table 3: Table represents the serum leptin levels (ng/ml) in Syrian hamsters fed only day time for one month in 14L photoperiod.

Weeks	Day Leptin Mean $\pm$ SE	Night Leptin Mean $\pm$ SE
1.	13,40 $\pm$ 0,40	8,30 $\pm$ 0,21
2.	12,80 $\pm$ 0,35	7,80 $\pm$ 0,20
3.	14,60 $\pm$ 0,41	9,10 $\pm$ 0,25
4.	13,50 $\pm$ 0,32	8,60 $\pm$ 0,20

Table 4: Table represents the serum leptin levels (ng/ml) in Syrian hamsters fed only night time for one month in 14L photoperiod.

Weeks	Day Leptin Mean $\pm$ SE	Night Leptin Mean $\pm$ SE
1.	12,50 $\pm$ 0,90	9,50 $\pm$ 0,90
2.	11,70 $\pm$ 0,80	8,60 $\pm$ 0,80
3.	13,50 $\pm$ 0,90	10,20 $\pm$ 0,90
4.	11,80 $\pm$ 1,10	9,70 $\pm$ 0,80

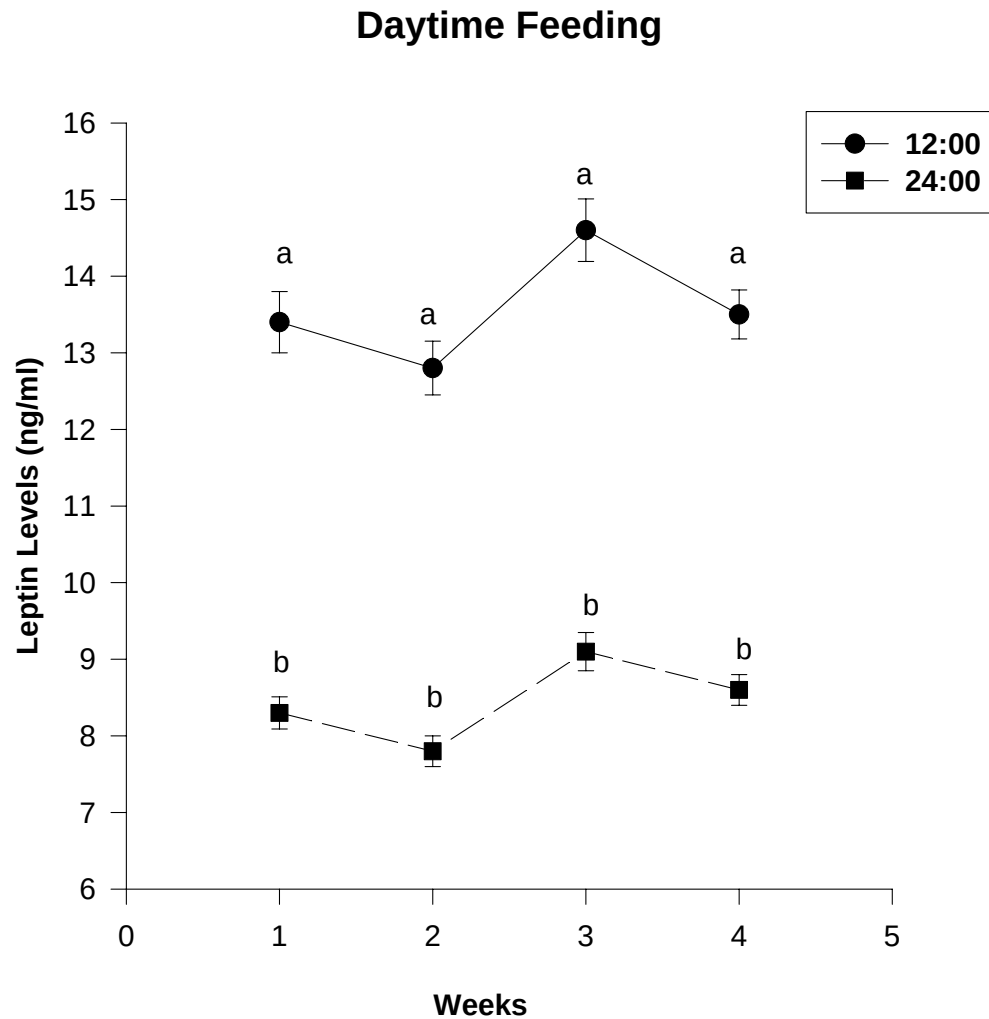


Fig. 9: Serum leptin level in day time fed hamsters in 14L photoperiod. Blood samples were collected every 6 h from the same animal (n=10) throughout the day. Letters indicate the statistical difference. The black bar indicates the period of darkness.

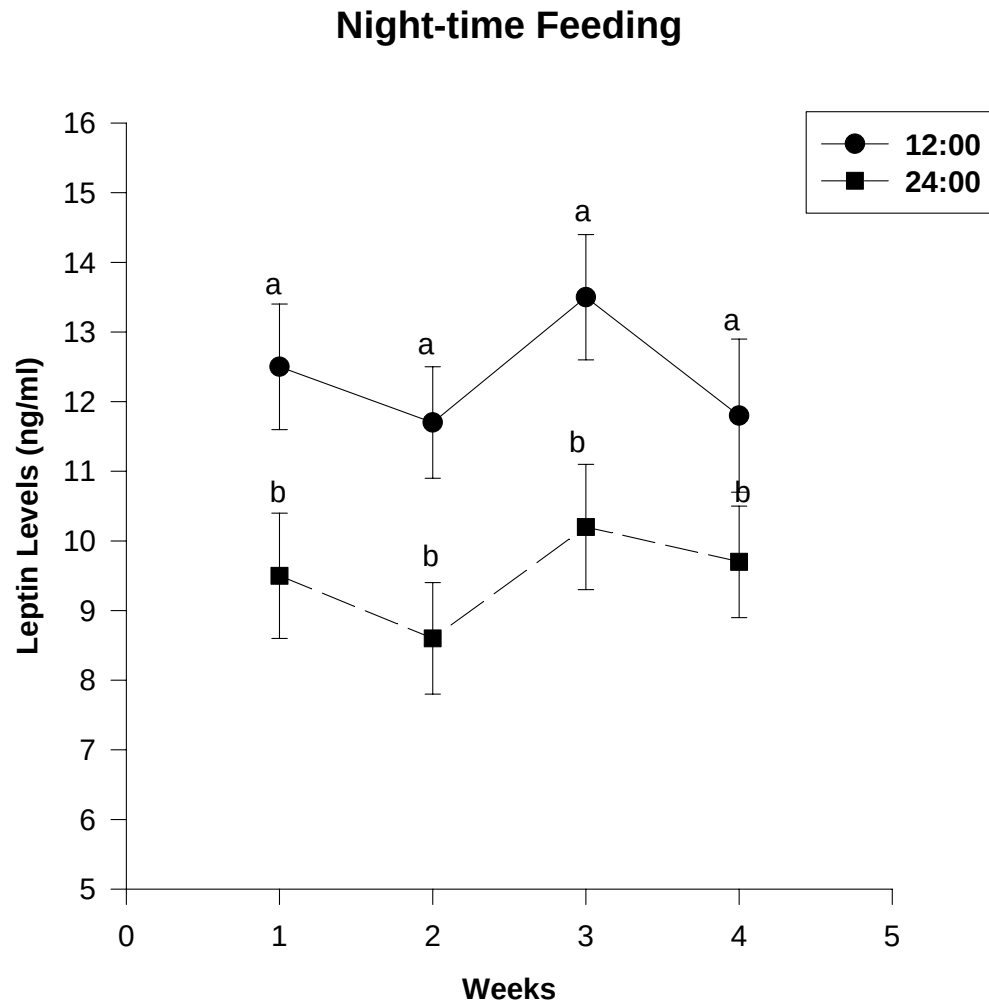


Fig. 10: Serum leptin level in night time fed hamsters in 14L photoperiod. Blood samples were collected every 6 h from the same animal (n=10) throughout the day. Letters indicate the statistical difference. The black bar indicates the period of darkness

Grup 2:

i) Table 5 shows leptin values in day-time fed with SCN lesioned animals.

SCN lesion abolished the rhythm of leptin release. When animals were fed only at the day time, leptin levels at 1200h and 2400 h did not change during the course of the experiment ($p>0,05$) (Fig. 11). Day time and night time leptin levels were very close to each other ($p>0,05$).

ii) Table 6 shows leptin values in night-time fed with SCN lesioned animals.

SCN lesion abolished the rhythm of leptin release. When animals were fed only night time, leptin levels did not change during the course of the experiment ($p>0,05$) (Fig. 12). Day time and night time leptin levels were very close to each other.

Table 5: Table represents the serum leptin levels (ng/ml) in SCNx Syrian hamsters fed only day time for one month in 14L photoperiod.

Weeks	Day Leptin Mean $\pm$ SE	Night Leptin Mean $\pm$ SE
1.	12,30 $\pm$ 0,50	11,40 $\pm$ 0,50
2.	11,70 $\pm$ 0,40	11,80 $\pm$ 0,40
3.	11,50 $\pm$ 0,40	12,10 $\pm$ 0,50
4.	11,90 $\pm$ 0,50	11,90 $\pm$ 0,80

Table 6: Table represents the serum leptin levels (ng/ml) in SCNx Syrian hamsters fed only night time for one month in 14L photoperiod.

Weeks	Day Leptin Mean $\pm$ SE	Night Leptin Mean $\pm$ SE
1.	13,30 $\pm$ 0,50	12,40 $\pm$ 0,40
2.	13,80 $\pm$ 0,35	12,80 $\pm$ 0,35
3.	13,60 $\pm$ 0,38	13,00 $\pm$ 0,32
4.	13,50 $\pm$ 0,35	12,90 $\pm$ 0,34

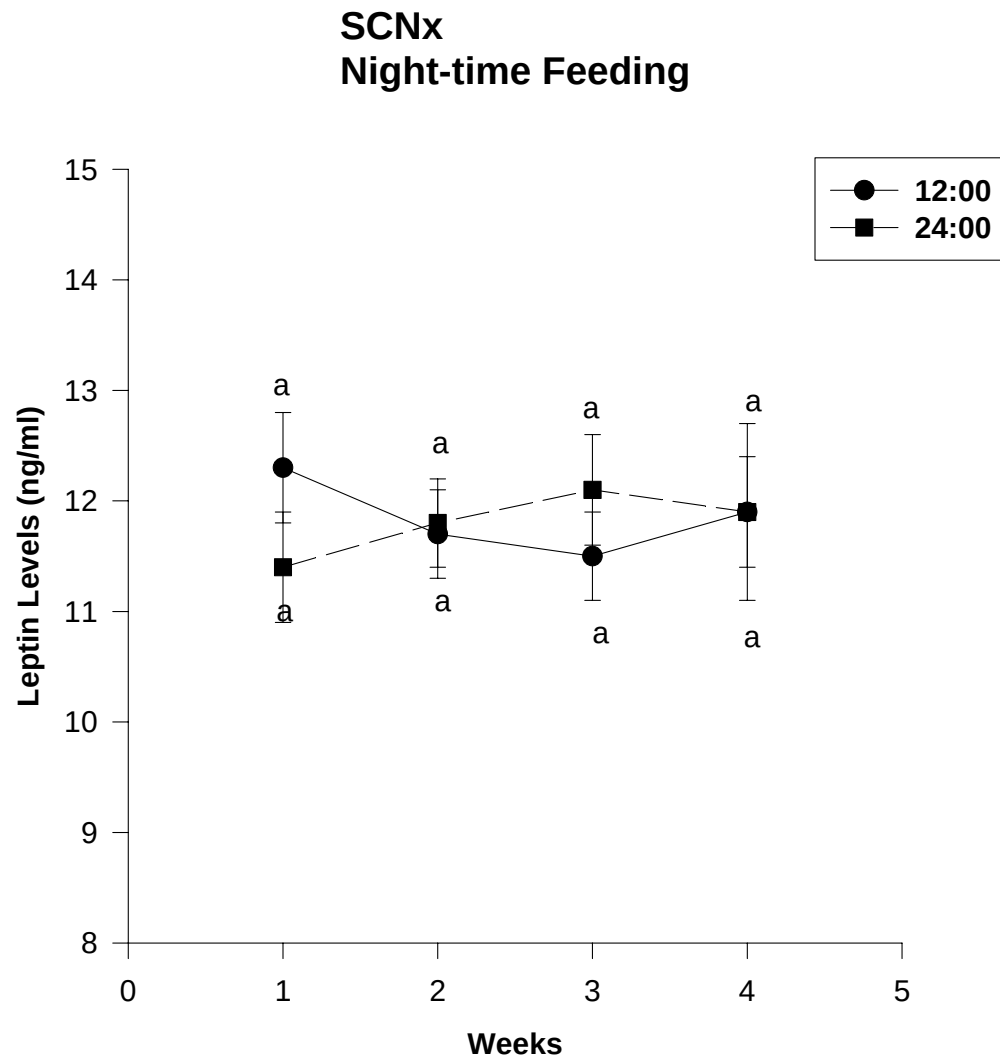


Fig. 11: Serum leptin level in night time fed SCN lesioned hamsters in 14L photoperiod. Blood samples were collected every 6 h from the same animal (n=10) throughout the day. Letters indicate the statistical difference. The black bar indicates the period of darkness.

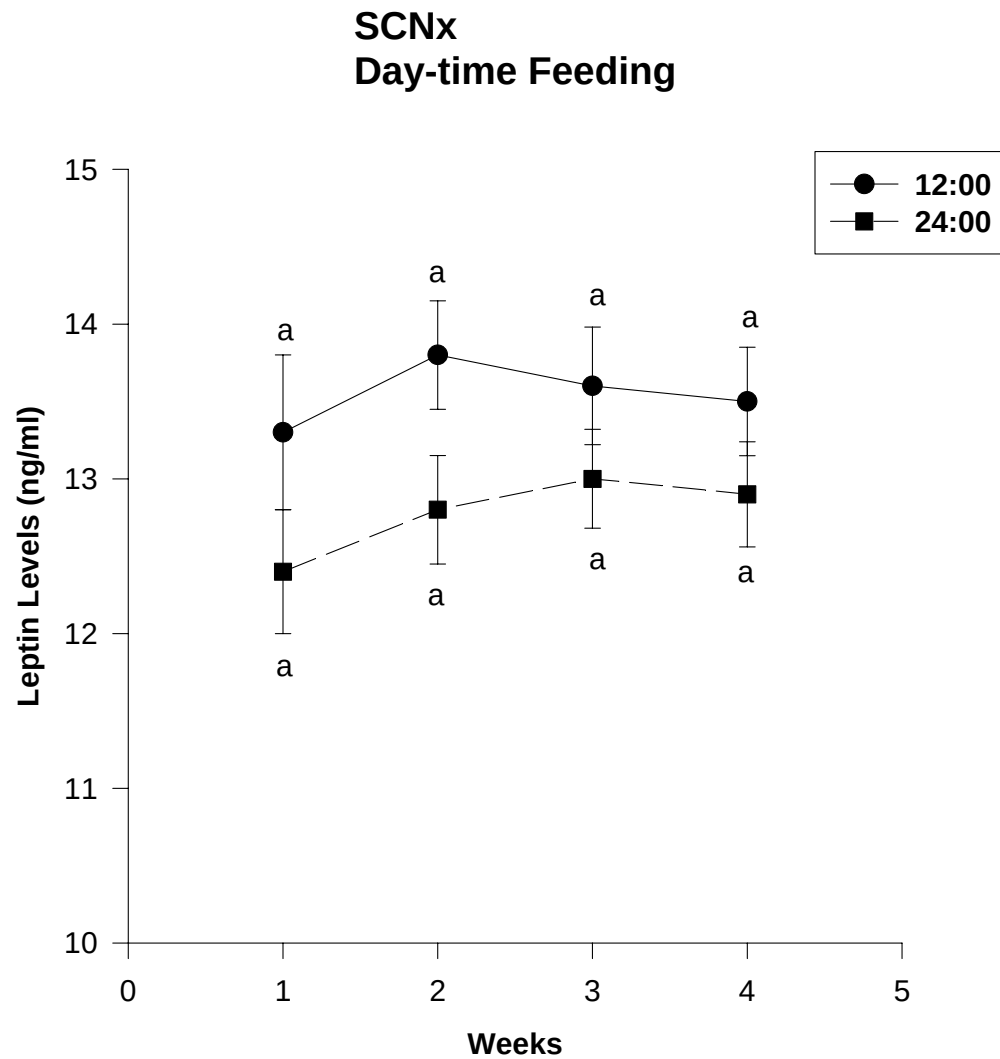


Fig. 12: Serum leptin level in day time fed SCN lesioned hamsters in 14L photoperiod. Blood samples were collected every 6 h from the same animal (n=10) throughout the day. Letters indicate the statistical difference. The black bar indicates the period of darkness.

Grup 3:

i) Table 7 shows leptin values in animals shifted from day-time to night-time feeding schedule. The shift in feeding time from day to night time did not change the rhythm of leptin release ($p > 0,05$). The day time (1200 h) leptin levels were higher than that of the night time (2400 h) leptin levels throughout the four weeks ($p < 0,05$) (Fig. 13).

ii) Table 8 shows leptin values in animals shifted from night-time to day-time feeding schedule. The shift in feeding time from night to day time did not change the rhythm of leptin release ($p > 0,05$) (Fig. 14). The day time (1200 h) leptin levels were higher than that of the night time (2400 h) leptin levels throughout the four weeks ($p < 0,05$).

Table 7: Table represents the serum leptin levels (ng/ml) in Syrian hamsters shifted in feeding time from day time to night time for one month in 14L photoperiod.

Weeks	Day Leptin Mean $\pm$ SE	Night Leptin Mean $\pm$ SE
1.	14,20 $\pm$ 0,60	8,45 $\pm$ 0,40
2.	13,90 $\pm$ 0,45	8,70 $\pm$ 0,48
3.	14,10 $\pm$ 0,48	8,50 $\pm$ 0,32
4.	14,40 $\pm$ 0,45	8,20 $\pm$ 0,35

Table 8: Table represents the serum leptin levels (ng/ml) in Syrian hamsters shifted in feeding time from night time to day time for one month in 14L photoperiod.

Weeks	Day Leptin Mean $\pm$ SE	Night Leptin Mean $\pm$ SE
1.	14,20 $\pm$ 0,45	7,60 $\pm$ 0,43
2.	13,90 $\pm$ 0,32	7,70 $\pm$ 0,41
3.	14,00 $\pm$ 0,41	7,50 $\pm$ 0,40
4.	13,80 $\pm$ 0,35	7,60 $\pm$ 0,40

Shift from Day-time to Night-time

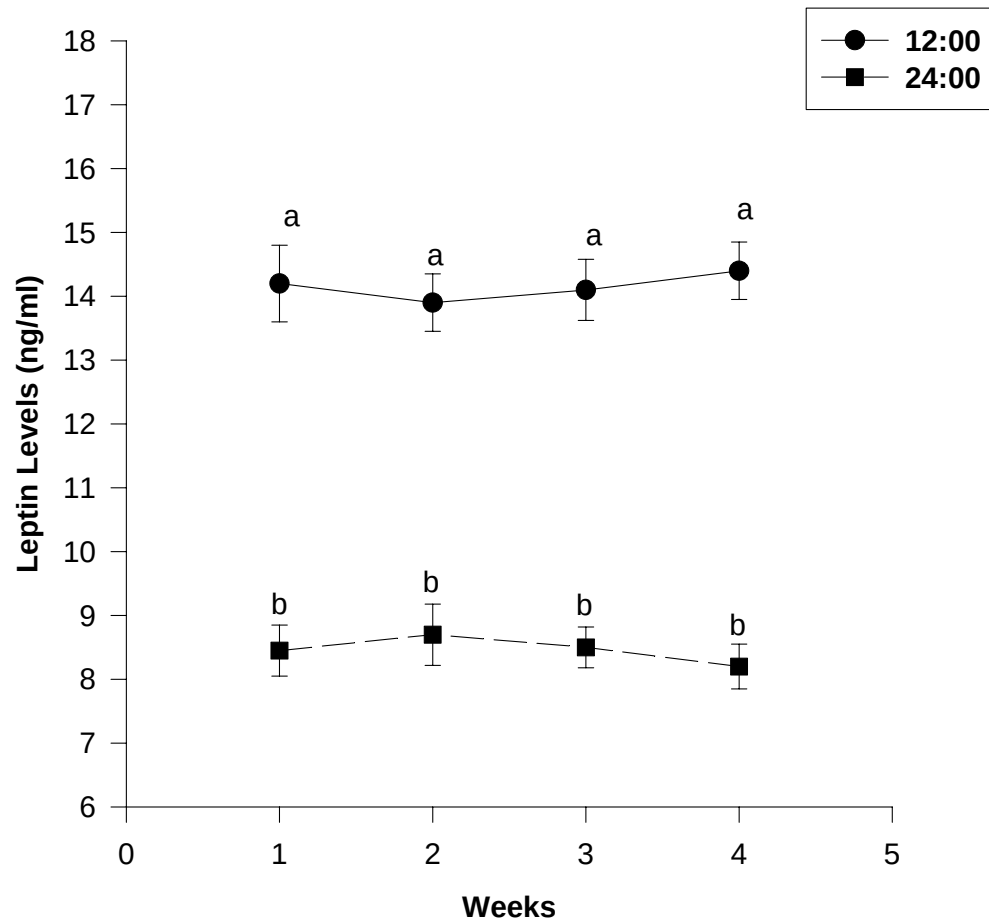


Fig. 13: Serum leptin level in feeding schedule shifted from day to night time hamsters in 14L photoperiod. Blood samples were collected every 6 h from the same animal (n=10) throughout the day. Letters indicate the statistical difference. The black bar indicates the period of darkness.

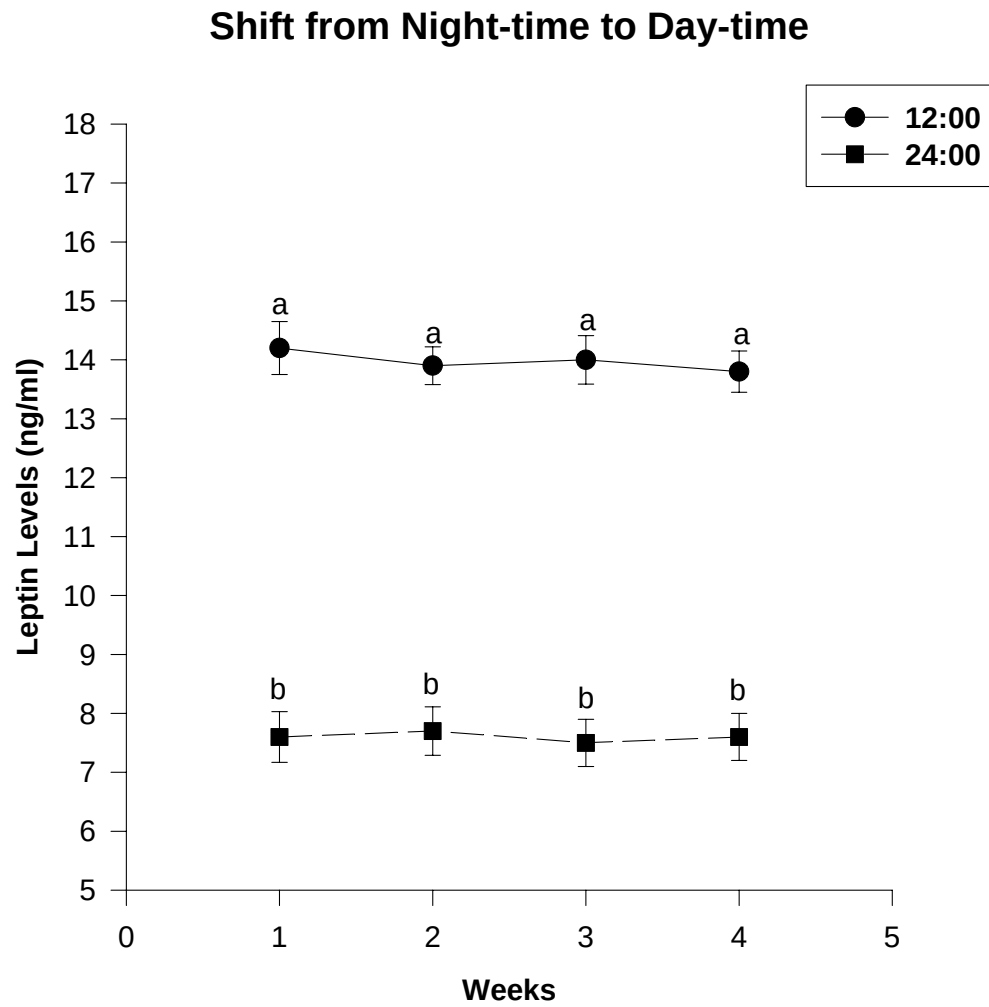


Fig. 14: Serum leptin level in feeding schedule shifted from night to day time hamsters in 14L photoperiod. Blood samples were collected every 6 h from the same animal (n=10) throughout the day. Letters indicate the statistical difference. The black bar indicates the period of darkness.

3.1.3 Experiment 3:

Leptin levels of the sham adrenalectomized and adrenalectomized hamsters were given in Table 9. The results have shown that the rhythm of leptin release was the same in both experimental groups (Fig. 15 and Fig. 16). Constant release of glucocorticoid did not change the leptin rhythm ($p>0,05$) (Fig. 16). Leptin levels were higher at day time than night time levels in both sham adrenalectomized and adrenalectomized hamsters.

Table 9: Table represents the serum leptin levels (ng/ml) in Syrian hamsters implanted with glucocorticoid and empty implants in 14L photoperiod.

	Sham ADx + Empty Implant	ADx + Glucocorticoid Implant
12:00	14,20 ± 0,40	15,50 ± 0,50
18:00	12,80 ± 0,31	13,80 ± 0,40
24:00	9,50 ± 0,21	10,40 ± 0,30
06:00	13,30 ± 0,38	14,20 ± 0,40

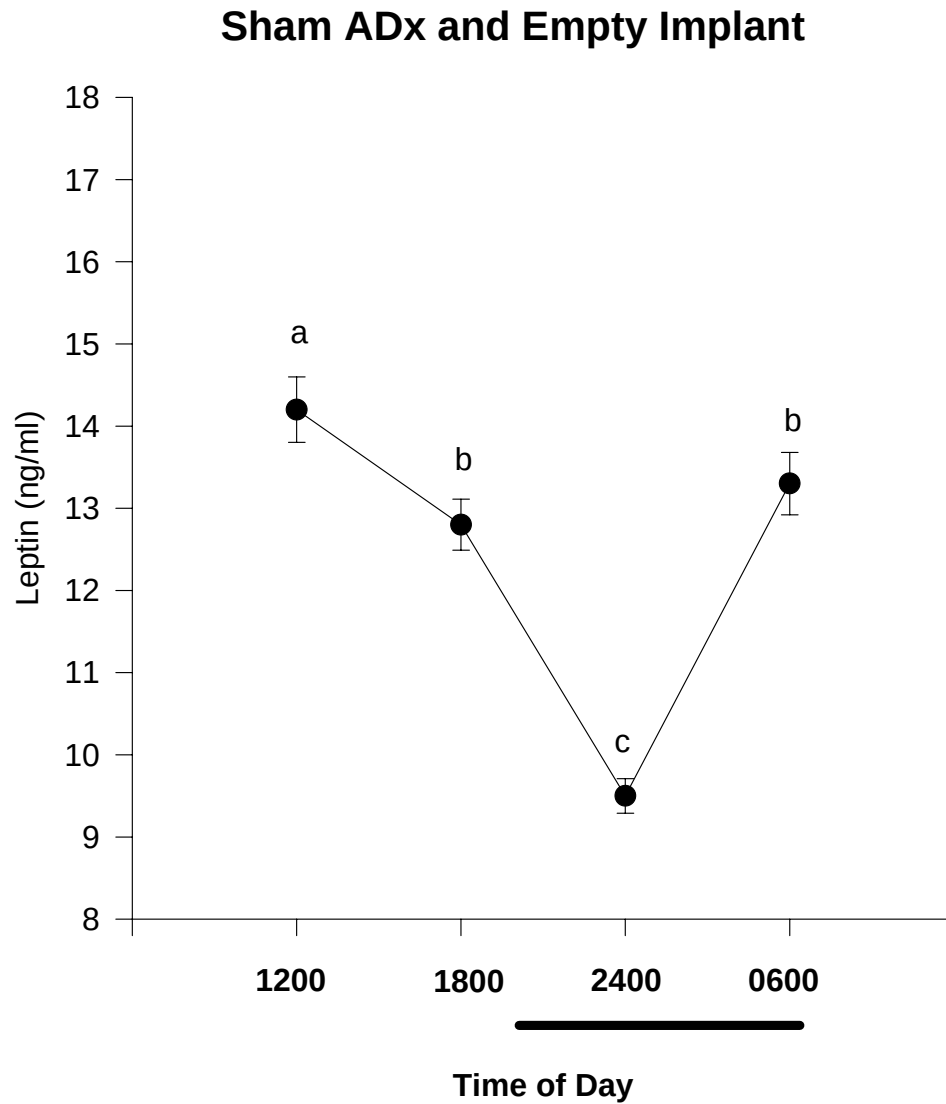


Fig. 15: Serum leptin level in adrenalectomized and implanted with empty implant hamsters in 14L photoperiod. Blood samples were collected every 6 h from the same animal (n=10) throughout the day. Letters indicate the statistical difference. The black bar indicates the period of darkness.

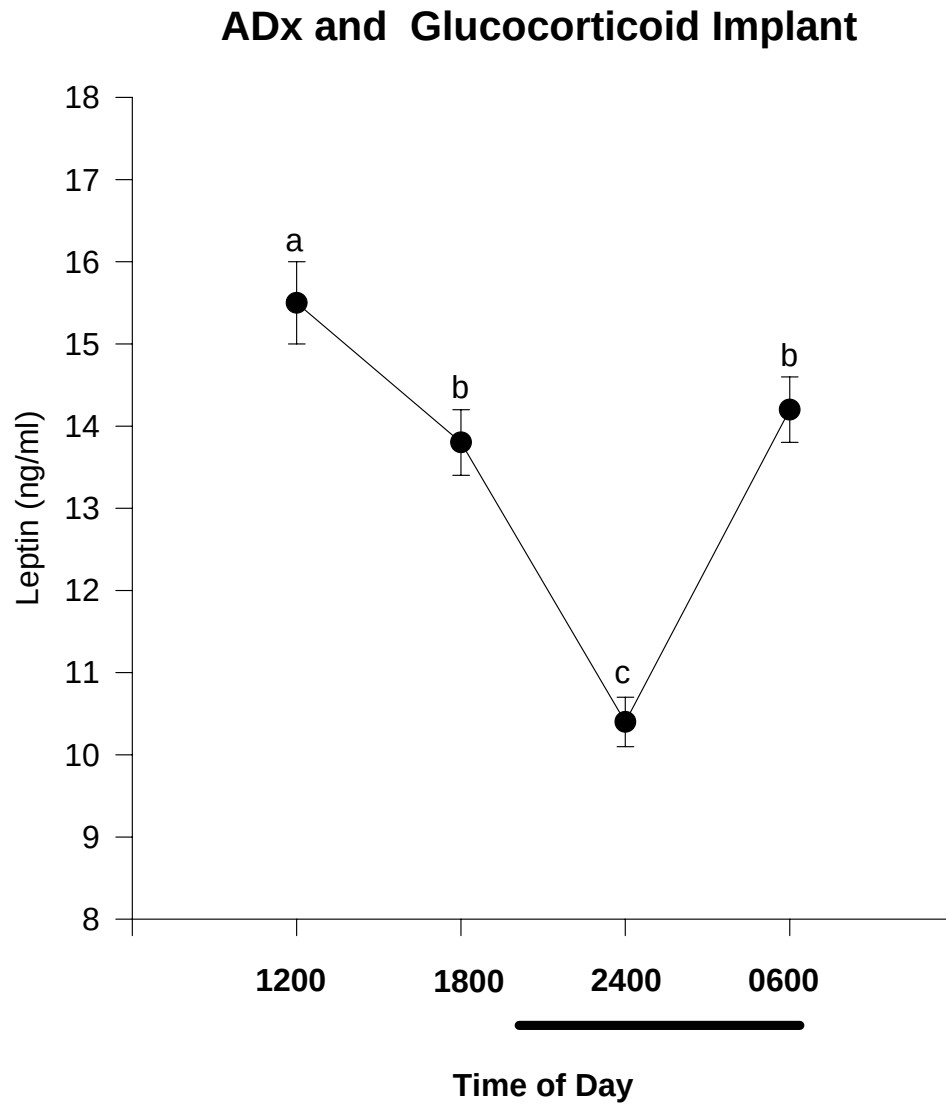


Fig. 16: Serum leptin level in adrenalectomized and implanted with glucocorticoid tubes hamsters in 14L photoperiod. Blood samples were collected every 6 h from the same animal (n=10) throughout the day. Letters indicate the statistical difference. The black bar indicates the period of darkness.

3.2 STUDY - 2

Normal leptin rhythm was abolished by the SCN lesions and pinealectomy prevented the internal synhronization. Both one and ten hour melatonin infusions suppressed the leptin levels ($p<0,05$) (Table 10). Leptin were app. 12 ng/ml in one hour melatonin infused group (Fig. 17) and app. 11 ng/ml in ten hour melatonin infused group (Fig. 18). Melatonin infusion did not reentraine the leptin rhythm.

Table 10: Table represents the serum leptin levels (ng/ml) in SCN lesioned and 1 and 10 hour melatonin infused Syrian hamsters in 14L photoperiod.

14 L	1 Hour Mel Infusion	10 hour Mel Infusion
12:00	12,40 $\pm$ 0,30	11,20 $\pm$ 0,20
18:00	12,60 $\pm$ 0,35	11,30 $\pm$ 0,20
24:00	12,00 $\pm$ 0,25	11,00 $\pm$ 0,18
06:00	12,50 $\pm$ 0,25	11,30 $\pm$ 0,17

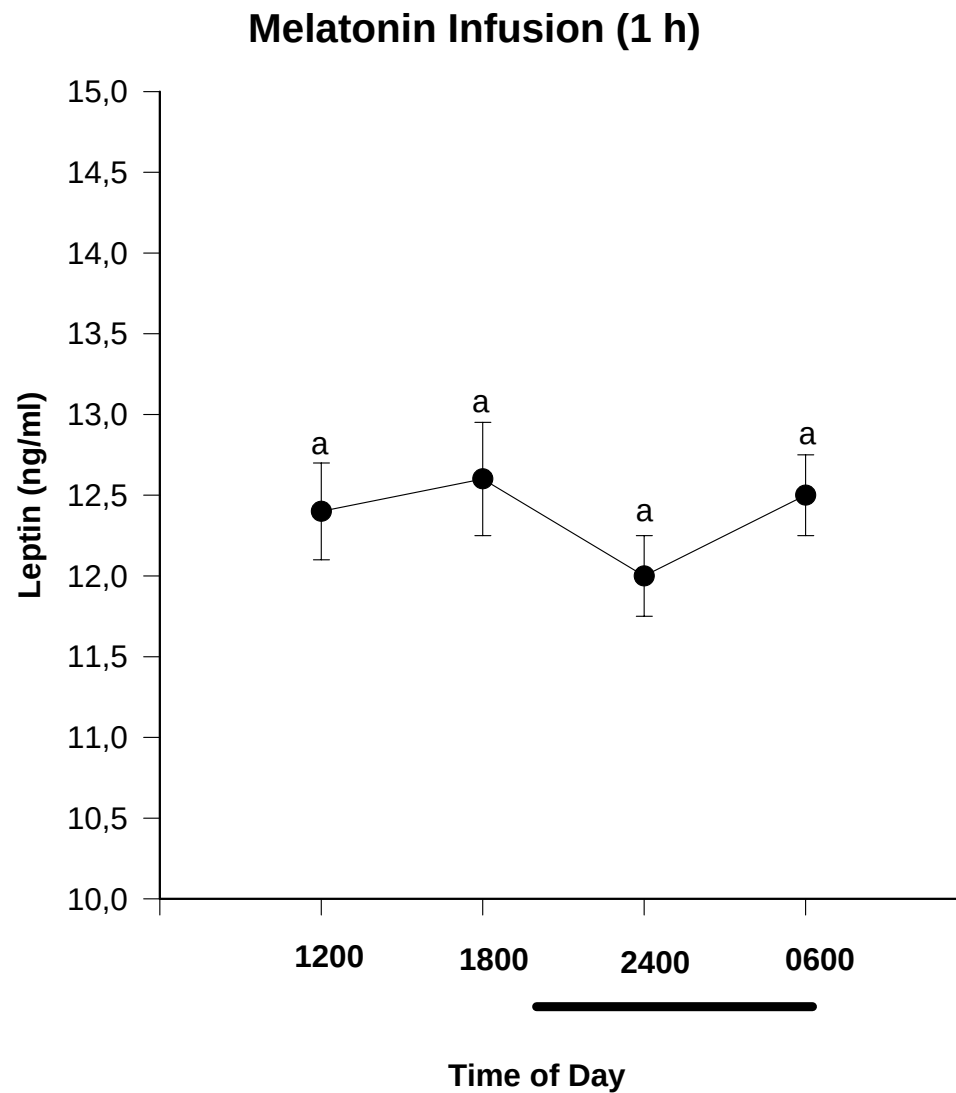


Fig. 17: Serum leptin level in SCN lesioned and 1 hour melatonin infused hamsters in 14L photoperiod. Blood samples were collected every 6 h from the same animal (n=10) throughout the day. Letters indicate the statistical difference. The black bar indicates the period of darkness.

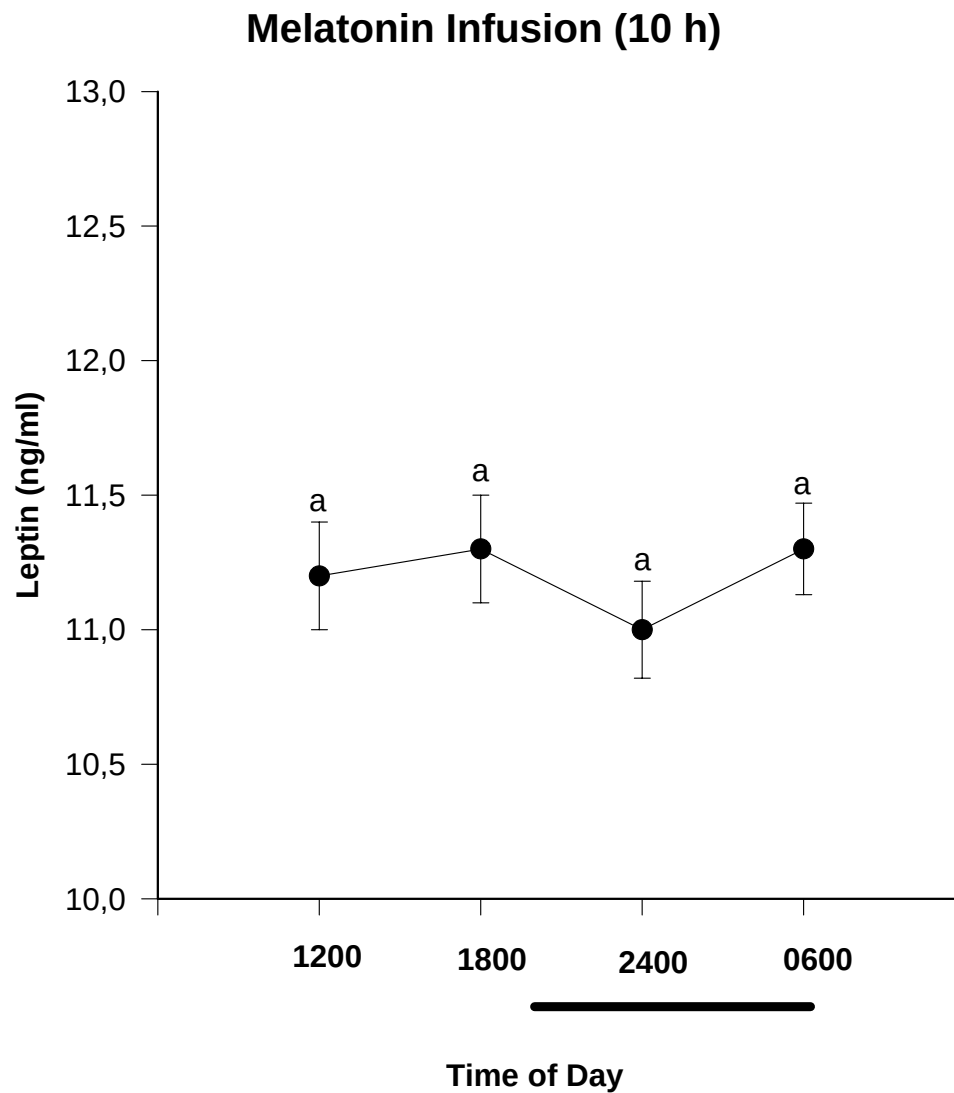


Fig. 18: Serum leptin level in SCN lesioned and 10 hour melatonin infused hamsters in 14L photoperiod. Blood samples were collected every 6 h from the same animal (n=10) throughout the day. Letters indicate the statistical difference. The black bar indicates the period of darkness.

3.3 STUDY - 3

3.3.1 Experiment 1:

Fig. 19 shows the activity and Fig. 20 illustrates the periodogram of the control hamsters. The duration of the activity in controls was 24:15 h. The average wheel turn in controls was 125,63. The control hamsters started to their activity at circadian time (CT) 12.

The Fig. 21 shows the locomotor activity and Fig. 22 shows the periodogram of the hamsters which were injected with 2 µg/kg leptin hormone. Hamsters were exposed to 14L photoperiod for the first 15 days to check the normal periodicity. Then, hamsters were exposed to constant light for the rest of the experiment. In constant light, hamsters showed free-run. Locomotor activity levels in both groups were suppressed by the constant light photoperiod. After ten days, 2 µg/kg leptin injections started. Leptin did not change the rhythm of the locomotor activity ($p>0,05$) (Fig. 22). The duration of the activity in controls was 24:10 h. The average wheel turn in controls was 45,32. The activity rhythm did not change but the activity level changed significantly ($p<0,05$).

Fig. 23 shows the locomotor activity and Fig. 23 shows the periodogram of the hamsters which were injected with 4 µg/kg leptin hormone. 4 µg/kg leptin injection did not change the rhythm of the locomotor activity ($p>0,05$). The duration of the activity in controls was 23:50 h. The average wheel turn in controls was 38,74. The activity rhythm did not change but the activity level changed significantly ($p<0,05$).

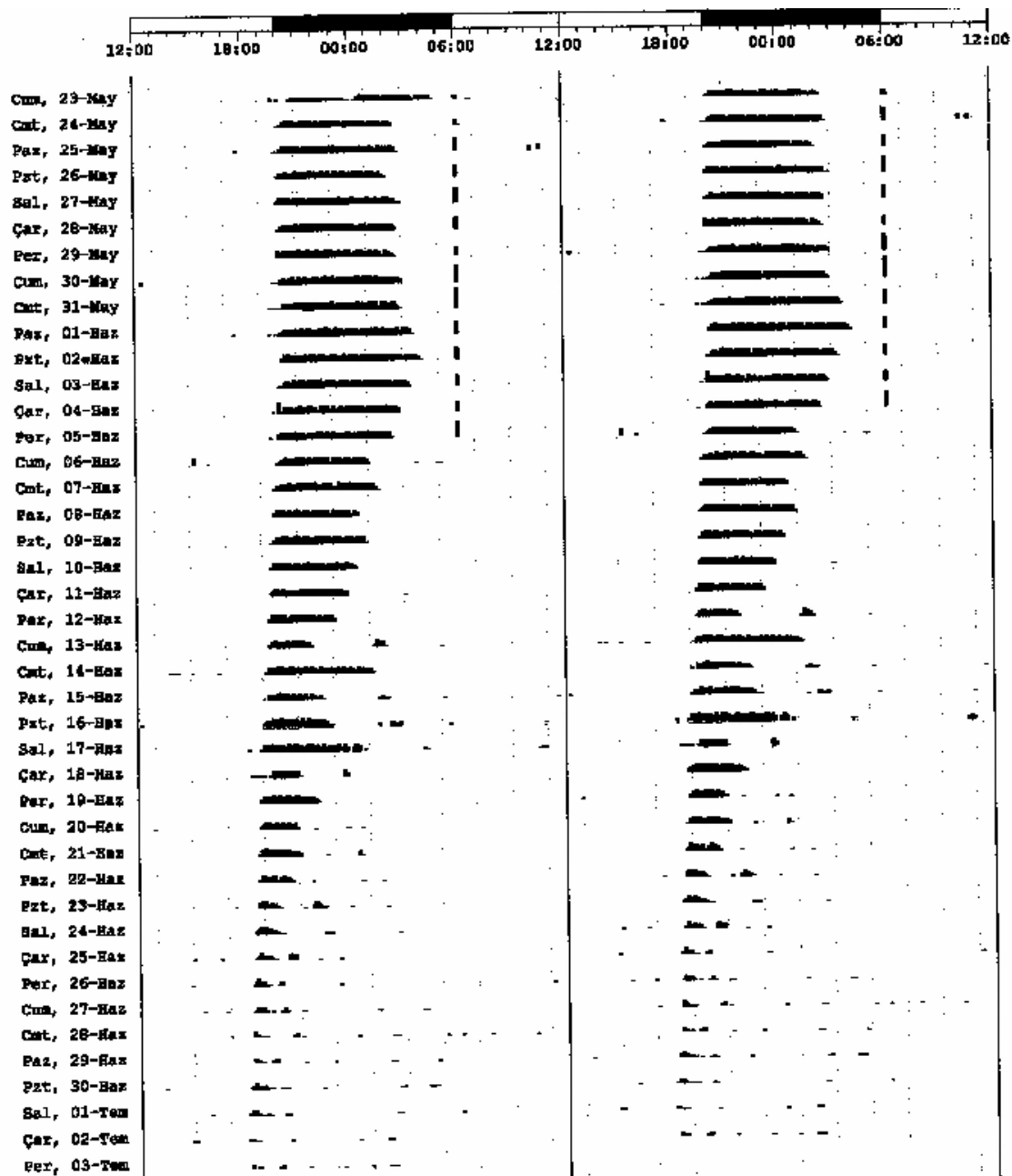


Fig. 19: The actogram of the control group in constant light..

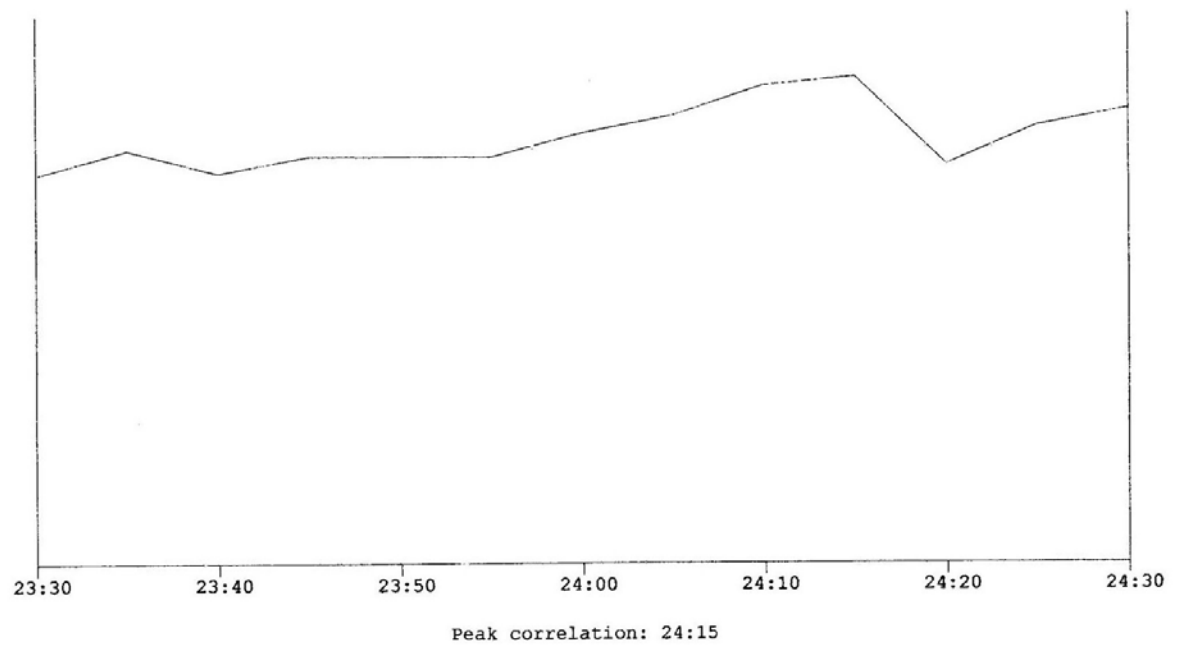


Fig. 20: The periodogram of the control hamster in constant light.

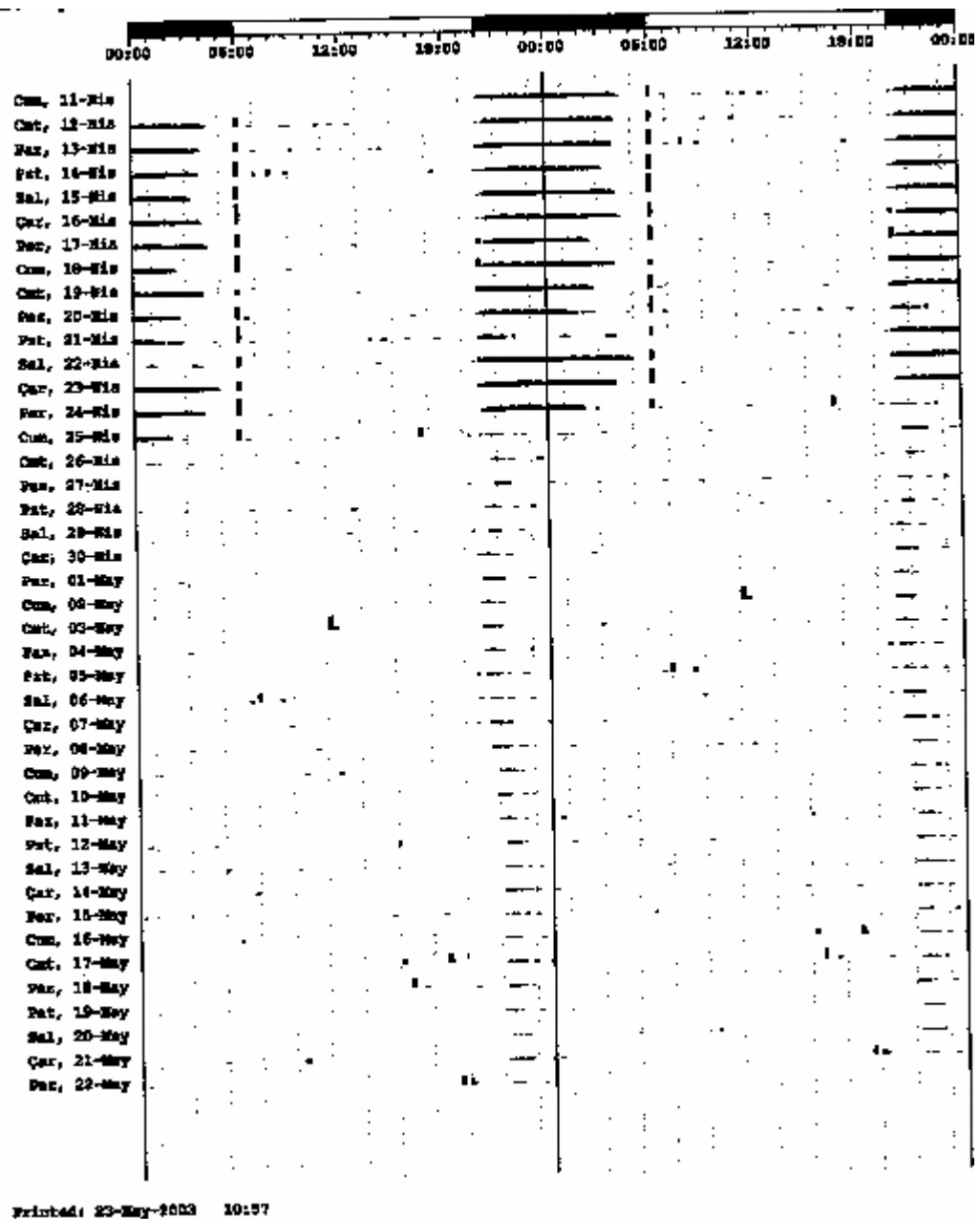


Fig. 21: The actogram of the 2 µg/kg leptin injected group in constant light.

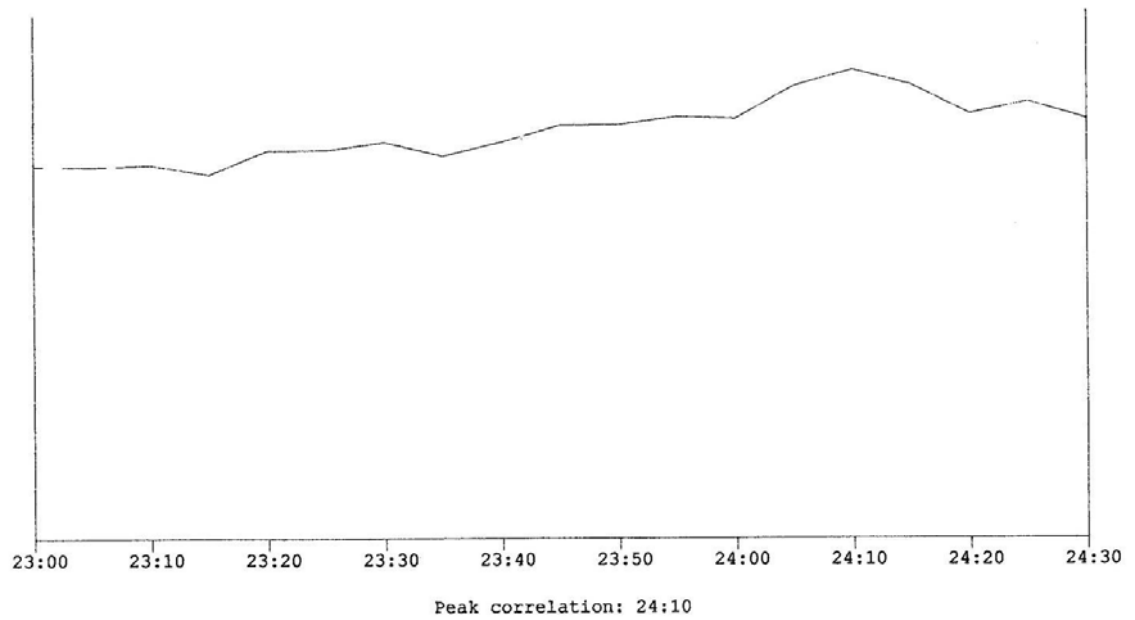


Fig. 22: The periodogram of the 2 $\mu\text{g/kg}$ leptin injected hamster in constant light.

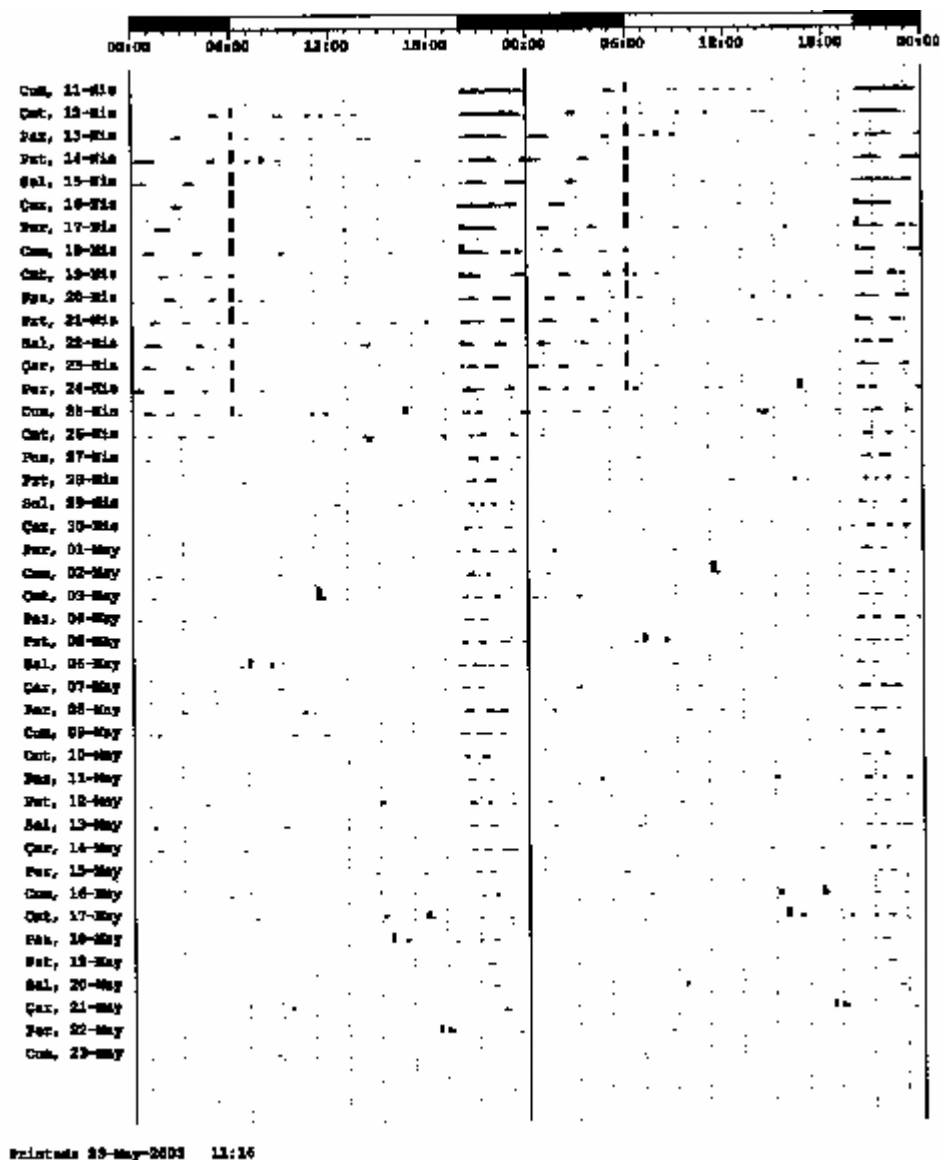


Fig. 23: The actogram of the 4 µg/kg leptin injected group in constant light.

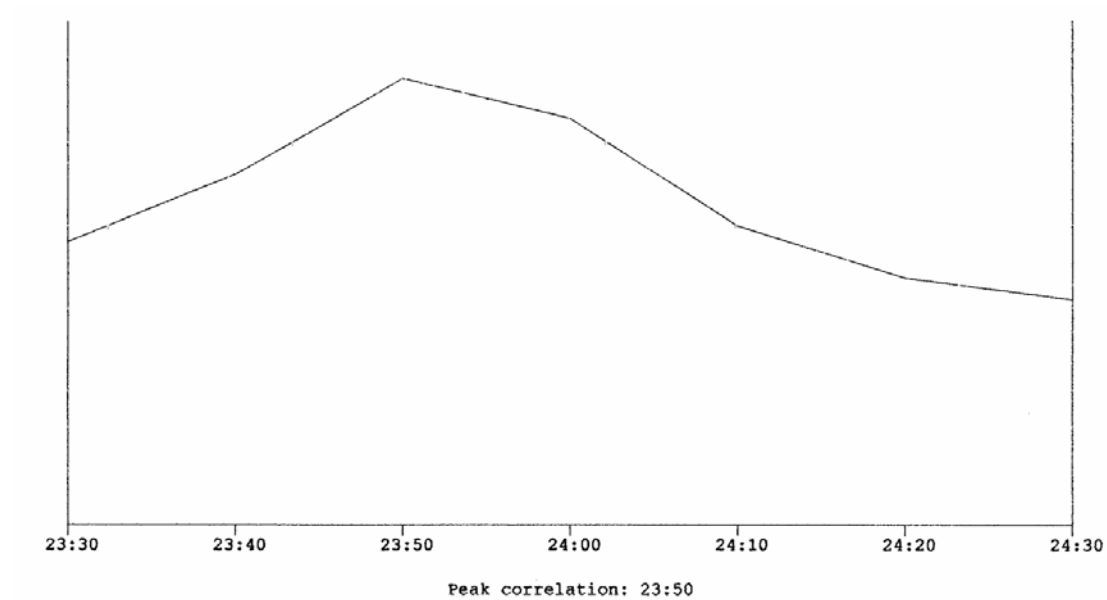


Fig. 24: The periodogram of the 4 $\mu\text{g/kg}$ leptin injected hamster in constant light.

3.3.2 Experiment 2:

Fig. 25 shows the activity and Fig. 26 illustrates the periodogram of the control hamsters. The duration of the activity in controls was 23:50 h. The average wheel turn in controls was 112,23. The control hamsters started to their activity at circadian time (CT) 12.

Fig. 27 shows the locomotor activity and Fig. 28 shows the periodogram of the hamsters which were injected with 2 µg/kg leptin hormone. Hamsters were exposed to 14L photoperiod for the first 15 days to check the normal periodicity. Then, hamsters were exposed to constant darkness. In constant darkness, hamsters showed free-run. After ten days, 2 µg/kg leptin injection started. Injection did not change the rhythm of the locomotor activity ($p>0,05$). The duration of the activity in controls was 23:55 h. The average wheel turn in controls was 92,87.

Fig. 29 shows the locomotor activity and Fig. 30 shows the periodogram of the hamsters which were injected with 4 µg/kg leptin hormone. 4 µg/kg leptin injection changed significantly the rhythm of the locomotor activity and phase advanced the activity onset $1,12 \pm 0,15\text{h}$ ($p<0,05$). The duration of the activity in controls was 24:00 h. The average wheel turn in controls was 89,12.

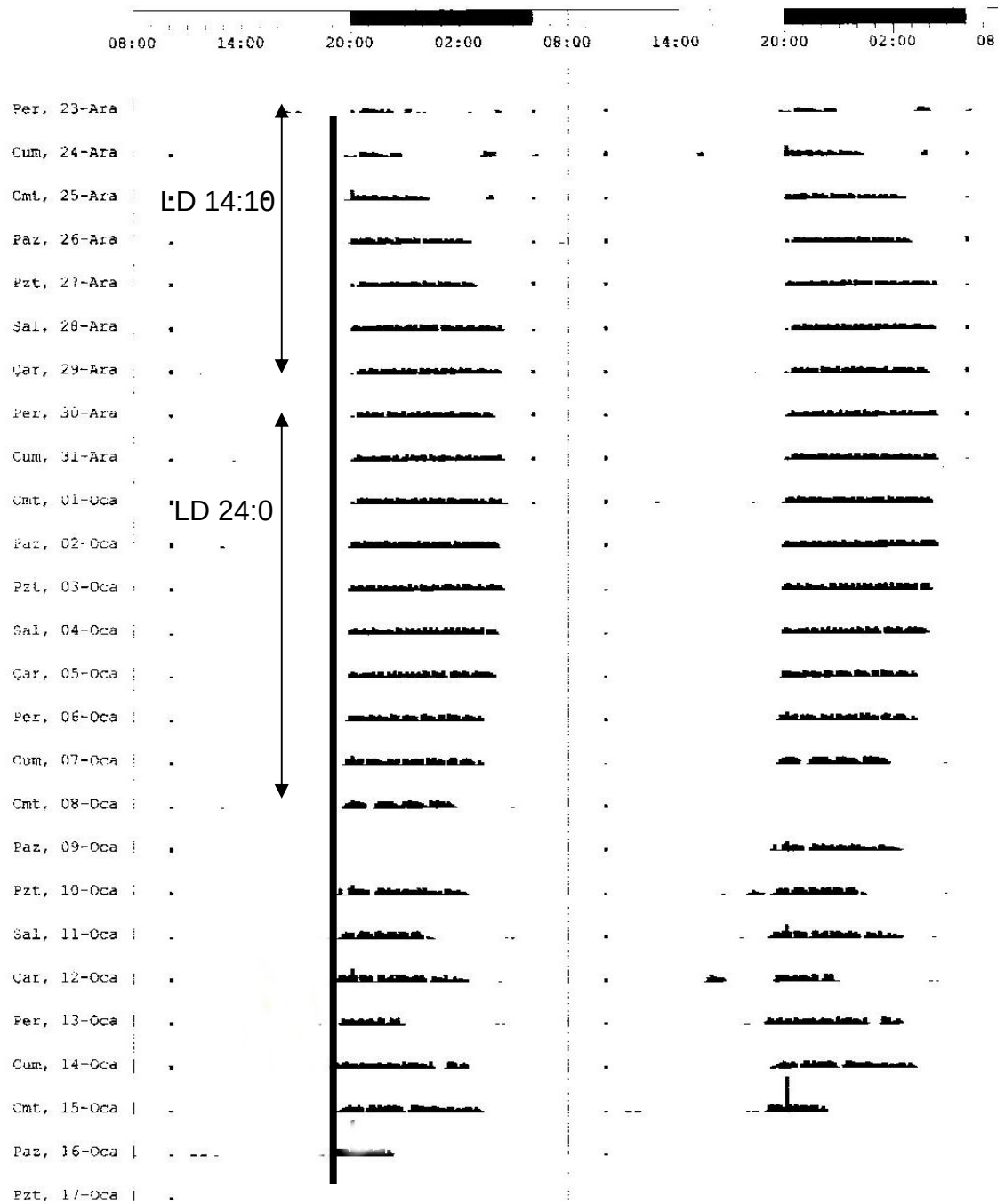


Fig. 25: The actogram of the control hamsters in constant darkness.

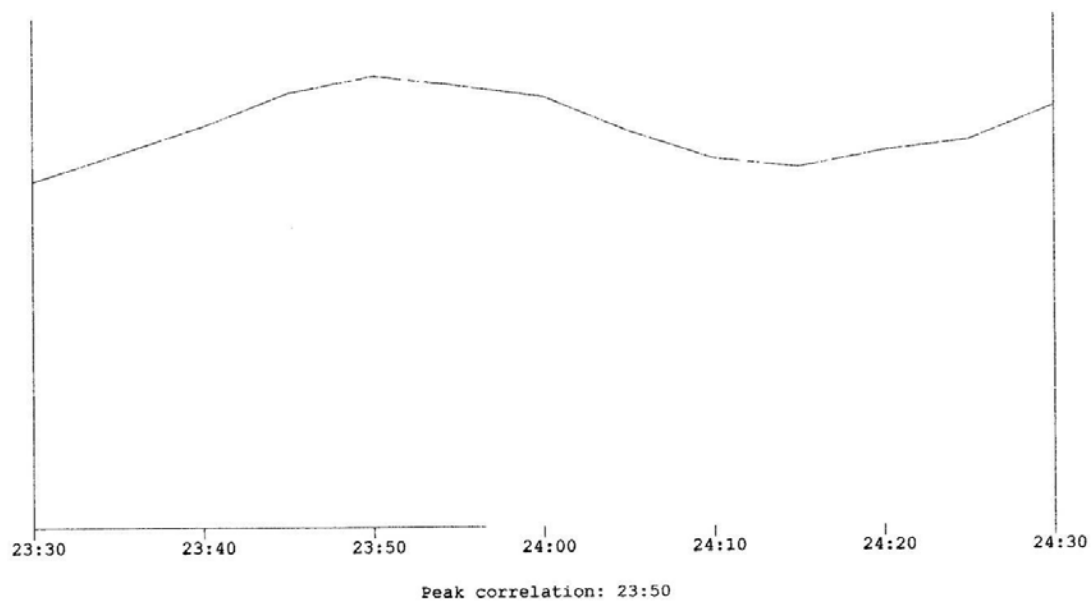


Fig. 26: The periodogram of the control hamster in constant darkness.

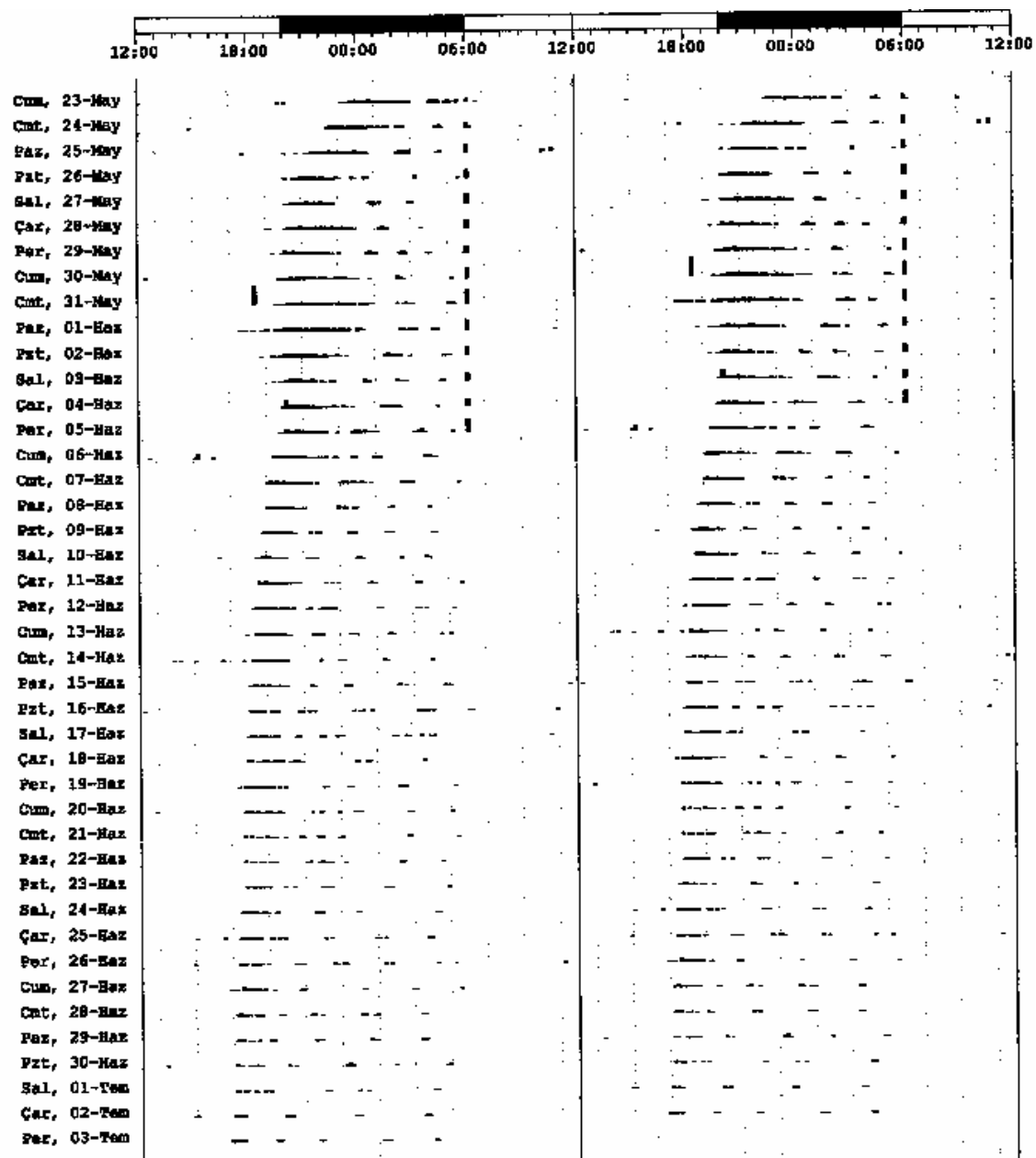


Fig. 27: The actogram of the 2 µg/kg leptin injected group in constant darkness.

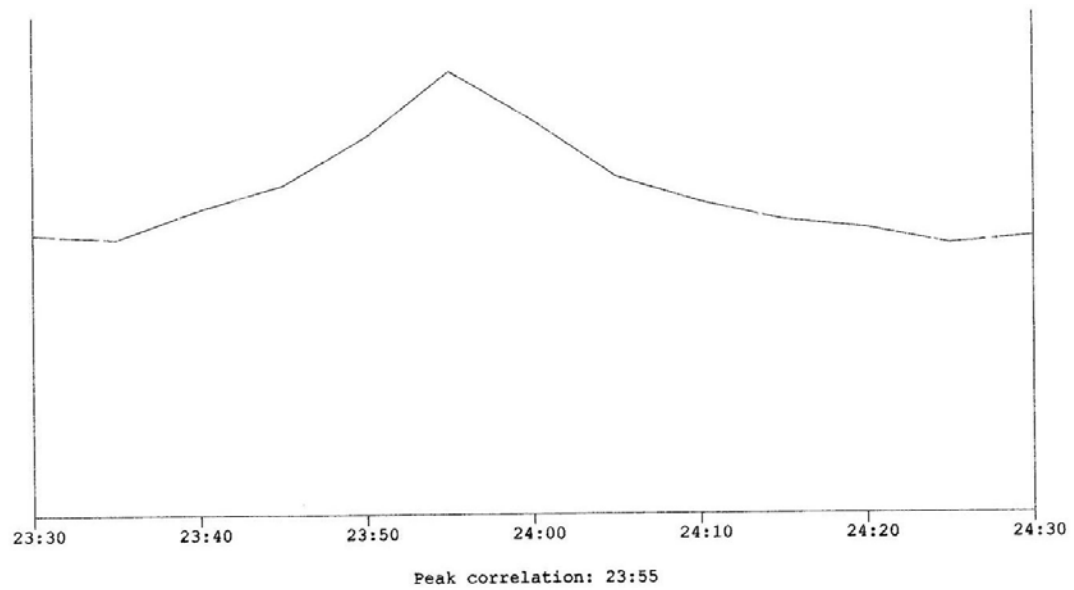


Fig. 28: The periodogram of the 2 $\mu\text{g/kg}$ leptin injected hamster in constant darkness.

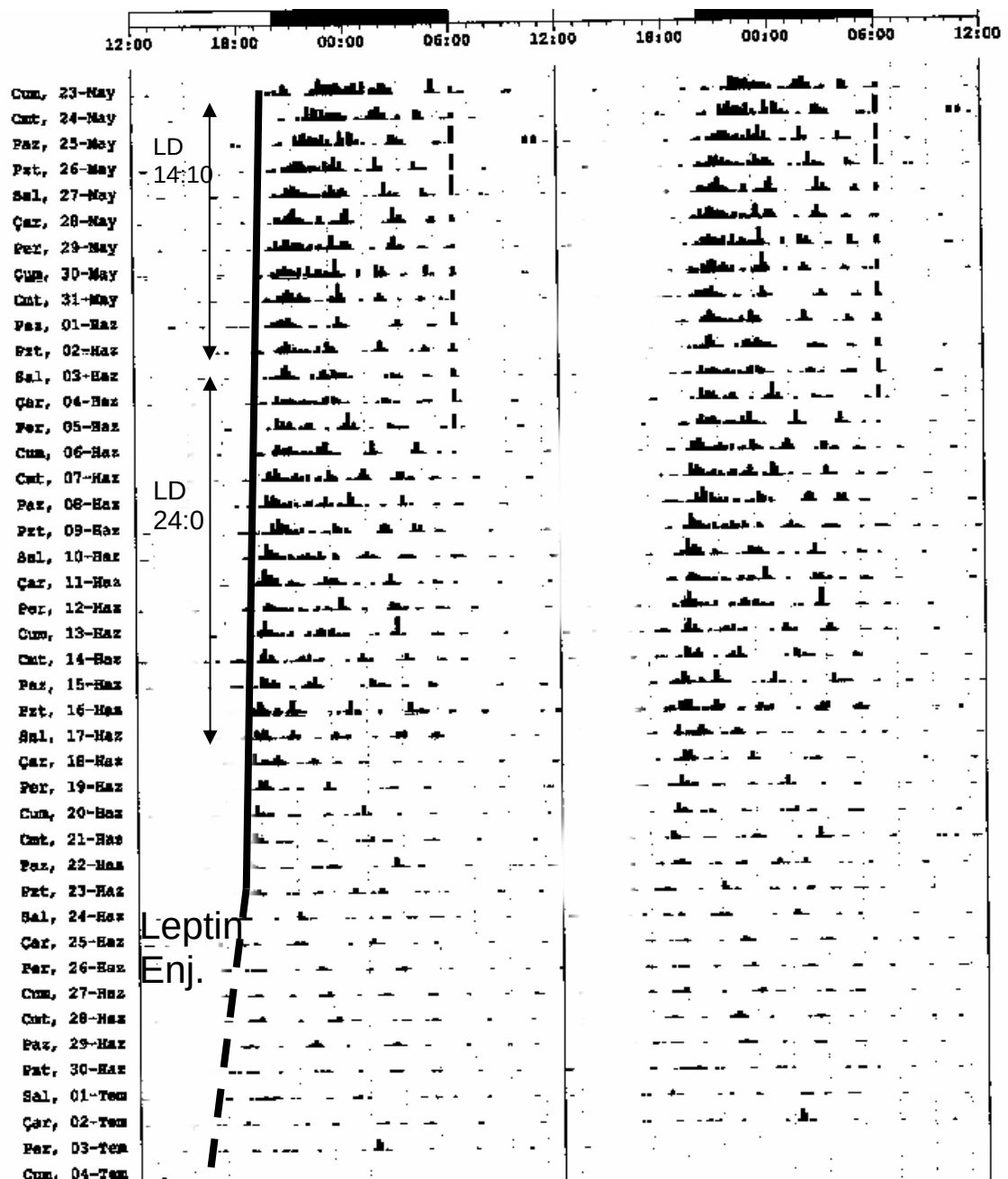


Fig. 29: The actogram of the 4 µg/kg leptin enjected hamsters. Note presence of the shift.

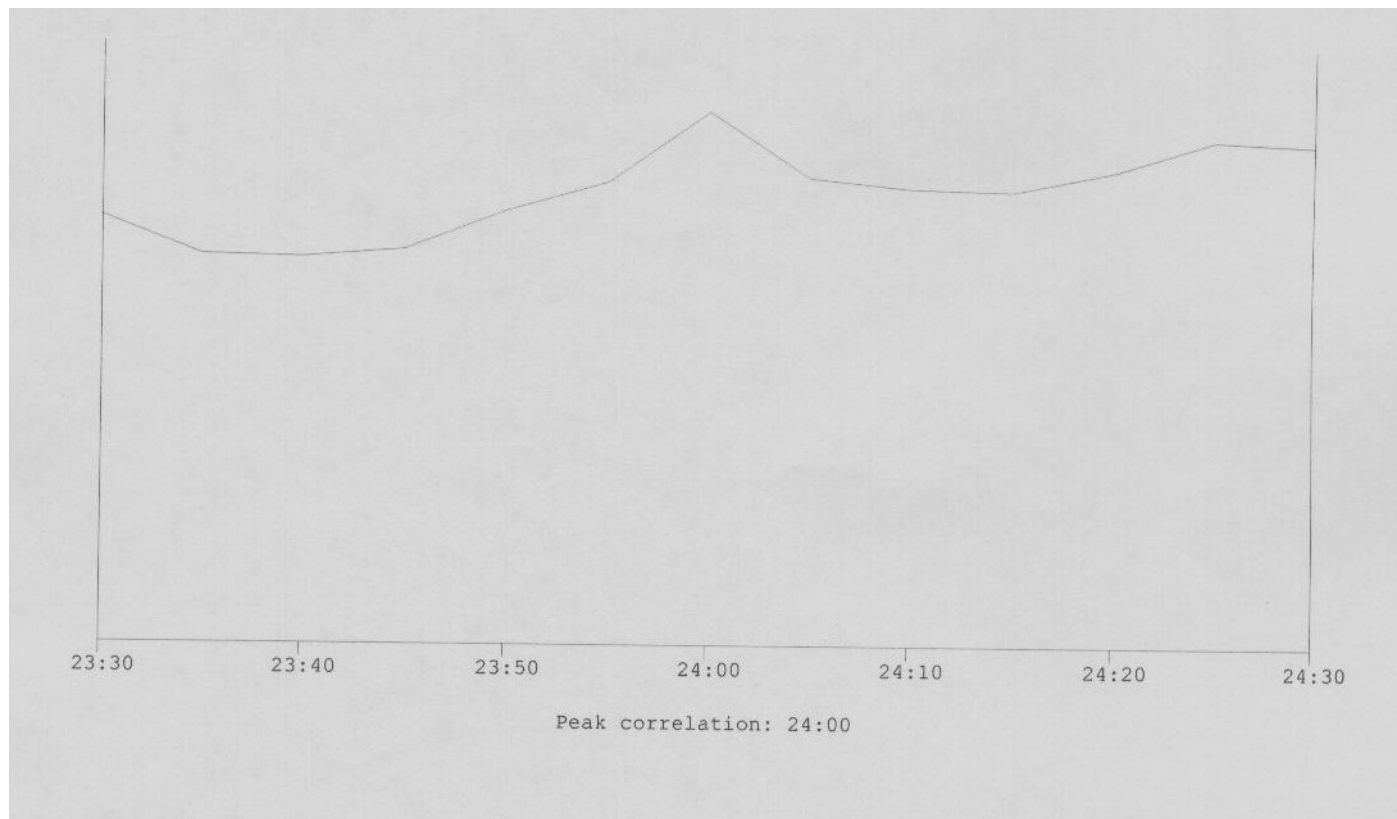


Fig. 30: The periodogram of the 4 µg/kg leptin injected hamster.

3.3.3 Experiment 3

Fig. 31 shows the activity and Fig. 32 illustrates the periodogram of the 4 $\mu\text{g/kg}$ leptin infused hamsters. The duration of the activity in controls was 23:30 h. The average wheel turn in controls was 120,55. The hamsters started to their activity at CT 12. Hamsters were exposed to 14L photoperiod for the first 15 days to check the periodicity. Then hamsters were exposed to constant darkness. In constant darkness, hamsters showed free-run. After ten days, 4 $\mu\text{g/kg}$ leptin infusions started. Infusion changed the rhythm of the locomotor activity and phase advanced the onset of activity $2,15 \pm 0,20$ h ($p < 0,05$).

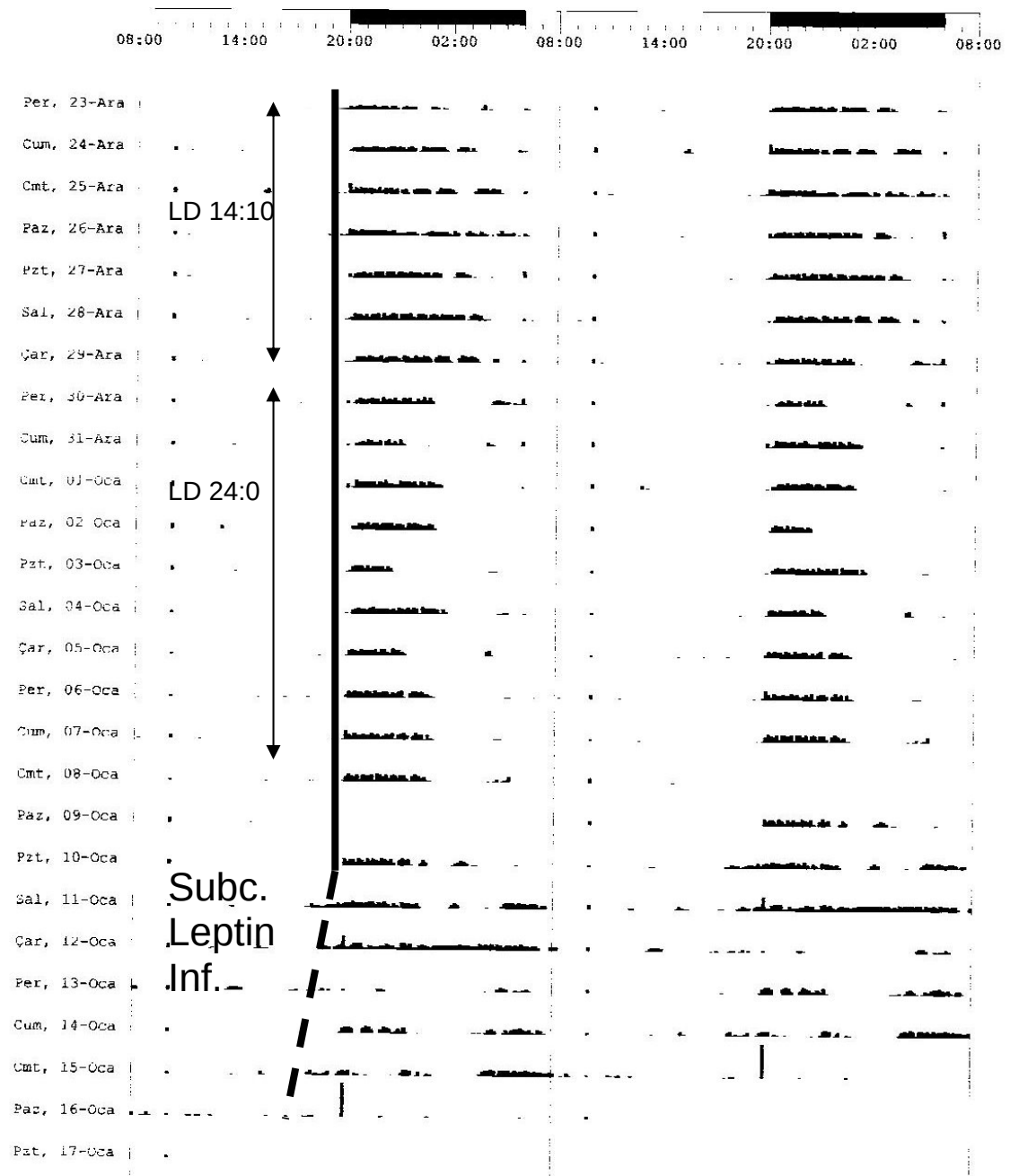


Fig. 31: The actogram of the subcutaneous 4 μ g/kg leptin infused hamsters in constant darkness. Note presence of the shift.

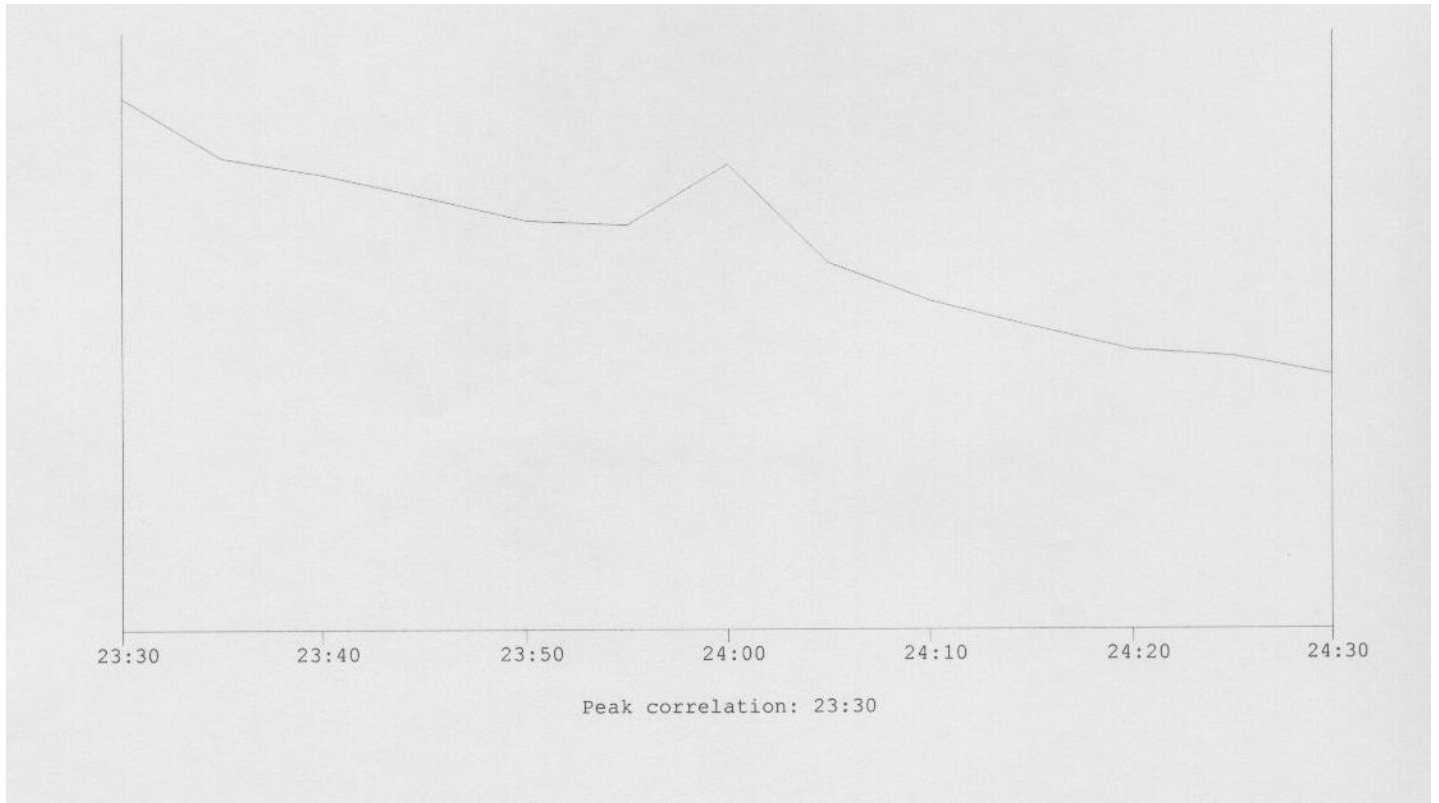


Fig. 32: The periodogram of the the subcutaneous 4 $\mu\text{g/kg}$ leptin infused hamster in constant darkness.

3.3.4 Experiment 4

Fig. 33 shows the activity and Fig. 34 illustrates the periodogram of the 0,4 µg/kg intracerebroventricular (icv) leptin infused hamsters. The duration of the activity in controls was 23:55 h. The average wheel turn in controls was 100,42. The hamsters started to their activity at CT 12. Hamsters were exposed to 14L photoperiod for the first 15 days to check the periodicity. Then, hamsters were exposed to constant darkness. In constant darkness, hamsters showed free-run. After ten days, 0,4 µg/kg leptin infusions started. Icv infusion changed the rhythm of the locomotor activity and phase advanced the onset of activity $2,48 \pm 0,24$ h ($p < 0,05$).

Table 11 and Fig. 35 show phase advances of locomotor activity in injected, infused and icv infused animals in constant darkness.

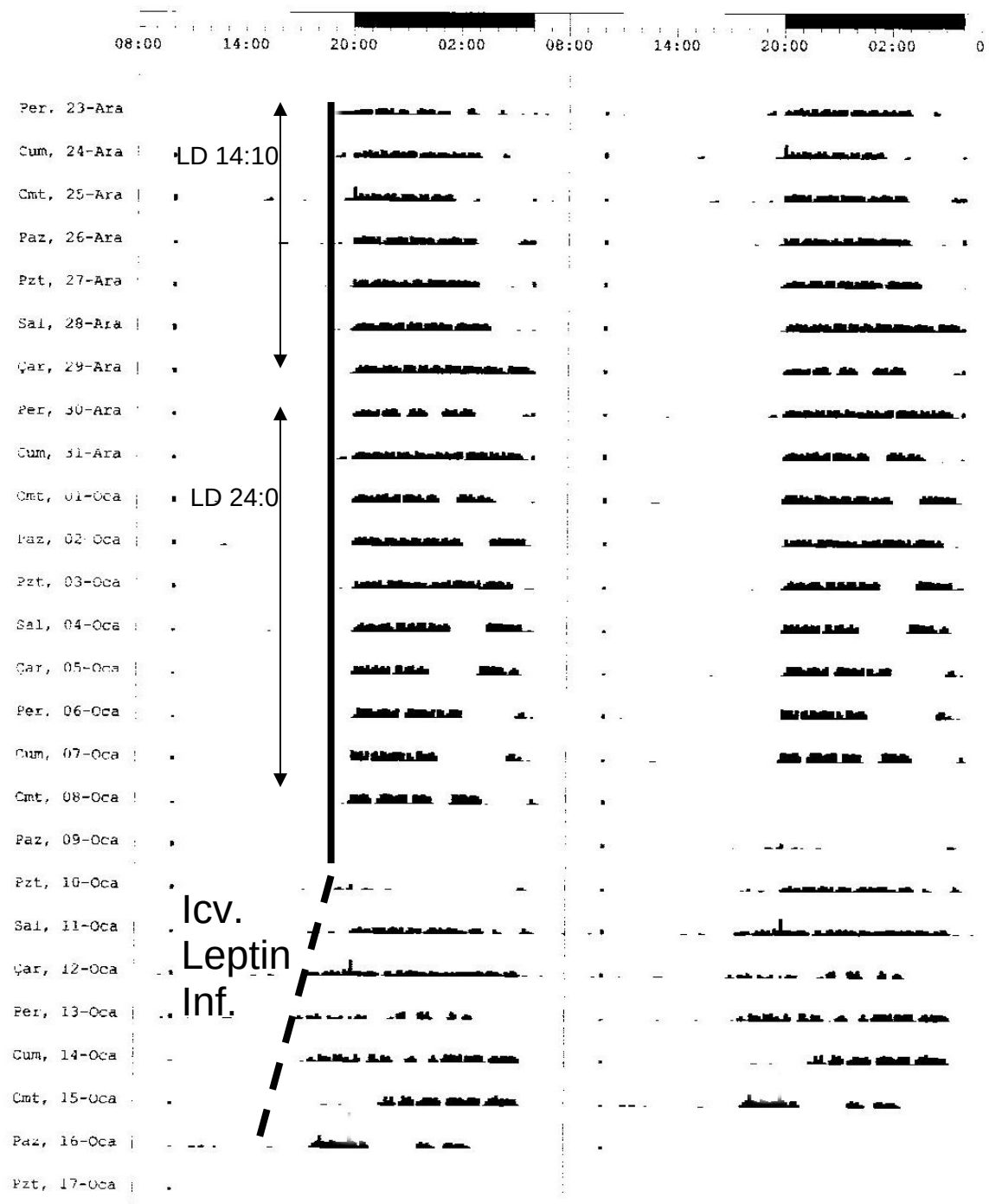


Fig. 33: The actogram of the intracerebroventricular 0,4 $\mu\text{g/kg}$ leptin infused hamsters in constant darkness. Note presence of the shift.

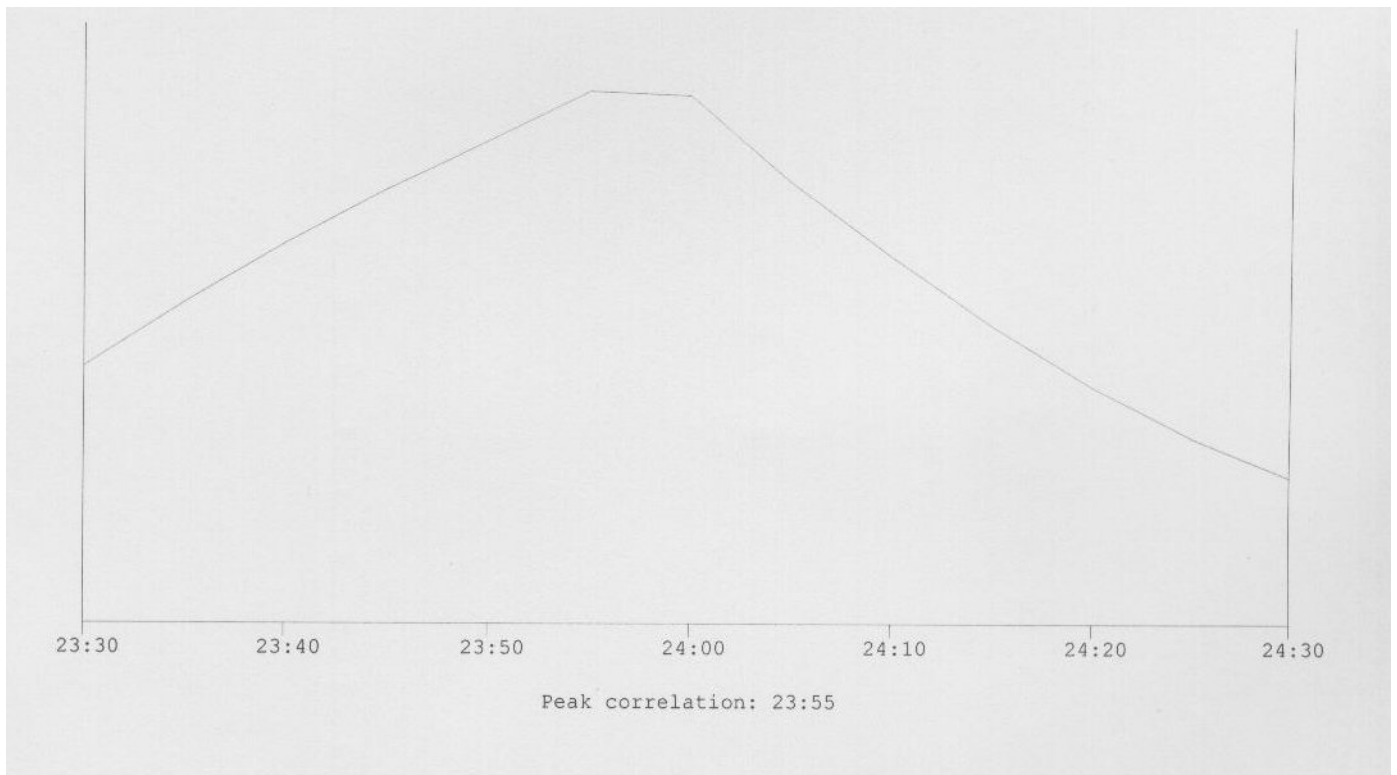


Fig. 34: The periodogram of the intracerebroventricular 4 $\mu\text{g/kg}$ leptin infused hamster in constant darkness.

Table 11: The table represents the phase advances of the different leptin administrations.

	Phase Advances
Injection (4 µg/kg)	1,12 ± 0,15 h
Subcutaneous Infusion (4 µg/kg)	2,15 ± 0,20 h
Intracerebroventricular Infusion (0,4 µg/kg)	2,48 ± 0,24 h

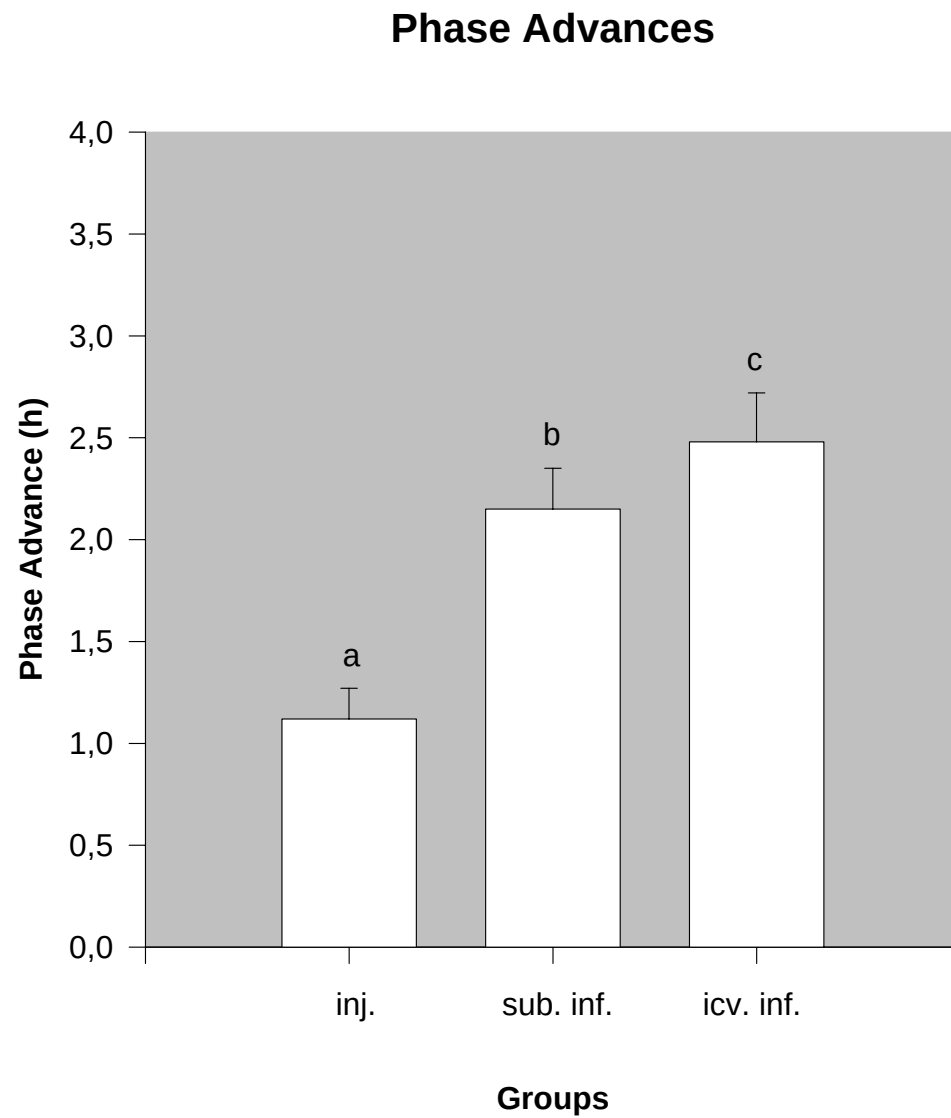


Fig. 35: The figure represents the phase advances of the different leptin administrations

3.3.5 Experiment 5:

Male hamsters (n:5) were SCN lesioned. The success of the lesions were checked by the locomotor activity. Fig. 36 shows the double plotted locomotor activity of the SCN lesioned hamsters. After lesion, hamsters were infused with 4 $\mu\text{g/kg}$ leptin for ten days. The onset of activity phase advanced day by day throughout the infusions. Fig. 37 shows the restoration of the locomotor activity with the daily timed leptin infusions.

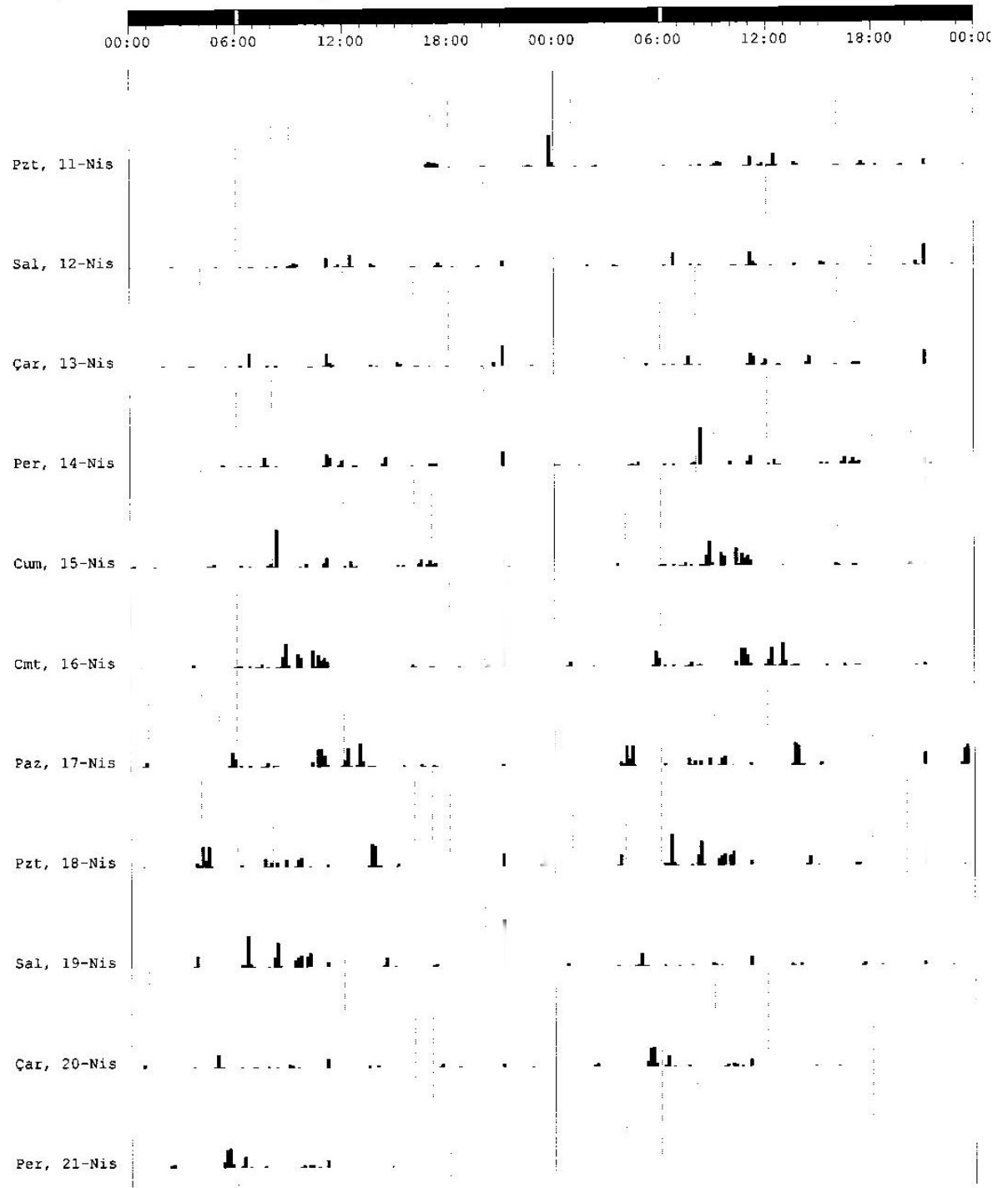


Fig. 36: The double plotted actogram of the SCN lesioned hamsters.

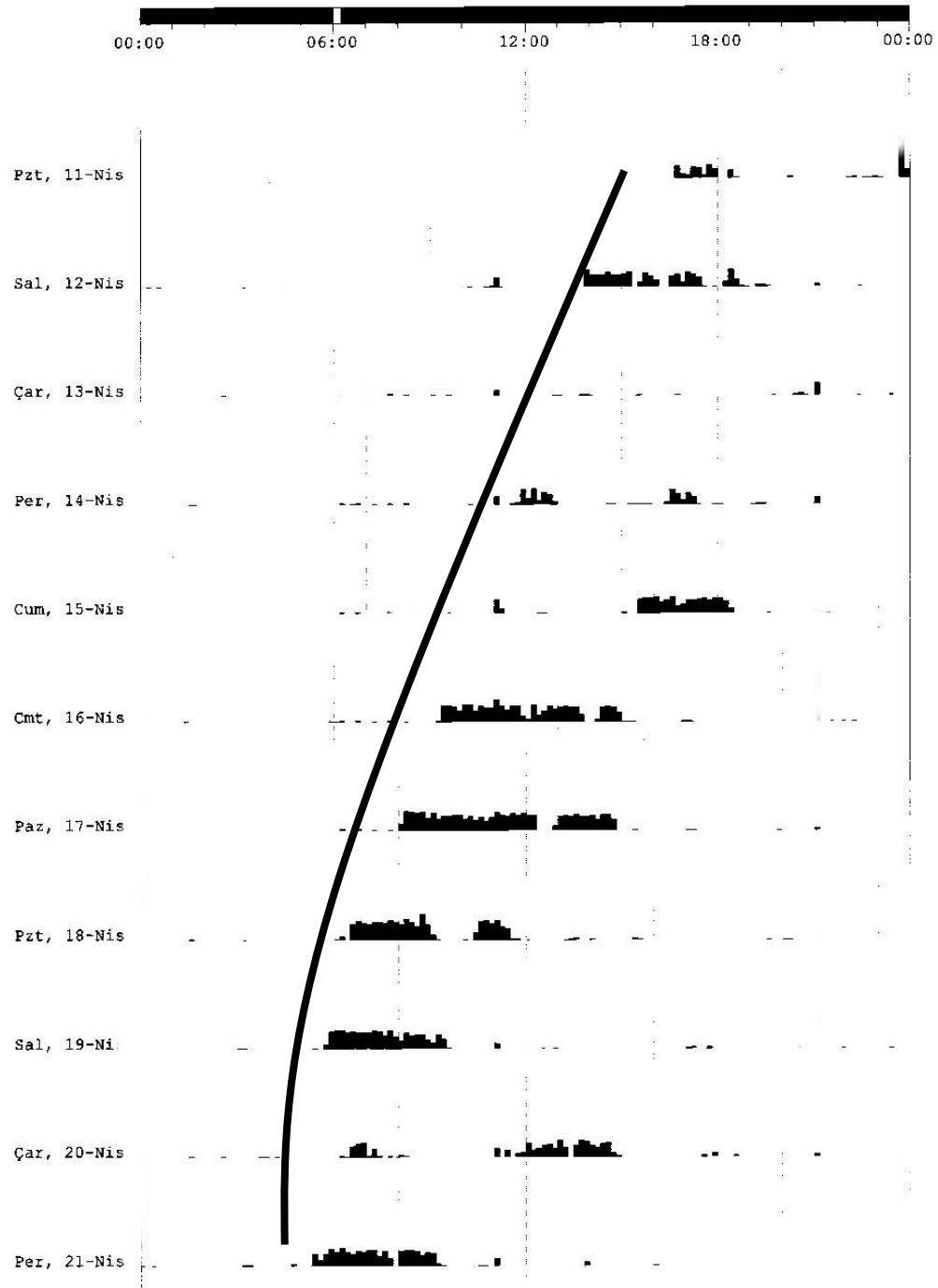


Fig. 37: The actogram of the SCN lesioned and daily leptin injected hamsters.
Note the restoration of the rhythm by the timed leptin injections.

3.4 STUDY - 4

3.4.1 Experiment 1:

In this experiment, pregnant Syrian hamsters were injected with saline, leptin, melatonin or insulin. After parturition and weaning, the body (Fig. 38), adipose (Fig. 39), epididymis (Fig. 40), seminal vesicle (Fig. 41) and testis weights (Fig. 42) of the prepubertal hamsters were examined for 8 weeks. All the parameters were higher in juvenile hamsters borned to leptin injected mothers ($p < 0,05$). The development of these parameters were similar in juveniles borned to melatonin injected, saline injected and intact mothers ($p > 0,05$) and they were slower in juveniles borned to insulin injected mothers. The results were given in Table 12. Testis histologies of the hamsters borned to leptin injected (Fig. 43), control (Fig. 44) and melatonin injected (Fig. 46) mothers were normal however hamsters borned to insulin injected (Fig. 45) mothers have abnormalities.

Table 12: The table represents the body, adipose, epididymis, seminal vesicle and testis weights of the intact, saline, leptin, melatonin and insulin injected Syrian hamsters.

	Body Weights (g) Mean $\pm$ SEM	Adipose Weights (g) Mean $\pm$ SEM	Epididymis Weights (g) Mean $\pm$ SEM	Seminal Vesicle Weights (g) Mean $\pm$ SEM	Testis Weights (g) Mean $\pm$ SEM
Intact	90,00 $\pm$ 6,80	1,80 $\pm$ 0,10	0,90 $\pm$ 0,08	1,80 $\pm$ 0,10	2,74 $\pm$ 0,21
Saline Injection	94,00 $\pm$ 7,00	1,83 $\pm$ 0,10	0,83 $\pm$ 0,07	1,75 $\pm$ 0,10	2,76 $\pm$ 0,25
Leptin Injection	110,00 $\pm$ 7,50	2,20 $\pm$ 0,14	1,30 $\pm$ 0,09	2,10 $\pm$ 0,13	3,20 $\pm$ 0,28
Melatonin Injection	95,00 $\pm$ 6,90	1,70 $\pm$ 0,10	0,80 $\pm$ 0,07	1,83 $\pm$ 0,10	2,68 $\pm$ 0,23
Insulin Injection	75,00 $\pm$ 5,20	1,50 $\pm$ 0,10	0,60 $\pm$ 0,05	1,65 $\pm$ 0,09	1,90 $\pm$ 0,11

Body Weight

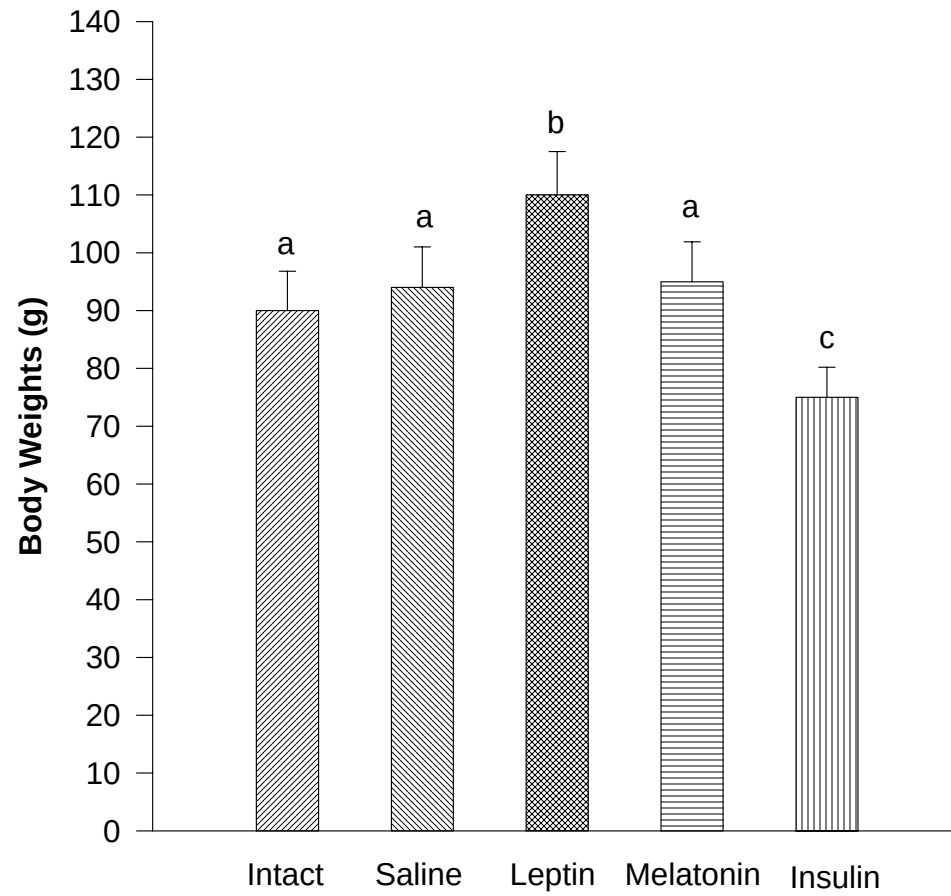


Fig. 38: The figure illustrates the body weights of the intact, saline, leptin, melatonin and insulin injected Syrian hamsters.

Adipose Weight

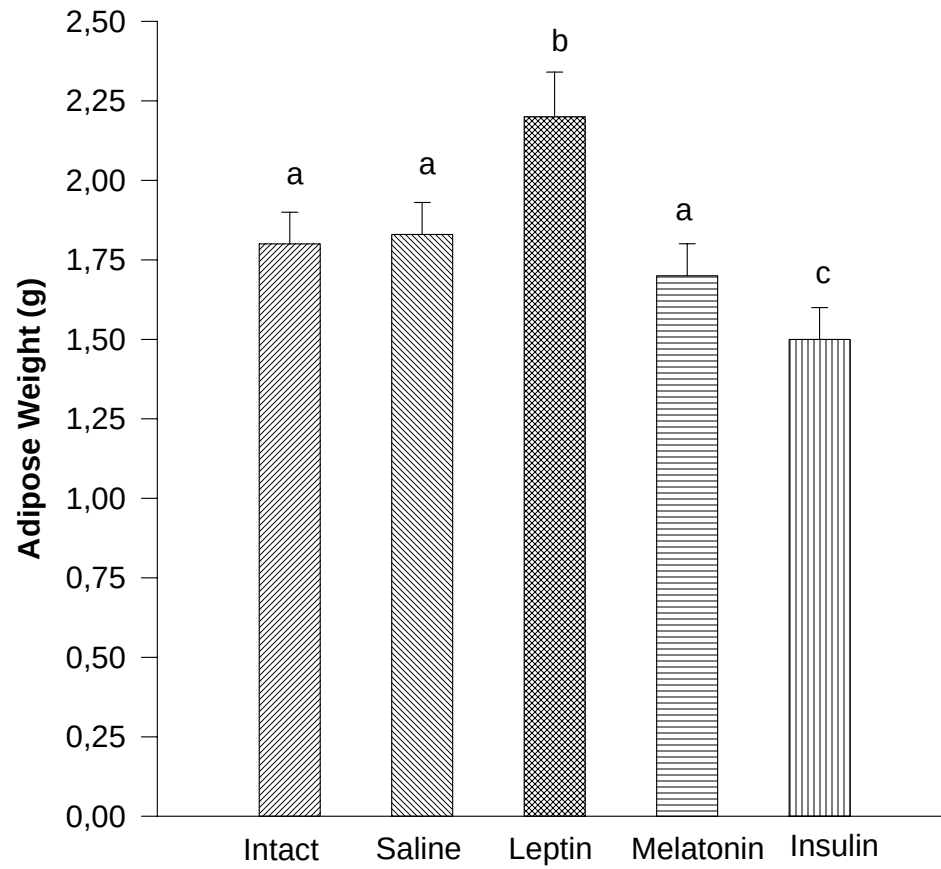


Fig. 39: The figure illustrates the adipose weights of the intact, saline, leptin, melatonin and insulin injected Syrian hamsters.

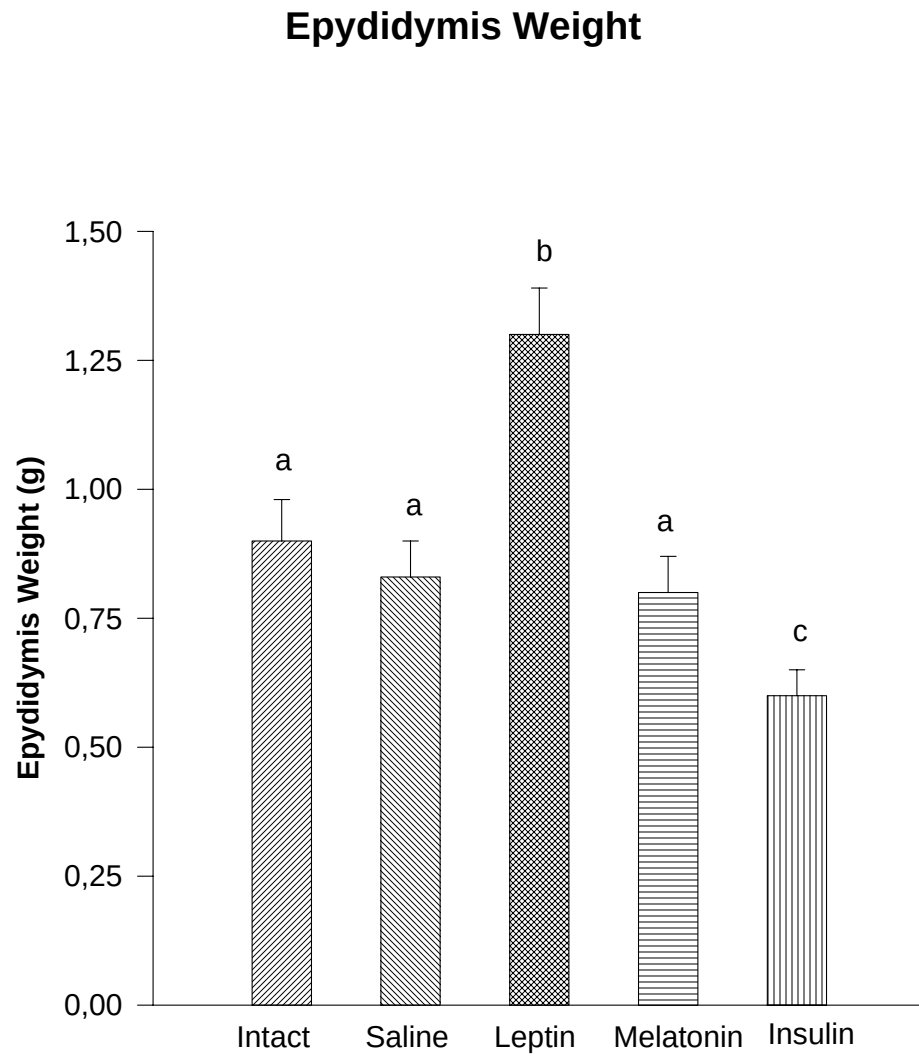


Fig. 40: The figure illustrates the epididymis weights of the intact, saline, leptin, melatonin and insulin injected Syrian hamsters.

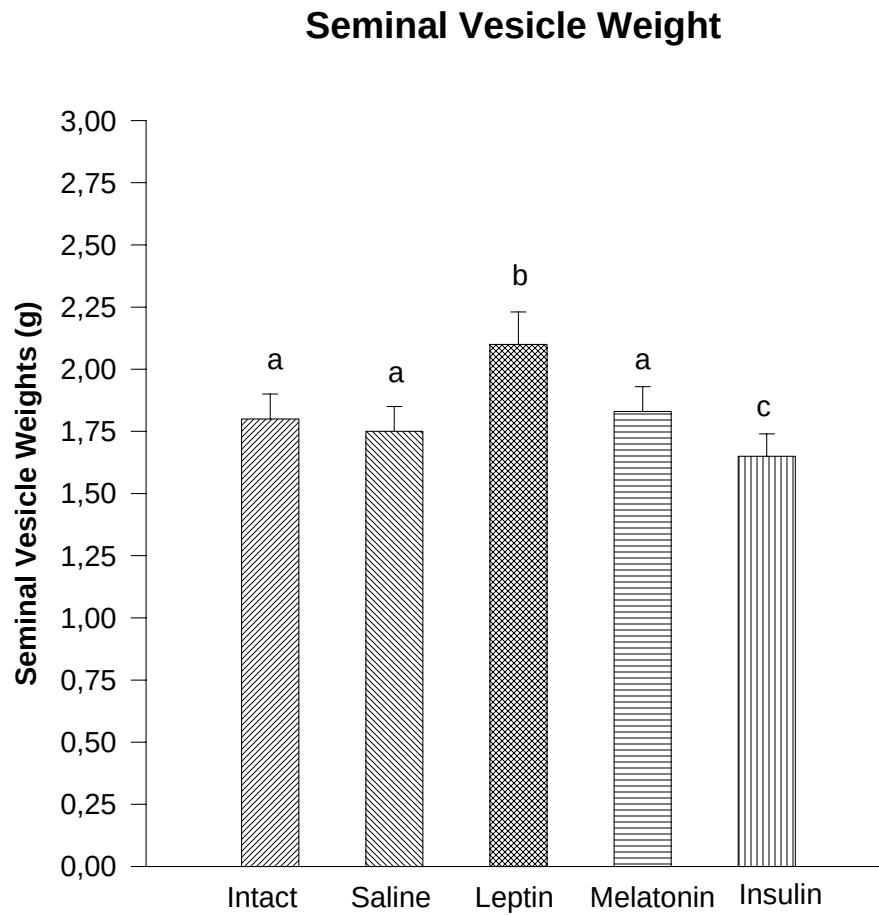


Fig. 41: The figure illustrates the seminal vesicle weights of the intact, saline, leptin, melatonin and insulin injected Syrian hamsters.

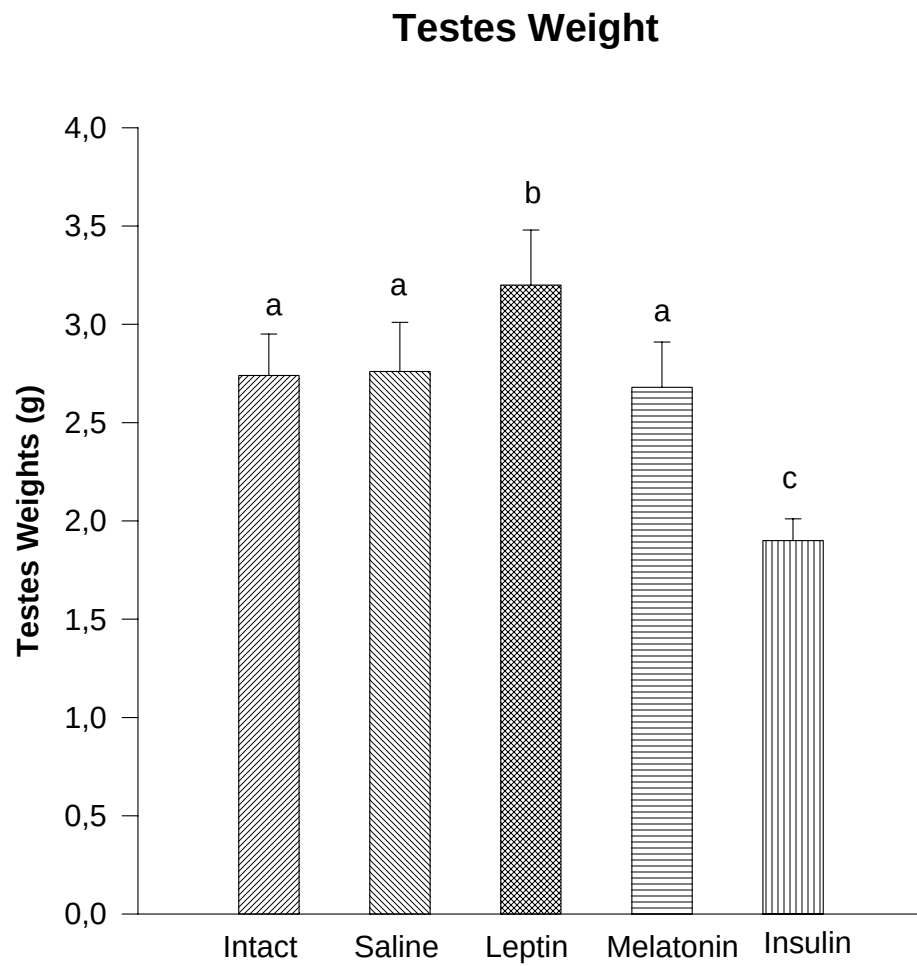


Fig. 42: The figure illustrates the testes weights of the intact, saline, leptin, melatonin and insulin injected Syrian hamsters.

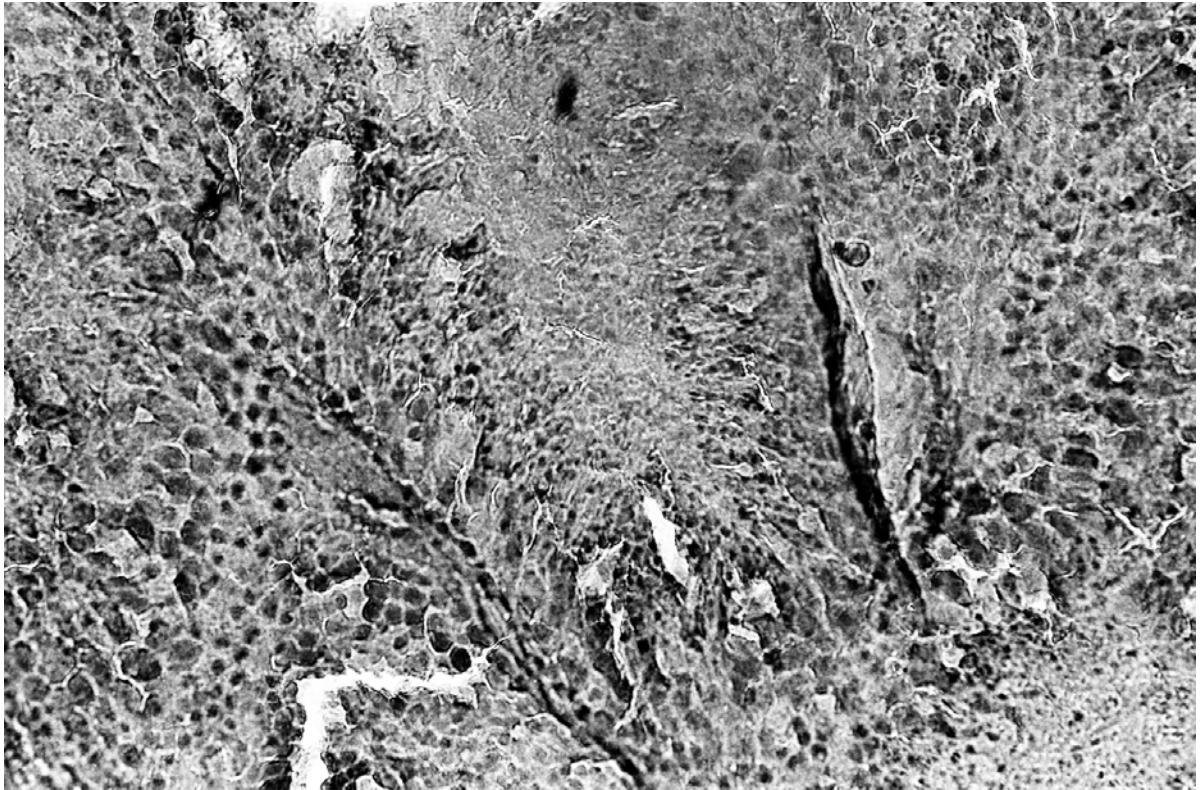


Fig. 43: The figure illustrates the testis of the hamster which borned to leptin injected mother. Note presence of various stages of the spermatogenesis (x 600).

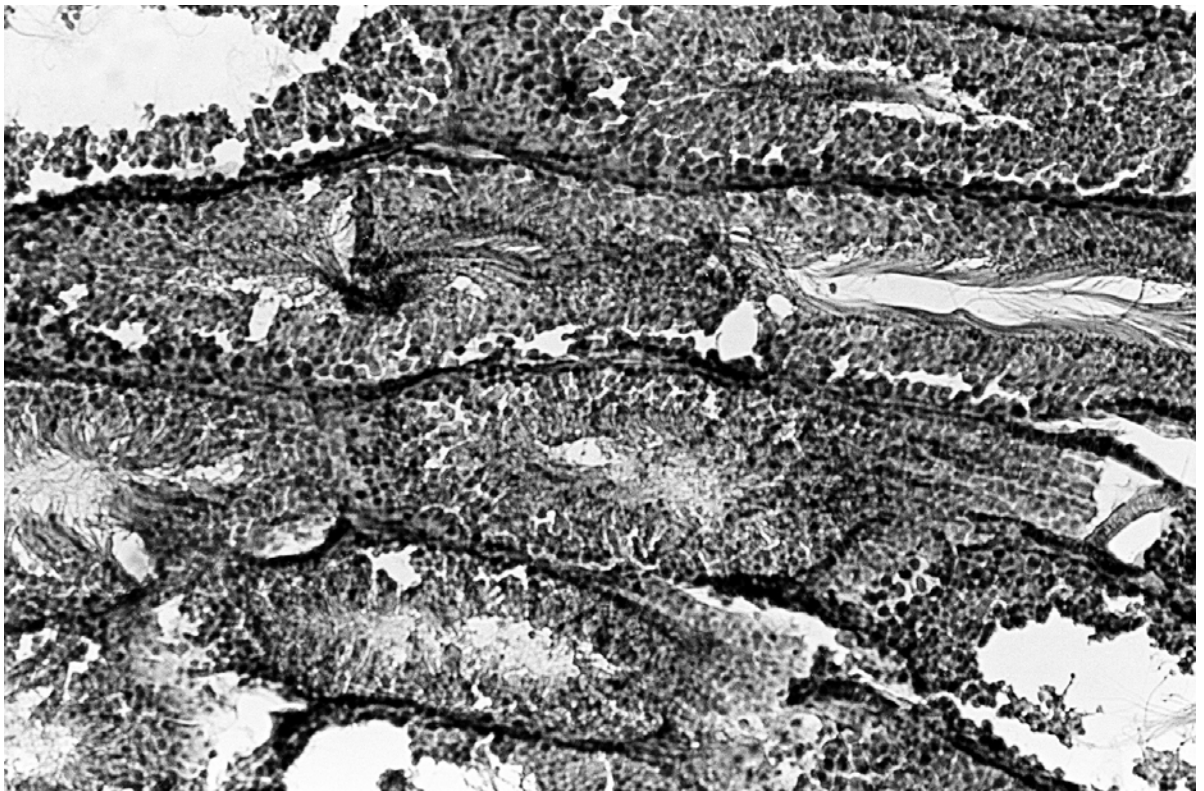


Fig. 44: The figure illustrates the testis of the hamster which borned to control mother. Note presence of various stages of the spermatogenesis (x 400).

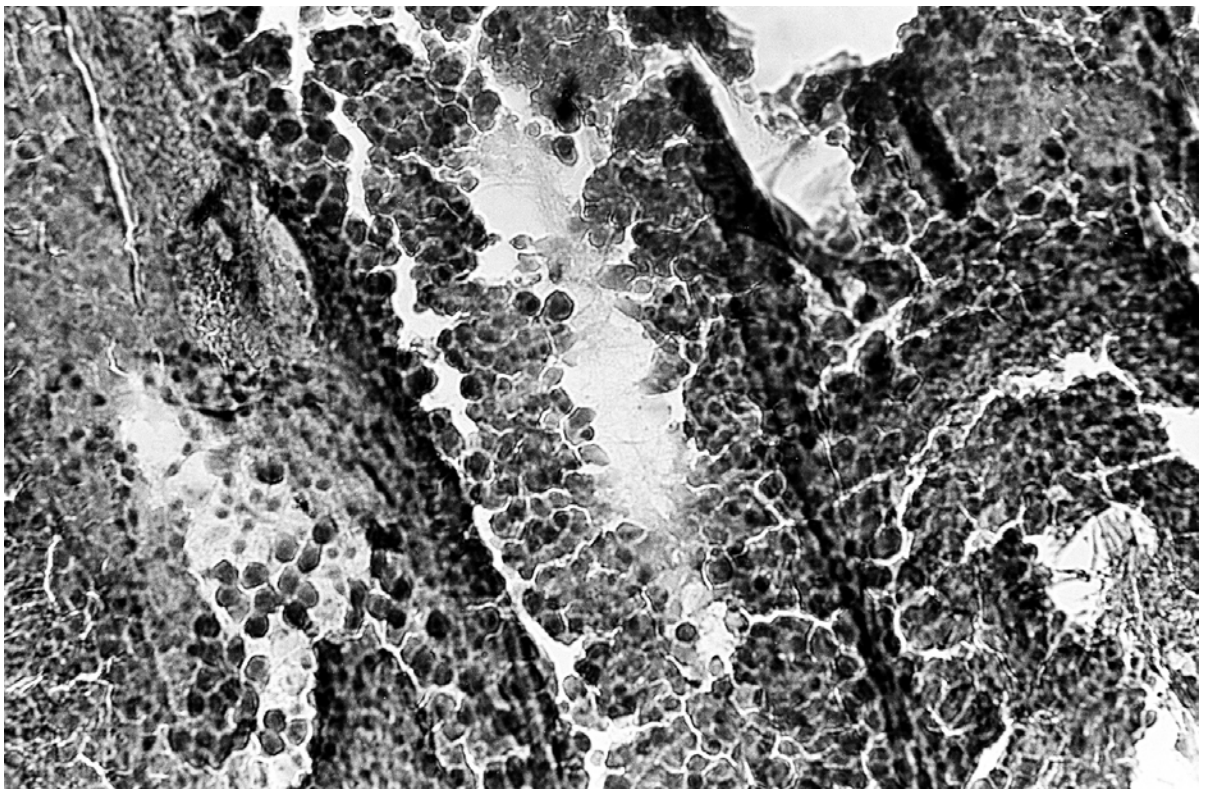


Fig. 45: The figure illustrates the testis of the hamster which borned to insulin injected mother. Note arrest of spermatogenesis, smaller leydig cells (x 600).

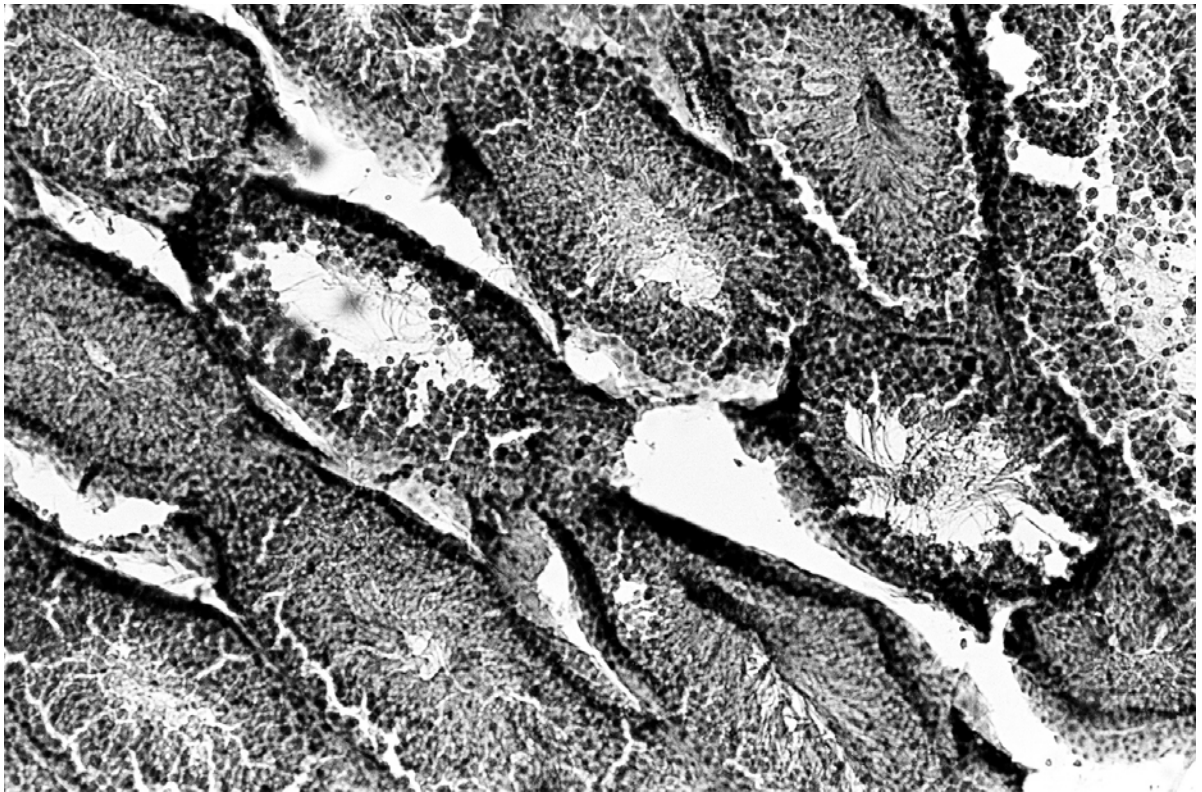


Fig. 46: The figure illustrates the testis of the hamster which borned to melatonin injected mother. Note arrest of spermatogenesis, smaller leydig cells (x 400).

3.4.2 Experiment 2:

Body weight (Figure 47), brain weight (Figure 48), volume (Figure 49), neurons per mm³ (Figure 50) and total neuron numbers (Figure 51) were higher in hamsters borned to leptin injected mothers. Brain growth of the hamsters was significantly increased by leptin when compared with the controls (Table 13). A significant increase in brain volume was obvious in hamsters borned to leptin injected mothers. The cerebral cortex of the leptin (Fig. 52, 54) group was noticeably larger than that of controls (Fig 53, 55). The results of the stereological measurements for the cerebral cortex in both hemispheres were presented in Table 13. The volume of the neocortex was % greater in leptin group compared to controls. This increase in volume was due to significant increases in both the pial surface area of the cortex and the mean cortical thickness.

Table 13: Morphometric variables of the cerebral cortex in both hemispheres for leptin and control hamster on postnatal day 1. All values are presented as the mean $\pm$ standard error of the mean (SEM).

	Control (n=8)	Leptin (n=8)
Body Weight (g)	2.83 $\pm$ 0.25	3.26 $\pm$ 0.33
Brain Weight (mg)	150.75 $\pm$ 8.24	178.56 $\pm$ 9.55
Volume (mm³)	35.42 $\pm$ 2.51	52.15 $\pm$ 4.45
Neurons/mm³	85,360 $\pm$ 2,451	92,750 $\pm$ 4,115
Total Neurons	3,023,451 $\pm$ 75,652	4,836,912 $\pm$ 92,887

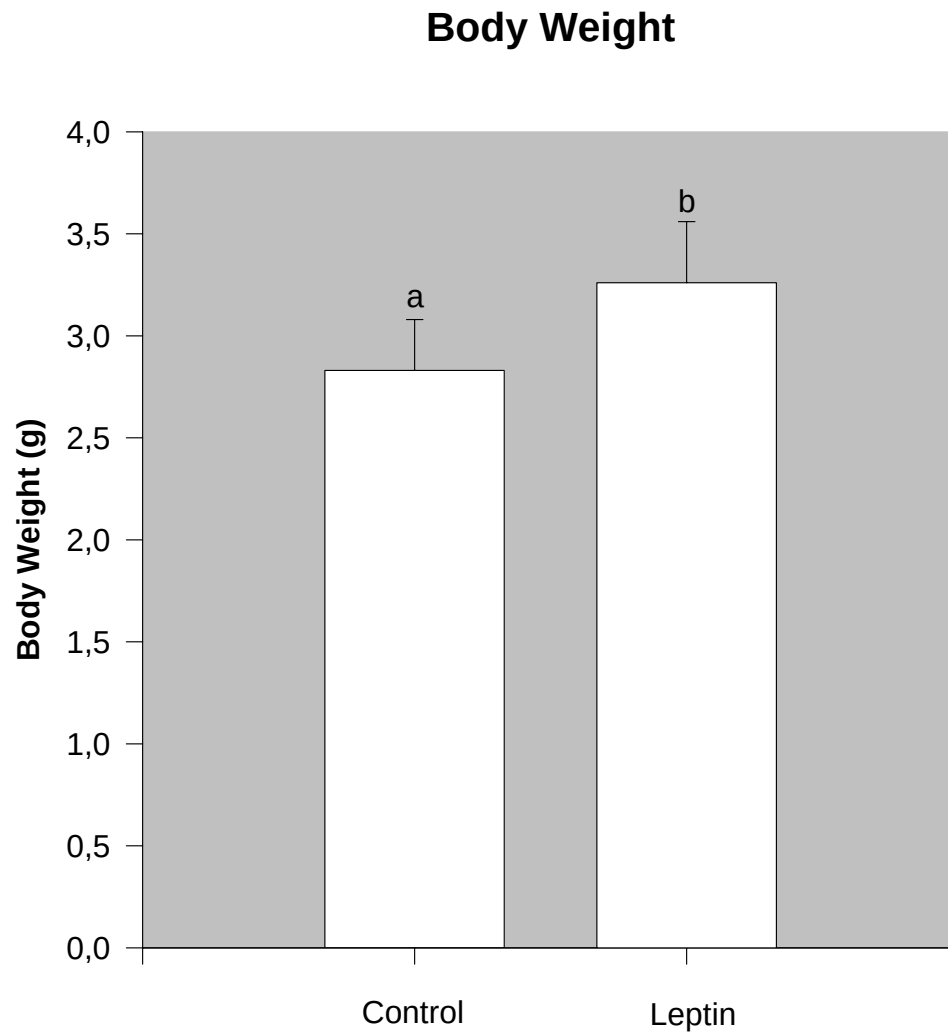


Fig. 47: The figure illustrates the body weights of postnatal hamsters borned to control and leptin injected mothers.

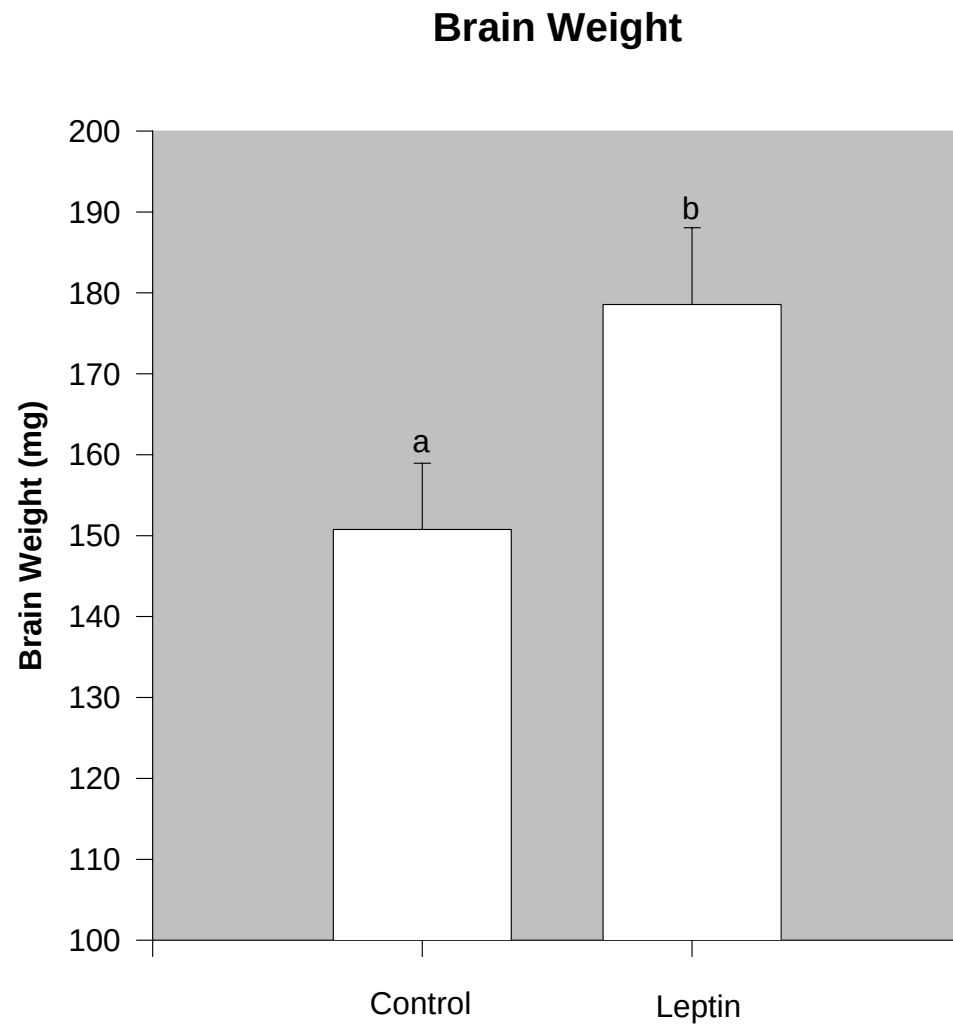


Fig. 48: The figure illustrates the brain weights of postnatal hamsters born to control and leptin injected mothers.

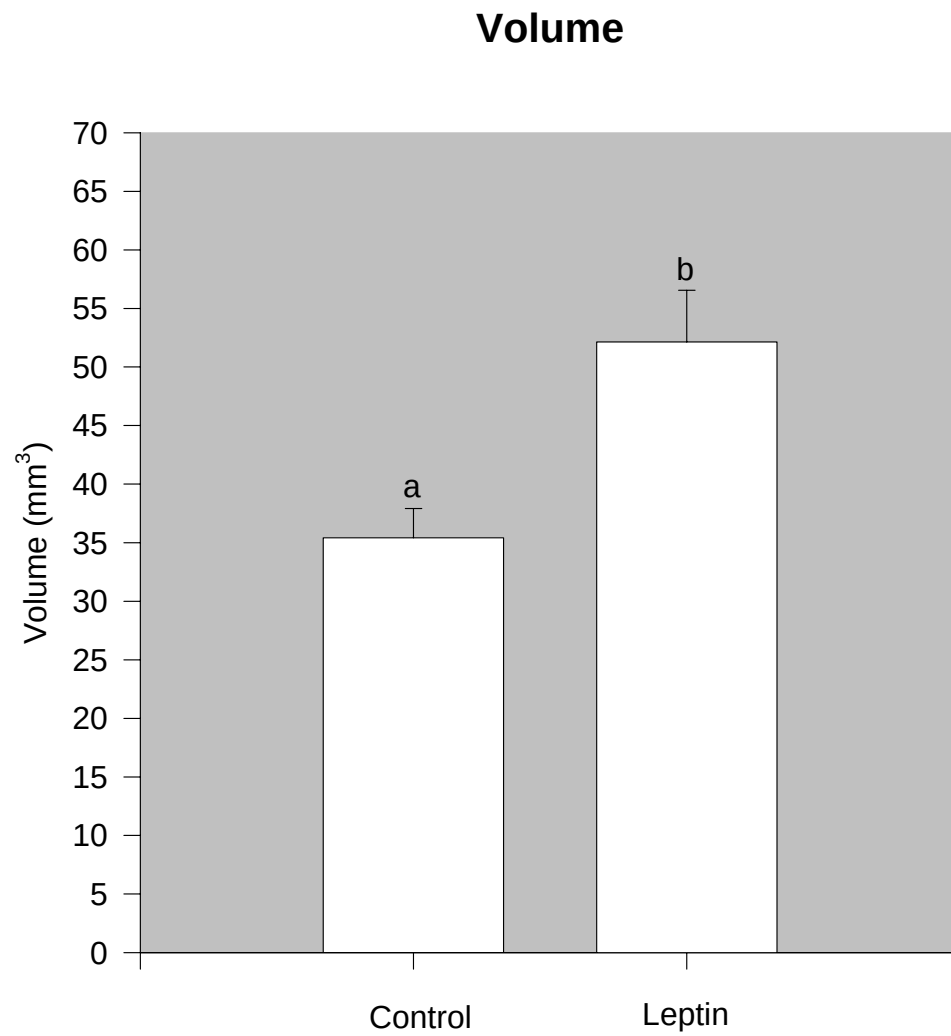


Fig. 49: The figure illustrates the cortex volume of postnatal hamsters born to control and leptin injected mothers.

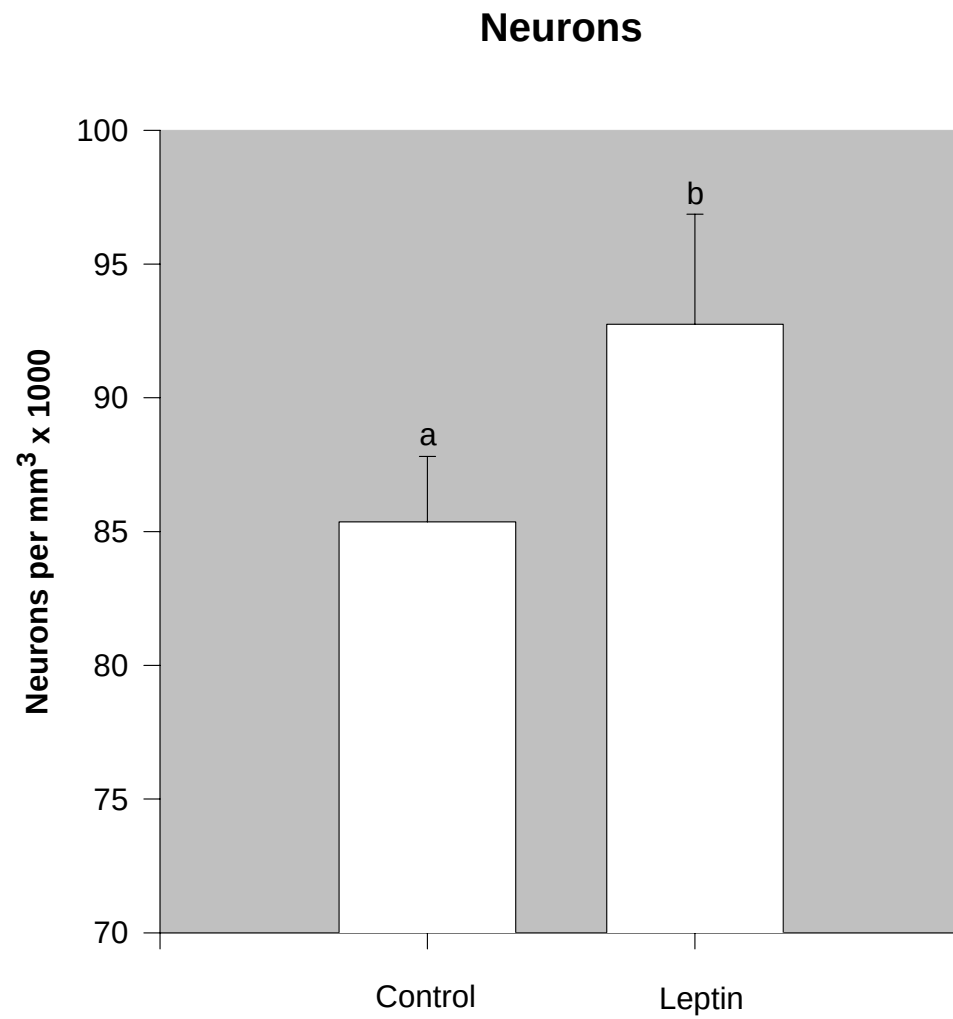


Fig. 50: The figure illustrates the neurons of postnatal hamsters born to control and leptin injected mothers.

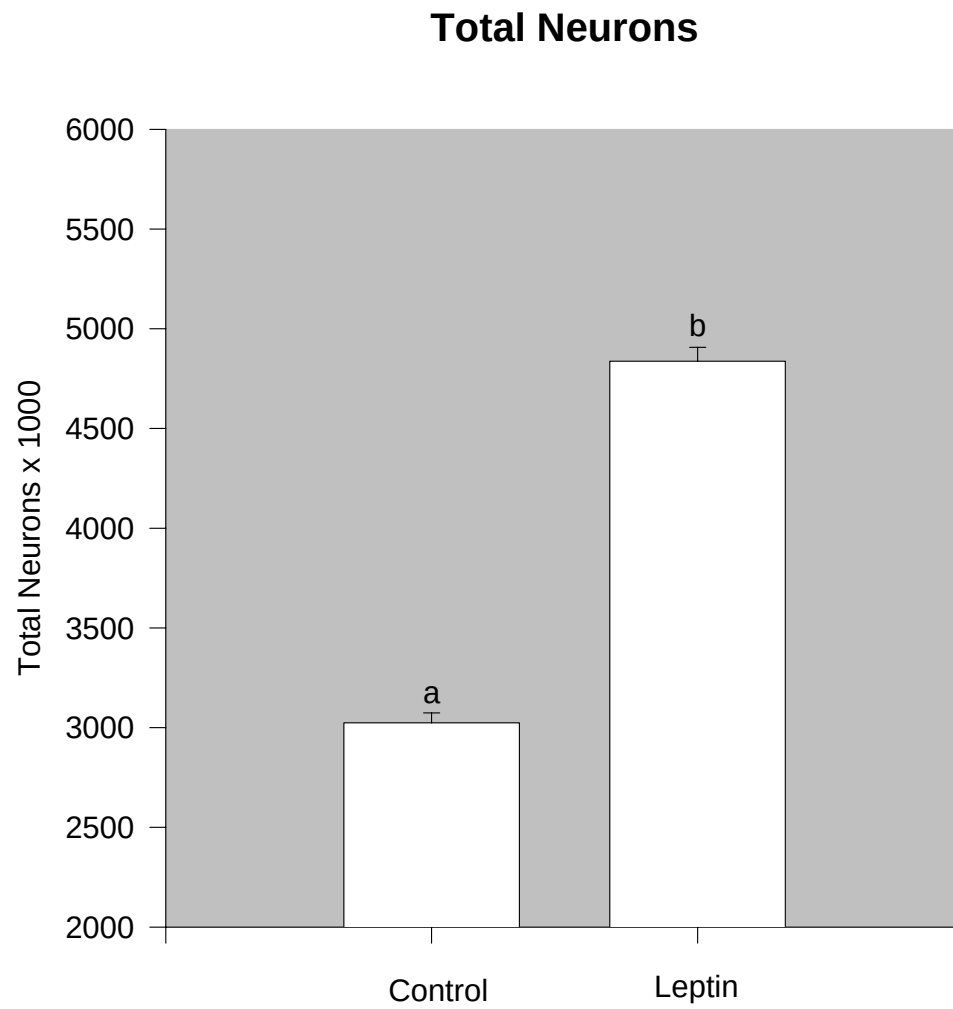


Fig. 51: The figure illustrates the total neuron number of postnatal hamsters born to control and leptin injected mothers.

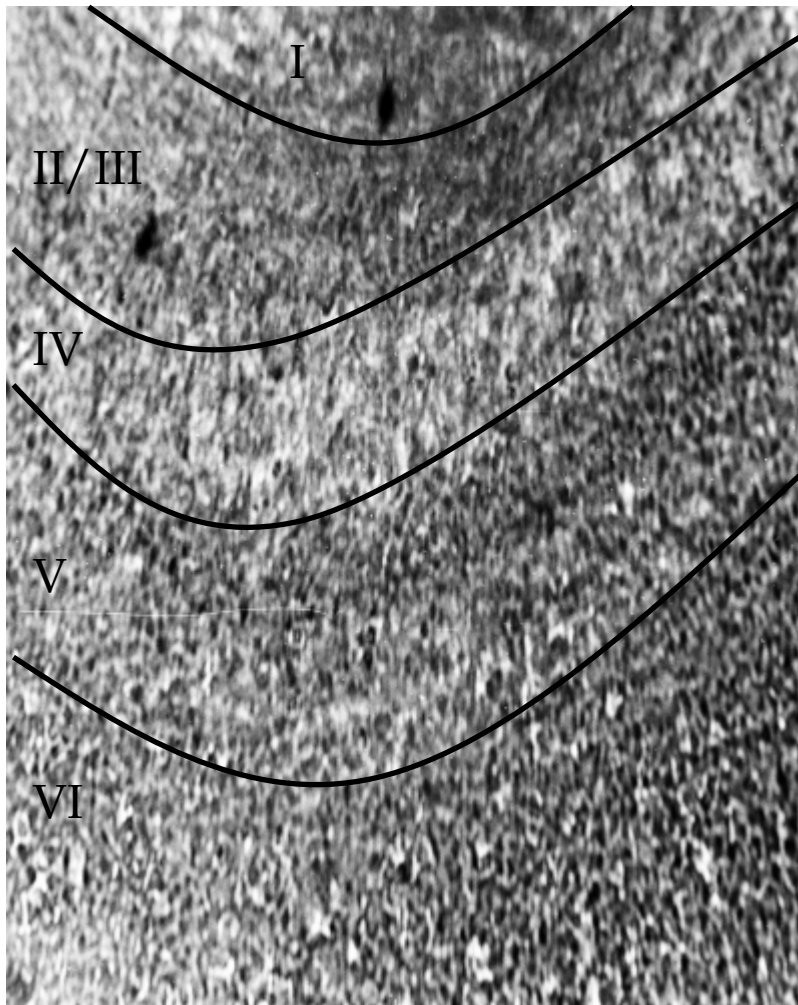


Fig. 52: Photomicrographs of the primary motor cortex in hamsters born to control mothers. Note that the cortical lamination and the neuronal packing density appear normal. (x800)

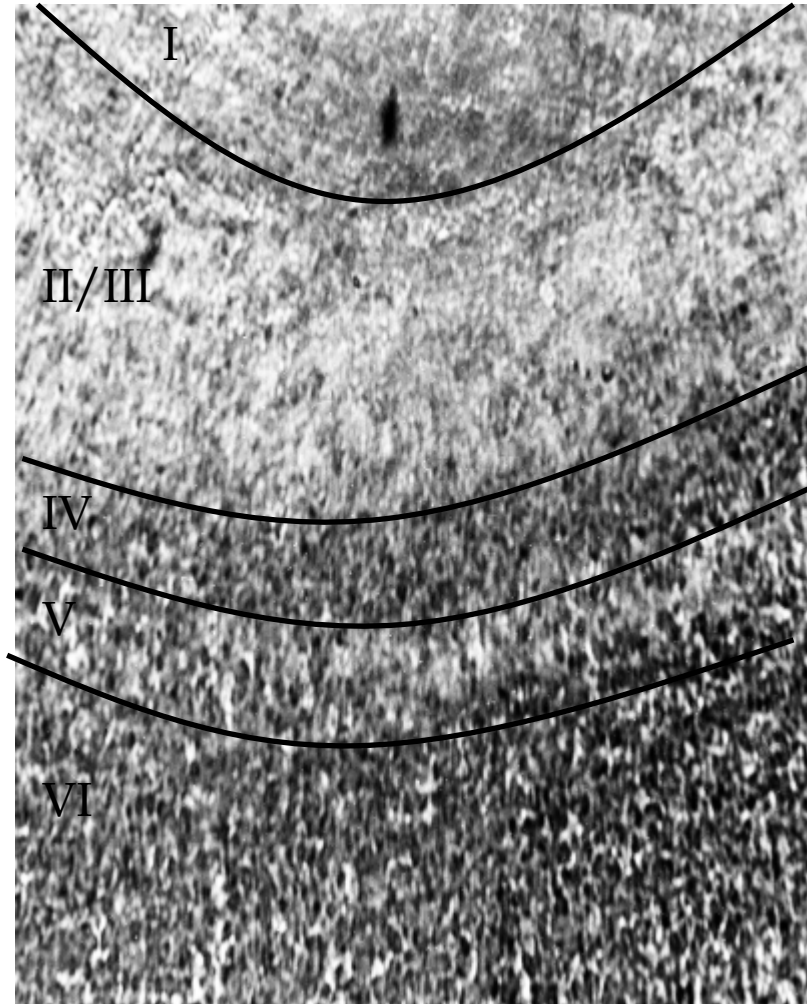


Fig. 53: Photomicrographs of the primary motor cortex in hamsters born to leptin-injected mothers. Note that the cortical lamination and the neuronal packing density appear more developed than normals. (x800)



Fig. 54: Photomicrographs of the motor cortex in hamsters born to control mother. Note that the cortical lamination and the neuronal packing density appear normal. (x400)

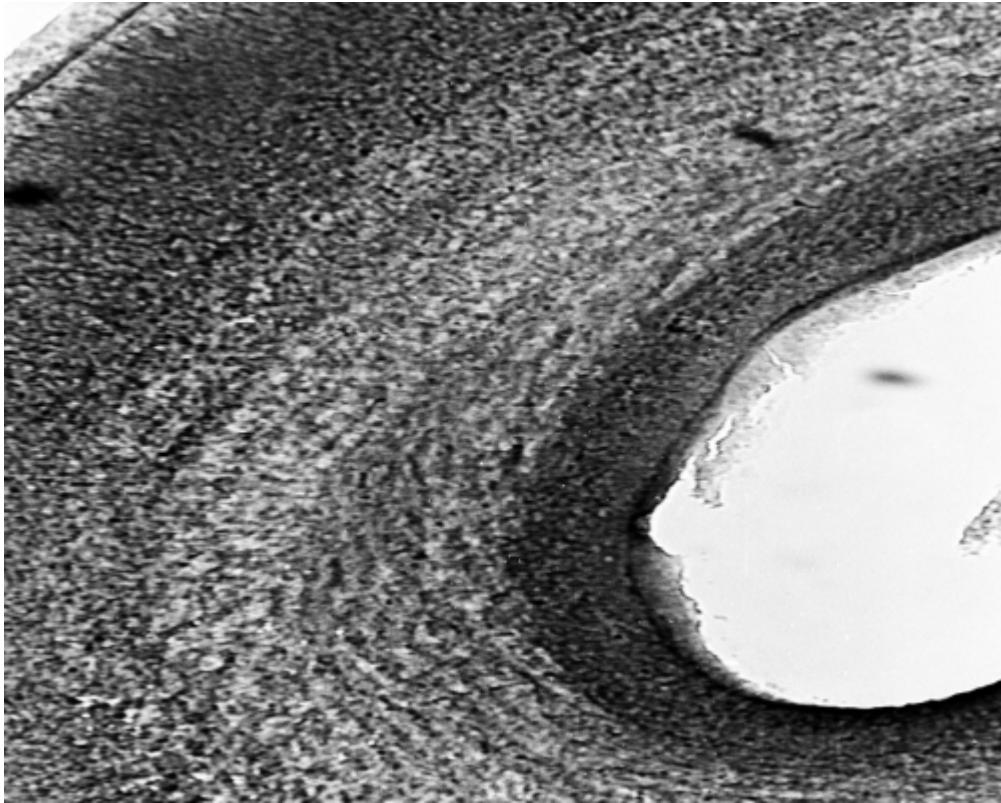


Fig. 55: Photomicrographs of the motor cortex in hamsters born to leptin injected mother. Note that the cortical lamination and the neuronal packing density appear more developed than normals. (x400)

3.5 Study 5

Table 14 shows leptin values in all groups at first week at day and night times. In the first week, day time serum leptin levels of the leptin injected group was very close to the leptin levels of the controls in all groups ($p>0,05$) (Fig. 56). Serum leptin levels of the lipectomized hamsters were lower compared to controls and leptin injected groups ($p<0,05$). There was not a significant difference among the 14L control, 14L pinealectomy, 10L control and 10L pinealectomy groups ($p>0,05$) (Fig. 56). Lipectomy decreased the leptin levels significantly whereas leptin injections elevated the leptin levels ($p<0,05$). The night time leptin levels differed when compared with the day time levels especially in 10L control group ($p<0,05$) (Fig. 57). Leptin levels of the leptin injected group were very close to the controls in all groups ($p>0,05$). The night time leptin levels of the lipectomized hamsters were lower than controls and leptin injected hamsters ($p<0,05$). However, the leptin levels of controls, leptin injected and lipectomized hamsters decreased in 10L control group ($p<0,05$) (Fig. 57).

Table 15 shows leptin values in all groups at eighth week at day and night times. In the eighth week, the day time and night time leptin levels of the leptin injected and lipectomized hamsters were significantly lower than the controls ($p<0,05$) (Fig. 58). The levels of the leptin were very close to each other in leptin injected and lipectomized groups. The night time leptin levels of the leptin injected and lipectomized hamsters were significantly lower than the

controls ($p < 0,05$) (Fig. 59). There was not a significant difference between the leptin levels of the leptin injected and the lipectomized hamsters ($p > 0,05$).

Table 14: First week, day and night time leptin levels of the control, leptin injected and lipectomized hamsters in 14 L and 10 L photoperiods.

	First Week 1200			First Week 2400		
	Cont.	Lep. Enj.	Lipx	Cont.	Lep. Enj.	Lipx
14L Control	14,75 ± 1,3	15,63 ± 1,5	4,3 ± 0,3	12,29 ± 1,1	14,71 ± 1,4	3,56 ± 0,3
14L Pinx	14,23 ± 1,25	16,42 ± 1,55	5,62 ± 0,33	15,34 ± 1,4	16,1 ± 1,5	4,83 ± 0,4
10 L Control	13,33 ± 1,13	14,56 ± 1,24	3,62 ± 0,25	9,33 ± 0,9	10,28 ± 1,1	3,17 ± 0,3
10 L Pinx	12,57 ± 1,15	13,63 ± 1,2	3,21 ± 0,2	13,16 ± 1,3	15,43 ± 1,5	5,1 ± 0,5

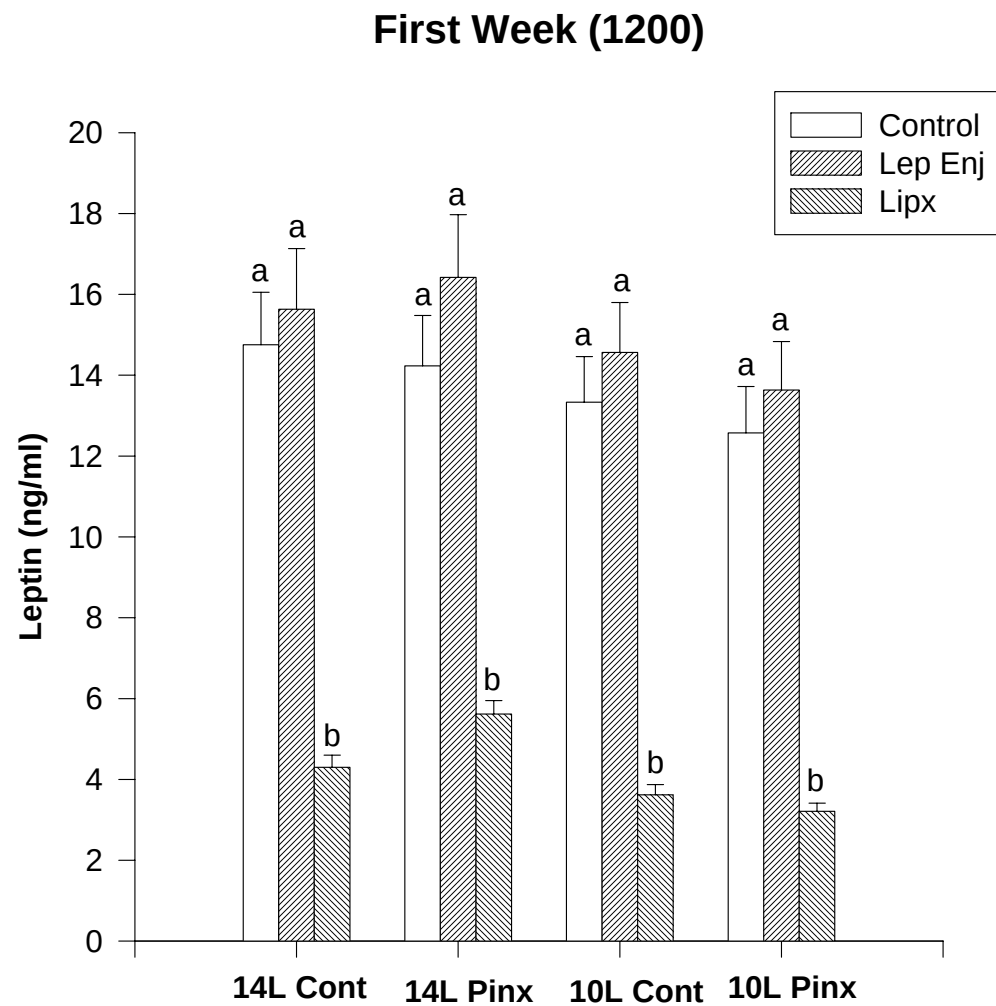


Fig. 56: The figure illustrates the first week day time leptin levels in control, leptin injected and lipectomized hamsters.

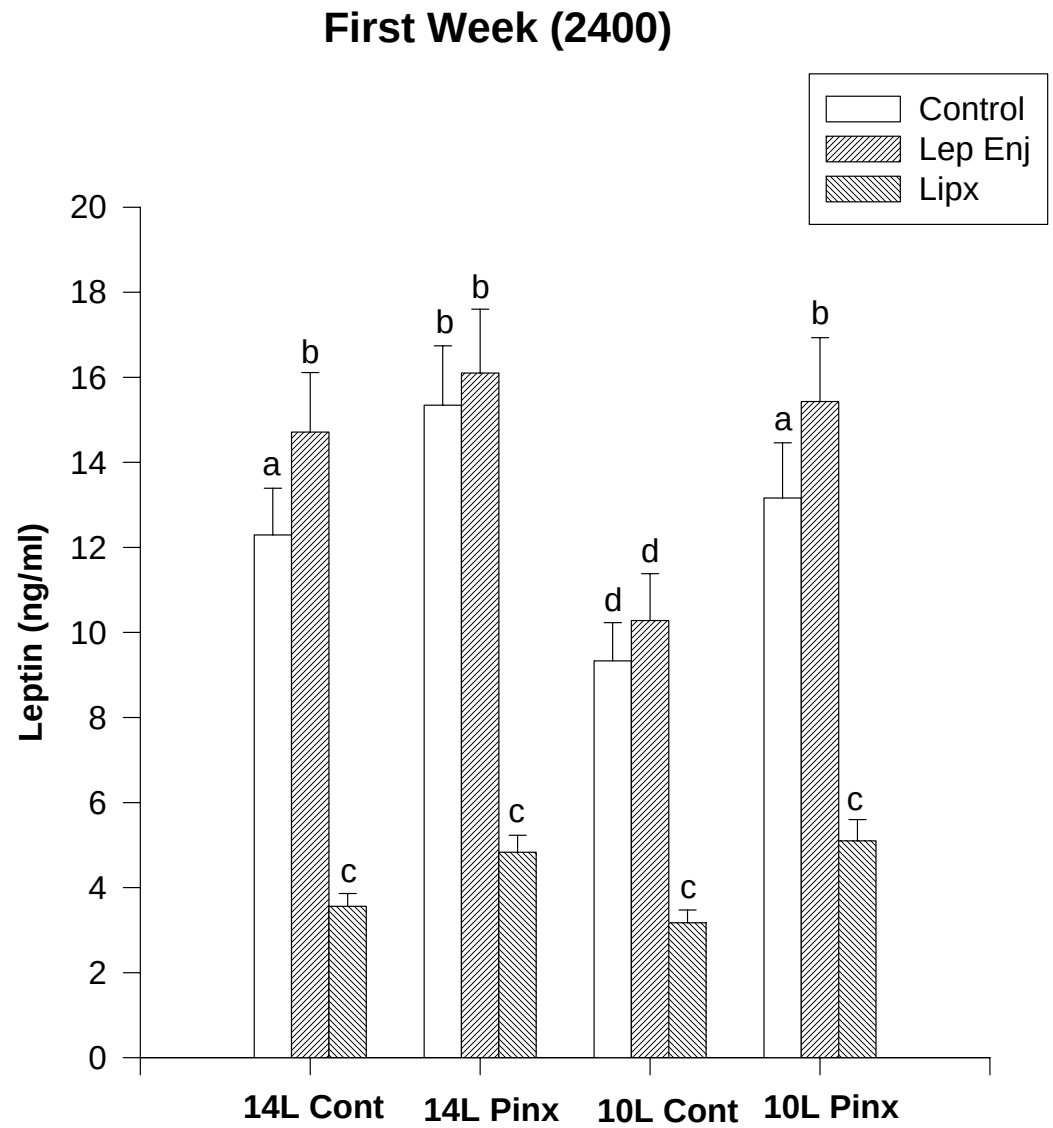


Fig. 57: The figure illustrates the first week night time leptin levels in control, leptin injected and lipectomized hamsters

Table 15: Eighth week, day and night time leptin levels of the control, leptin injected and lipectomized hamsters in 14 L and 10 L photoperiods.

	Eighth Week 1200			Eighth Week 2400		
	Cont.	Lep. Enj.	Lipx	Cont.	Lep. Enj.	Lipx
14L Control	16,3 ± 1,6	8,63 ± 0,8	8,14 ± 0,8	13,1 ± 1,3	7,63 ± 0,7	6,48 ± 0,6
14L Pinx	15,76 ± 1,5	9,45 ± 0,9	7,83 ± 0,7	14,87 ± 1,4	8,76 ± 0,8	7,65 ± 0,7
10 L Control	15,28 ± 1,5	9,33 ± 0,9	8,62 ± 0,8	13,88 ± 1,3	8,64 ± 0,8	7,93 ± 0,75
10 L Pinx	14,5 ± 1,4	8,76 ± 0,8	8,35 ± 0,8	14,42 ± 1,4	9,45 ± 0,9	7,4 ± 0,63

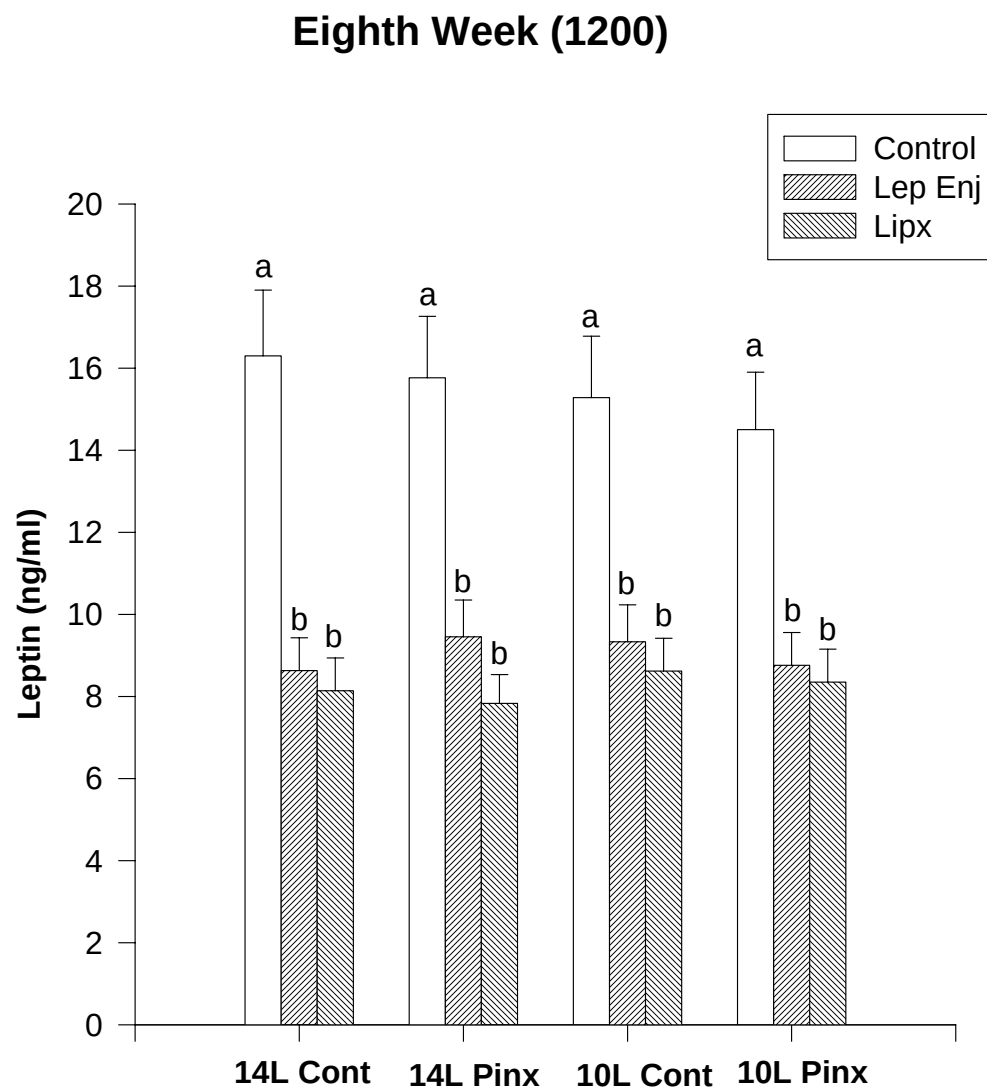


Fig. 58: The figure illustrates the eighth week day time leptin levels in control, leptin injected and lipectomized hamsters

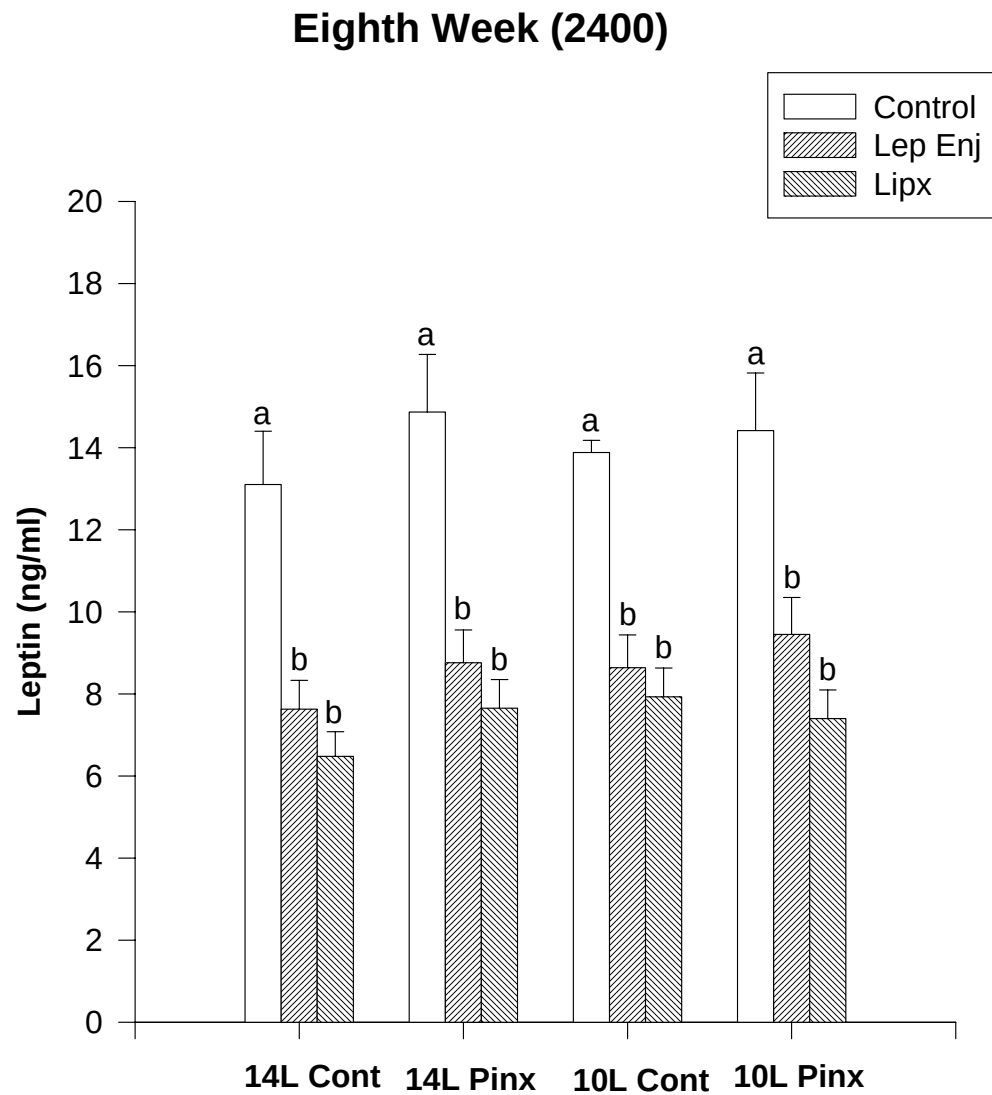


Fig. 59: The figure illustrates the eighth week night time leptin levels in control, leptin injected and lipectomized hamsters

4. DISCUSSION

4.1 Study 1

The present study indicated that the serum leptin concentration showed diurnal variations being directly associated with SCN in hamsters. Leptin production is known to be influenced by hormones, which have specific temporal relationships with feeding behaviour. For example, Wabitsch et al (1996) reported that cortisol promotes leptin production in cultured human fat cells. In contrast with such in vitro observations, administration of constant release cortisol did not induce a significant change in circulating leptin level in our hamster model. Our data demonstrate that the biological clock (SCN) controls the diurnal rhythm of plasma leptin levels directly independently of other clock-controlled rhythms. It is shown that the plasma leptin rhythm in adrenalectomized animals with constant cortisol release implants is identical to that observed in control group with a diurnal leptin peak at noon (12:00 h). Although chronic cortisol exposure is shown to increase leptin (Li et al., 1997; Masuzaki et al., 1997), we have shown that cortisol replacement did not modify the diurnal rhythm of leptin synthesis and release. Taken together glucocorticoids seem unlikely to be the major factor for diurnal variations of leptin in the hamster species. Moreover, modification of the normal daily feeding pattern of animals by introducing a fixed 24 h feeding schedule (i.e. meal at 0600 h or meal at 2000 h or cross feeding pattern) does not remove the diurnal cyclicality of leptin levels, in contrast with a clear circadian leptin synchronized by food intake in rodents (Saladin et al., 1995; Ahren, 2000) but in agreement with no circadian leptin rhythm in rat (Mistlberger, 1994). In addition, 60% of ad libitum food restriction has lowered plasma leptin levels in Mongolian gerbils (Karakaş et al., 2005).

We showed that the endogenous concentration of leptin in blood varied at different times of the day and followed a diurnal pattern, being highest (15 ng/ml) at 1200 h, and lowest (10 ng/ml) at 2400 h. A robust daily variation in leptin levels has repeatedly been found in rats (Dallman et al., 1999; Kalsbeek et al., 2001), mice (Ahima et al., 1998), dogs (Ishioka et al., 2005) and human (Sinha et al., 1996; Licinio et al., 1998), while the results from Syrian and Siberian hamsters are inconsistent (Drazen et al., 2000; Horton et al., 2000; Gündüz, 2002) and no circadian rhythm has been detected in sheep (Tokuda et al., 2000; Daniel et al., 2002). The exact causes of the diurnal rhythm are not clear and seems very complex. Possible influences on this diurnal rhythm include obesity and body fat distribution, feeding schedule, insulin and glucose levels, glucocorticoids and hormones (i.e., melatonin), indicating neuroendocrine control.

Melatonin decreased visceral adiposity, leptin and insulin without altering food intake (Wolden-Hansen et al., 2000). Pinealectomy increases leptin secretion therefore melatonin exerts an inhibitory effect on leptin release (Gündüz, 2002; Canpolat et al., 2001). Both anatomical and physiological techniques have demonstrated that the SCN control the sympathetic input to the pineal gland by contacting those PVN neurons. PVN transduces the leptin signal to autonomic and endocrine control sites involved in maintenance of homeostasis. For example, medial PVN control secretion of hormones, including melatonin, TSH, GH and ACTH. The PVN may also been implicated in control of feeding behaviour, as lesions the PVN induce hyperphagia and obesity. Therefore, it is possible that, opposite to the rhythm in melatonin release, SCN control of leptin release is mainly stimulatory, and thus removal of this stimulatory SCN control results in increased leptin levels. However, pinealectomized hamsters also showed an

increased plasma leptin levels (Gündüz, 2002). The neural connections from the SCN to white adipose tissue through the sympathetic nervous system support the view that lighting conditions can control fat metabolism directly. Therefore, it must be elucidated that whether SCN can control the adipose tissue directly or through melatonin rhythmic release.

The 24-h rhythm of serum melatonin exhibits peak levels during the dark period and lowest levels during the light period in both nocturnal and diurnal species. Because the reported nocturnal increase in serum leptin in humans and murine rodents is similar but it is opposite in Syrian hamster to the pattern of melatonin secretion, the 24-h rhythm of leptin levels has been hypothesized to be controlled by the circadian system.

It is believed that leptin establishes a feedback loop between the energy reserves and the hypothalamic centers that control food intake (Prolo et al., 1998). Leptin receptors are widely distributed throughout the brain including the choroid plexus, cerebral cortex, hippocampus and thalamus. In the hypothalamus, especially strong immunoactivity for leptin receptors was found in the VMH, ARN, LHR and SCN (Guan et al., 1997). As well as participatory in the expression of CRH in the hypothalamus leptin also interacts with ACTH in the adrenals and its levels are regulated by glucocorticoids. Adrenal steroids may modulate the activity of hypothalamic NPY neurons (Hisano et al., 1988). Cortisol pellets may stimulate NPY-containing neurons directly to increase NPY content. However, results show in Fig. 4 indicated no difference in leptin secretion between ADx + cortisol implant and sham-operated animals, revealing the non-involvement of adrenal corticoids.

The absence of a nocturnal rise in leptin is in sharp contrast with reports in humans, rats and mice (Prolo et al., 1998; Trayhurn et al., 1999). The differences among

rats, mice and hamsters may be related to species-specific difference in feeding behaviour or metabolism. Rats and mice consume most of their food during the night; thus the nocturnal rise in circulating leptin levels in rats and mice may be primarily due to increased food consumption at night, whereas caloric consumption in hamsters is more uniformly distributed throughout the day and night. Syrian hamster consume approximately equal amounts of food throughout the day and night (Rowland, 1985).

The identification of significant temporal variation in the 24-h leptin rhythm in animals suggests that the regulation and secretion of leptin may be under multifactorial control. This is consistent with numerous hormonal and gene expression *in vivo* and *in vitro*; many of those factors are regulated primarily by circadian system but not at least by meals and glucocorticoids.

The SCN plays a key role in the induction, synchronization and entrainment to the environmental light/dark cycle of circadian rhythms in behavioral, metabolic and endocrine functions (Rusak and Zucker, 1979). The disappear of the diurnal leptin rhythm after SCN lesion does not prove the direct control of leptin release because by the SCN lesions not only the leptin rhythm but also the other clock controlled rhythms of the physiological parameters such as feeding and release of glucocorticoids are abolished. Therefore, to investigate separately the respective roles of the SCN, adrenal glucocorticoids and feeding rhythms in the control of leptin rhythmicity, we examined daily plasma leptin levels in three different experimental groups; SCN lesioned, different feeding scheduled, adrenalectomized and implanted with cortisol implants.

Many studies have shown that changes in the daily pattern of food intake can affect plasma leptin levels (Schoeller et al., 1997; Simon et al., 1998). A

phase delay in the timing of the meals may cause a phase delay in the nocturnal leptin peak (Schoeller et al., 1997). Mice show an extra leptin surge during the day time when one day time meal was restricted to 4 hour. On the other hand, in human, the diurnal leptin rhythm does not change after a continuous enteral feeding (Simon et al., 1998). The results of the present study are consistent with the results of the human experiments. These results show that in addition to food intake, other factors are involved in the diurnal regulation of leptin secretion.

Syrian hamster is an animal with strong seasonality and photoperiodism. Although Syrian hamster has been used extensively as an animal model for the study of the physiological mechanisms that underlie photoperiodic control of the circadian rhythms, there is not much information in the literature about the relationship between the leptin hormone and the SCN. Kalsbeek et al (2001) showed that the biological clock (SCN) controls the diurnal rhythm of plasma leptin levels directly, independently of other clock-controlled rhythms (corticosterone, insulin, feeding) in rats. Ablation of SCN eliminated the diurnal rhythmicity of circulating plasma leptin levels. Our results agree with findings reported by Kalsbeek et al (2001) in rats. However, hamsters and rats differ significantly in their responses to metabolic challenge. The Syrian hamster does not express hyperphagia in response to food deprivation, and can not adapt to restricted daily feeding schedules or alternating daily availability, even if supply during the feeding period is unlimited (Silverman and Zucker, 1976). It has been speculated that the inability of the Syrian hamsters to adapt their feeding behaviour in response to

these challenges reflects an insensitivity to some signal of energy depletion (Silverman and Zucker, 1976), or perhaps a species difference in the neuroendocrine regulation of feeding behaviour. The possibility that the failure of Syrian hamsters to compensate for food deprivation could be due to insensitivity to NPY has been addressed in studies in which the peptide was injected ICV into ad lib-fed animals. NPY, a potent stimulant of eating behaviour in many species, has been strongly implicated in the hypothalamic regulation of food intake and energy balance in the rat. Within the rat hypothalamus, NPY is synthesized predominantly in the arcuate nucleus from which axons project to the paraventricular nucleus. Food deprivation and attendant weight loss induce increases in NPY gene expression in the arcuate nucleus. In the rat, increases in NPY gene expression and synthesis in hyperphagic models are accompanied by elevated plasma glucocorticoids and reduced insulin titre or defects in insulin signaling. Syrian hamsters response to food deprivation with expected changes in body weight and plasma insulin titre.

The role of feeding as a zeitgeber is very well established. Entrainment of free-running circadian rhythms by the restriction of food or water availability to a few hours each day has been demonstrated in various species (Jilge, 1991; Kennedy et al., 1995). Feeding after several days of fasting has also been shown to cause phase shifts (Challet et al., 1997). Hunger is obviously a very strong motivational stimulus. Thus, when animals were placed under the conflicting condition of being exposed simultaneously to a light-dark cycle and a cycle of food availability, their behaviour showed a compromise between the two drives, and food was often found to be the dominant stimulus (Mistlberger,

1991). Animals under a restricted feeding regimen regularly anticipate the time of feeding because they become active several hours before the food becomes available each day (Abe and Rusak, 1992). This anticipation could be timed by the circadian system. However, animals will anticipate feeding even when they do not entrain to the feeding cycle (Kennedy et al., 1991). These results in a form of splitting of two activity components: one of them free running and the other apparently pseudo-entrained by the feeding cycle. Because of the long-lasting nature of this form of splitting, and because the feeding component clearly anticipates the availability of food independently of circadian time, a separate timing mechanism must be inferred. The data strongly suggest that there is an independent food-entrainable-pacemaker.

The stored fat in the white adipose tissue informs the central nervous system via leptin about the energy available for different purposes. Although leptin levels in many species is determined by the amount of fat deposits present in the body, in the Syrian hamster it has been demonstrated that plasma leptin levels do not accurately reflect the body fat content in all conditions (Scheneider et al., 2004). Data obtained in rats and mice suggest that serum leptin concentrations and leptin mRNA content in adipose tissue are elevated during the dark phase relative to the light phase (Ahima et al., 1998). It has been shown that leptin concentration is changed with photoperiod in adult Syrian hamsters and leptin level exhibited circadian rhythm in a previous study (Gündüz, 2002). There is a reverse relationship between plasma melatonin and level of leptin connected to photoperiod and/or pineal gland; leptin levels of hamsters in light phase high but melatonin levels are low, on the contrary that

happens opposite in the dark phase (Gündüz, 2002). Although the exact mechanism is not clear, melatonin may inhibit leptin release directly from adipose tissue. It was demonstrated that daily rhythm of leptin changes in rodents (Ahima et. al., 1996) and humans (Sinha et. al., 1996). Studies on humans especially in children showed serum leptin levels increased in dark phase and decreased in the morning (Schoeller et. al., 1997). It was demonstrated that plasma leptin level reaches its highest value at 2400 h and remains low between 09:00 and 12:00 in young humans. In other study, it was demonstrated that leptin shows circadian rhythm and remains high level in mornings and low level in midday (Flier, 1998). It was exhibited that, leptin secretion profile in human has a similar relationship with prolactin, triotropin and melatonin, on the contrary of that secretion profile of leptin has a reverse relationship with cortisol and adrenocorticotrophic hormones (Mantzoros and Moschos, 1998). Although, Mantzoros and Moschos (1998) found similar rhythmic relationship between melatonin and leptin in human examples, this rhythmic relationship is reverse in adult golden hamsters (Gündüz, 2002).

In rats and hamsters, food-anticipatory rhythms have been shown to persist after SCN (Abe and Rusak, 1992) and IGL lesions (Mistlberger, 1994). In intact and SCN ablated rodents, these rhythms exhibit several properties, including circadian limits of entrainment and persistence during food deprivation, that are consistent with regulation by a circadian pacemaker. Evidently this pacemaker is outside of the SCN and is entrained by input pathways independent of the IGL. The site of this pacemaker and its entrainment pathway remain to be determined (Mistlberger, 1994).

Although the most important zeitgeber of melatonin secretion is the light-dark cycle, there is some evidence that the daily schedule of food availability might be another important external synchronizer for the circadian system. In rodents, daily restricted food access schedules resulted in marked phase shifts of the circadian rhythmicity of many biological variables. For example, in most mammalian species timed food restriction entrained the circadian rhythm of plasma corticosterone, body temperature and locomotor activity (Challet et al., 1997). In addition, it has been reported in rats that food restriction decreased the amplitude or phase-advanced pineal melatonin rhythm (Challet et al., 1997) but increased serum melatonin concentrations. Although food restriction seems to have an effect on the circadian system, it remains unclear whether it is the access schedule or food restriction that is the most influential.

The autonomic nervous system has an important role in the regulation of the leptin hormone release. The decrease in noradrenaline release causes hyperleptinemia, on the other hand, β -adrenergic receptor agonists decrease plasma leptin levels and leptin mRNA expression (Trayhurn et al., 1995). The regulation of the leptin hormone release by the sympathetic nervous system activity looks like the control of the melatonin hormone release from pineal gland. However there is an inverse relationship between the peak levels of the leptin and the melatonin hormones (Gunduz, 2002). The sympathetic input to the pineal gland is controlled by the SCN (Kalsbeek et al., 1996). The results of the first study suggest that the similar mechanisms are controlling the diurnal release of the hormones melatonin and the leptin. Therefore, it is possible that

SCN control of leptin release is mainly stimulatory and thus removal of the SCN controlled pineal gland results in increased leptin levels.

4.2 Study 2

In the second study, SCN lesions abolished the diurnal leptin rhythm in both photoperiods. This result is consistent with the first study. The aim of this study was to investigate the leptin release in SCN lesioned, pinealectomized and timed melatonin infused hamsters. In a recent study, we have determined the antagonistic relationship between the hormones melatonin and leptin (Gündüz, 2002). Melatonin suppresses the serum leptin levels and in pinealectomized hamsters serum leptin level remains high. The results agree with the results of the previous study. Melatonin infusions suppressed the serum leptin levels in both photoperiods.

The other interesting point is the similar effects of the 1- and 10- hour melatonin infusions on the leptin levels. Infusion times were selected to differentiate two hypothesis of the melatonin action; coincidence and duration. Both infusions have given similar effect; low leptin levels. However, the suppressional effect was significantly bigger in 10-hour infusion group.

Concerning the nature of the melatonin message, there is still a debate about how melatonin signal is read by animal. Duration hypothesis depends on the fact that the changing photoperiod likewise alters the duration of the daily melatonin peak and this signals the organism as to daylength and the appropriate endocrine adjustments are made. Coincidence hypothesis depends on the synchronization of elevated melatonin levels with the sensitivity of particular organ system to the melatonin peak; when this occurs the organ responds

accordingly. Our results support both hypothesis but we are unable to give a clear explanation why both infusions showed a similar effect. One possibility is that both infusions started at the same CT time; CT12. This time is the most sensitive time for melatonin action in reproduction. On the other hand this is a new data that mel infusion does not restore the leptin rhythm. It has been shown that the rhythms such as locomotor activity can be reentrained by the timed melatonin administrations. The possible reason may be the duration of the experiment. It might be possible to reentrain the leptin rhythm by longer melatonin administrations.

The results of the second study support the findings that pineal gland and/or melatonin hormone have an important regulatory role in leptin hormone release.

4.3 Study 3

The results of the third study show that leptin administrations advanced the locomotor activity rhythm when applied at CT 10. Before the administrations, animals started their activity at CT 12 (2000). After the administrations, animals started their activity before CT 12. These results provide the first evidence that in vivo leptin treatment is an important regulatory factor affecting the SCN to modulate locomotor activity rhythms of adult male Syrian hamsters. The rhythm of the locomotor activity is driven by the SCN, thus, an early onset of activity in the locomotor activity due to an early administration of the leptin hormone could have a phase-advancing effect on the SCN clock. Our in vivo results are consistent with the results of the in vitro leptin administration (Prosser and Bergeron, 2003).

Constant light was used to make the activity as free-run. Locomotor activity levels decreased in all groups because of the suppressional effect of the constant light. Period lengths of the control and injection groups were very close to each other; around 24 hours. These results indicate that leptin injections does not act on locomotor activity rhythm in constant light photoperiod. One of the possible explanation was that the functional pinealectomy effect of the constant light. The melatonin hormone rhythm and the feedback of this hormone to the SCN are abolished. Hence, SCN continuous with the rhythm before the constant light.

Since the results from the first experiment was not clear, we have decided to use constant darkness condition. Constant darkness is generally used to obtain a constant environmental light condition. Understanding the influence of a chemical on rhythm it must be applied in the constant conditions. It has been shown for the first time that in vivo leptin administration would affect the locomotor activity rhythm. Constant darkness was the key point of the influence because this photoperiod did not block the SCN and the pineal gland and also did not suppress the locomotor activity levels. The doses of the hormone was very important. Only 4 $\mu\text{g/kg}$ leptin injections affected the locomotor activity rhythm. Dose should be adequate because of the half life and the saturation of the transporter system in blood-brain barrier.

It is clear that infusions are much more effective than injections. One hour leptin infusions caused bigger phase shifts in the locomotor activity onsets. The biggest phase advances were obtained by the icv leptin infusions. According to these results, icv administration is much more effective.

In the next step, we studied whether the arrhythmic locomotor activity rhythm due to the SCN lesions might be reentrained by the leptin infusions. Infusions corrected arrhythmic activity. This is surprising result, because there must be other factors involving leptin's effect on rhythmicity besides SCN. We know that SCN controls the leptin rhythm. Therefore, if leptin is administered at the right time to SCN-lesioned animal, it will initiate a cascade involving melatonin release.

There may be several key components to the development of the concept of the central nervous system's effect for peripheral peptides. For example, peptides injected into the periphery can affect the brain, directly or indirectly. A single peptide can exert more than one action. An intact peptide can directly cross the blood-brain barrier (BBB) to exert such actions. Leptin is a satiety polypeptide which is produced by the adipose tissue in the periphery but exerts its effects in the brain. Leptin is able to enter the brain because of a saturable transporter (Banks et al., 1996) located at both the endothelial and the epithelial (blood-CSF barrier) arms of the blood-brain barrier. How these non-peptide and peptide-protein substances interact with one another is an area of investigation. One possible mechanism of interaction yet to be considered is that non-peptides control the rate at which leptin is transported into the brain.

4.4 Study 4

Growth of the fetus in utero is the result of maternal nutrition, nutrient transfer and metabolism across the placenta, and fetal metabolism, regulated by growth factors. The interaction among these processes is very complex and varies throughout the gestation. Fetal and placental nutritional needs are

determined by two major components: the formation of new tissues accompanied by fetal and placental growth, and the production of energy within both the fetus and placenta.

It has been shown that placenta produces high levels of leptin (Masuzaki et al., 1997) and foetal tissues also produces leptin and contain leptin receptors. Leptin deficiency or insensitivity decreases brain weight and protein content. On the other hand, postnatal administration of leptin normalizes the levels of these proteins and brain weight in ob/ob mice (Ahima et al. 1998). Ob/ob mice has been shown to display deficiencies in brain myelin, reduced neuronal soma size and altered dendritic orientation compared to control mice, supporting the view that leptin deficiency affects CNS development.

We have previously shown that leptin administration stimulates the gonadal and body weight development of the juvenile Syrian hamsters and the onset of these animals to puberty was faster than the control animals (Pehlivan and Gündüz, unpublished data). In the present study we have investigated the effects of maternal leptin administrations on fetal and prepubertal development of the Syrian hamsters. It has been shown that prepubertal photoperiods are important factors on reproductive development (Gündüz and Stetson, 1994). On the other hand, gestational photoperiods may also play an important role on reproductive development (Gündüz and Stetson, 1998). We have found similar effects in our studies and prepubertal hamsters borned to leptin injected mothers exhibited a rapid gonadal development whereas the prepubertal hamsters borned to control mothers exhibited a slow gonadal development.

The previous studies have shown that the environmental photoperiods during the gestation affects the postnatal reproductive system development of many species. This photoperiodic information is transmitted to the fetus by the hormone melatonin. However, the gonadal development of the prepubertal hamster does not depend on the environmental photoperiods. Thereby, in the first experiment, melatonin injections have no effect on the prepubertal gonadal development of the juvenile Syrian hamsters. On the other hand, leptin stimulated the development. In fact, leptin is a cytokin which stimulates the cellular growth and division. Insulin injections suppressed the prepubertal development. All investigated parameters were lower than the controls. Insulin stores the glucose into the cells and decreases the serum glucose levels. Therefore, fetus could not get enough food from mother.

It has been suggested that leptin may act as a growth factor in the fetus, directing growth and development via central or peripheral actions (Udagawa et al., 2000). Many studies have measured plasma leptin concentrations in newborn infants; however, the results from these studies have been highly inconsistent. These contrasting findings may be due to a number of confounding factors, including geographical location, maternal and social differences.

Many adaptations occur in maternal energy metabolism for the fetal growth in gestation period. Leptin may have a role in the coordination of maternal adaptation due to its regulatory role in energy metabolism. In some species like mouse, bat, rat and human, both serum leptin levels and food intake increase in gestation period. This situation exhibits that the leptin levels are

regulated without the effect of energy balance and body lipid content but this is not consistent with the effect of leptin in food intake. However in Syrian hamster decreasing leptin levels rise the appetite and food intake during the gestation. According to this data, opposite to other species, regulation of leptin concentrations in gestation period is achieved by energy balance and body lipid content.

The lack of consistent correlation between circulating leptin and body adiposity in pregnant animals, however, suggest that leptin is secreted during pregnancy from a non adipose site. Consistent with this hypothesis, human placentae and cell lines derived from human choriocarcinomas express leptin mRNA at comparable or greater levels than adipose tissue and they also produce leptin protein. In rodents, however, conflicting results have been reported. Kawai et.al. reported that leptin mRNA was expressed in adipose but not in placental tissue. Other investigators have observed low levels of leptin mRNA in rat placenta, which in one case, increased as pregnancy progressed. In mice, leptin protein content in the placenta is high, but whether leptin mRNA is expressed in mouse placenta is equivocal. Thus, whether the placenta contributes to hyperleptinemia of pregnancy in rodents remains unresolved. The factors responsible for up-regulation adipose leptin expression and secretion during pregnancy are unclear, but two possible candidates are estradiol and glucocorticoids. As with leptin, the circulating concentrations of these hormones increase during pregnancy. Both hormones also increase leptin gene expression in rodent and women adipose tissue. For example, adrenalectomy and ovariectomy decreases leptin mRNA levels in adipose tissue among rodents,

and these effects are reserved by glycocorticoids or estradiol replacement, respectively.

Histological examination of the testes showed that there was a complete spermatogenesis in hamsters borned to leptin injected, control and melatonin injected mothers. Leptin hormone accelerates the onset of puberty by stimulating GnRH from the hypothalamus. Since melatonin suppresses the onset of puberty in many species by decreasing the release of GnRH, this mechanism does not work in juvenile Syrian hamsters. On the other hand, the testes of the hamsters borned to insulin injected mothers were suppressed. Insulin suppresses all reproductive activity including spermatogenesis and ovulation. Thus, this hormone decreases the development of the testis functions.

Histological examination of the brains showed that leptin stimulated the brain development when compared with the controls. Leptin receptors are found nearly in all organs of the fetus including the brain. Leptin may affect the development of the brain by directly binding to it's own receptors.

4.5 Study 5

The results of the present study have shown that at the first week of the experiment, leptin levels of the injection group were high and the leptin levels of the lipectomized hamsters were low (Fig. 55, 56). However, at eight week, leptin levels of the injection group decreased and the leptin levels of the lipectomized hamsters increased when compared with the first week results (Fig.57, 58). Possible reason for a decrease in leptin levels may be the decrease in adipose tissue by the leptin injections. Increase in leptin levels may be

adipose weight gain during eight weeks. Lipectomized animals need to increase their food intake and/or decrease their energy expenditure in order to provide sufficient energy for compensation in lipid deposition. However, fat pad removal does not produce a significant increase in food intake in Syrian hamsters (Mauer et al., 2001).

Kennedy (1953) proposed for the first time the lipostatic hypothesis to explain the body fat regulation. According to this hypothesis, long term energy balance is achieved by controlling lipid energy stores. If the level of body fat is monitored, excess of body fat should lead to decreases in food intake and decreases in total body fat should stimulate food intake to facilitate lipid deposition.

Lipectomy is an effective and fast removal of the body fat. Lipectomy is the only means of directly reducing body fat content and therefore is useful for testing humoral, neural and behavioral responses specific to reductions in body fat. Lipectomy changes the number of adipocytes (Michel and Cabanac, 1998). Moreover, recovery from lipectomy is rapid, thus, changes in metabolism and behaviour can more readily be directly attributed to changes in adiposity. In Syrian hamster, partial lipectomy induces restoration of body fat to levels that are indistinguishable from those of non-or sham-operated controls.

The responses of the rodents to lipectomy differs. Day length not only affects body fat levels in Siberian hamsters, but recent findings from lipectomy experiments suggest that it also may affect their availability to compensate for surgically induced lipid deficits. Body fat recovery after lipectomy is incomplete in short day but not long day exposed Siberian hamsters (Bartness et

al., 1989). In contrast, short days do not inhibit body fat restoration after lipectomy in Syrian hamsters (Bartness et al., 1987). Because the overwintering strategy of the Syrian hamsters depends on body fat gain rather than loss.

Many systems have been suggested for the regulation of total body fat (Bray and York, 1979). The simplest of these is the dynamic equilibrium system in which body fat is self stabilizing due to obligatory increases in metabolic rate that occur with increases in body mass. More complicated body fat stabilizing systems have been proposed and these include a fat detector, through which body fat stores are monitored, and/or reference signal to which total body fat is compared so that adjustments can be made to maintain lipid stores at the appropriate level.

In conclusion, the partial lipectomy-induced lipid deficit is communicated to the brain via decreases in humoral input (decreased insulin, decreased leptin, free fatty acids, glycerol or lactate released from white adipose tissue) and/or via decreases in sensory nerve outflow from WAT to brain. Reception sites in the brain are unknown but may involve the brain stem and hypothalamus. In response to the signal of decreased body fat, the brain decreases the SNS outflow to brown adipose tissue (BAT), white adipose tissue (WAT) and the adrenal medulla, resulting in a host of responses including decreases in BAT thermogenesis, decreases in WAT lipolysis and increases in white adipocyte proliferation. These responses would retain the existing stored lipid, increase energy for lipid deposition and increase the number of fat cells available for lipid storage.

Results from the last study have clearly demonstrated that the regulation of the adipose tissue mechanism in Syrian hamster is a multifactorial event. This regulation consist of food intake, photoperiod, as an environmental influence, and pineal gland, as synchronizer and regulator of the environmental information. More studies are needed to solve this puzzle.

In conclusion, the results presented here demonstrate that the SCN circadian clock directly orchestrates the diurnal changes in plasma leptin levels. SCN is sensitive to phase modulation by the hormone, leptin. These studies therefore indicates the presence of an efferent pathway, probably mediated by the autonomic nerves system, between the central nervous system and adipose tissue involved in the control of leptin secretion.

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plasma leptin and insulin independent of food intake and total body fat.
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Positional cloning of the mouse obese gene and its human homologue.
Nature, (1994), 372: 425-432

6. CURRICULUM VITAE

Education

BS: Department of Biology, Faculty of Arts and Sciences, Canakkale Onsekiz Mart University (1998)

MSc : Department of Biology, Faculty of Arts and Sciences, Abant Izzet Baysal University (2001)

PhD : Department of Biology, Faculty of Arts and Sciences, Abant Izzet Baysal University (2005)

MSc Thesis: Regulation by photoperiod of seasonal changes in body mass and reproductive function in Mongolian gerbil (*Meriones unguiculatus*)

PhD Thesis: Interaction between suprachiasmatic nucleus (SCN) and leptin hormone and their effect on puberty

Publications

1- Gündüz B.; **Karakaş A.** “Effects of Photoperiod and Melatonin Infusions on Body Weight in Pinealectomized Juvenile Siberian Hamsters (*Phodopus sungorus*).” **Turkish Journal of Biology**, 25, 301-321, **2001**.

2- Gündüz B.; **Karakaş A** “Photoperiodic Control of Testis Activity in the Mongolian Gerbil (*Meriones unguiculatus*).” **The Sciences (J. Med. Sci.)**, Vol.1 No. 3, 121-124, **2001**.

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4- **Karakaş A.**; Gündüz B. “Testicular Status in Pinealectomized Adult Mongolian Gerbil (*Meriones unguiculatus*).” **Israel Journal of Zoology**, Vol 48, pp 189-196, **2002**.

5- Pehlivan F.; **Karakaş A.**; Gündüz B. “Leptin Hormone Profile in Syrian Hamster During Estrous Cycle .” **Genel Tıp Dergisi**, 13(2,Ek), p:62 **2003**

6- **Karakaş A.**; Gündüz B. “Melatonin Implants Have no Effect on the Testis Volume and Body Weight in Mongolian Gerbils.” **Turkish Journal of Biology**, 27, 209-214, **2003**

7- **Karakaş A.**; Soyuturk H.; Gündüz B. “The effect of Maternal Transfer on Reproductive System Development of the Mongolian Gerbils.” **Genel Tıp Dergisi**, 14(Ek), p: 37 **2004**

8- Karakaş A.; Çamsarı Ç.; Serin E.; Gündüz B. “*Effects of Photoperiod and Food Availability on Growth, Leptin, Sexual Maturation and Maintenance in Mongolian Gerbils (Meriones unguiculatus).*” **Zoological Science** Vol. 22 No.6, 665-670, **2005.**

9- Karakaş A.; Serin E Gündüz B. “*Circadian Locomotor Activity Rhythms in Mongolian Gerbils: Effects of Food Restriction.*” **Turkish Journal of Biology** (Submitted)

10- Karakaş A.; Gündüz B. “*Effects of One Hour Infusion on Locomotor Activity of the Syrian Hamsters.*” **Neuroanatomy (2005) 4 (Suppl.1):1-48**

11- Karakaş A.; Gündüz B. “*Suprachiasmatic nuclei directly regulate the rhythm of leptin hormone release in Syrian hamsters (Mesocricetus auratus).*” **Chronobiology International** (Submitted)

Abstracts

1) Gündüz B.; **Karakaş A.** "The Effects of Melatonin on the Development of Diabetes in Streptozotocin-Induced Diabetic Mice." **35th National Diabetes Congress and 4th International Obesity Symposium. 10-15 May 1999.** Istanbul, TURKEY

2) Karakaş A.; Gündüz B. "Photoperiods and Testicular Status in the Adult and Juvenile Mongolian gerbils, *Meriones unguiculatus*." **26th Congress of Turkish Physiological Sciences Society. 4-8 September 2000.** Eskisehir, TURKEY

3) Karakaş A.; Gündüz B. "The Role of pineal Gland on Testis Activity and Body Weight Regulation in Mongolian gerbils." **27th Congress of Turkish Physiological Sciences Society. 8-12 October 2001.** Istanbul, TURKEY

4) Karakaş A.; Gündüz B. "The effect of melatonin implantation on reproductive activity in Mongolian gerbils." **28th Congress of Turkish Physiological Sciences Society. 24-27 September 2002.** Izmir, TURKEY

5) Pehlivan F.; Soy Turk H.; **Karakaş A.;** Gündüz B. Ankara, TURKEY "Serum leptin hormone profiles in pinealectomized Syrian hamsters during gestation and lactation." **29th Congress of Turkish Physiological Sciences Society. 01-05 September 2003.**

6) Karakaş A.; Gündüz B "The effect of leptin hormone on locomotor activity rhythm of Syrian hamsters." . **29th Congress of Turkish Physiological Sciences Society. 01-05 September 2003.** Ankara, TURKEY

7) Soy Turk H.; Yılmaz H.; **Karakaş A.;** Gündüz B. " The effect of caffeine on prepubertal development of Juvenile Syrian hamsters." **30th Congress of Turkish Physiological Sciences Society. 31-03 September 2004.** Konya, TURKEY

- 8) Yılmaz H.; Soyuturk H.; **Karakaş A.**; Gündüz B. "*The effects of caffeine on motor activity rhythm in adult Syrian hamsters.*" **30th Congress of Turkish Physiological Sciences Society. 31-03 September 2004.** Konya, TURKEY
- 9) Çamsarı Ç.; **Karakaş A.**; Gündüz B. "*The effects of pineal gland and melatonin on activity rhythm in Mongolian gerbils.*" **30th Congress of Turkish Physiological Sciences Society. 31-03 September 2004.** Konya, TURKEY
- 10) Erdoğan B.; **Karakaş A.**; Gündüz B. "*Activity rhythm can be entrained by food restriction in Mongolian gerbils.*" **30th Congress of Turkish Physiological Sciences Society. 31-03 September 2004.** Konya, TURKEY
- 11) **Karakaş A.**; Çamsarı Ç.; Gündüz B. "*The effect of food restriction on body weight and reproductive activity of Mongolian gerbils.*" **17th National Biology Congress. 21-24 June 2004.** Adana, TURKEY
- 12) **Karakaş A.**; Serin E.; Gündüz B. "*Cerebral Ischemia Reperfusion damage: the effects of exercise, melatonin and pinealectomy.*" **4th National Free Radicals and Antioxidants Research Society Congress. 9-12 May 2005** Antalya/TURKEY
- 13) **Karakaş A.**; Karakaş F.; Uçar T.A.; Gündüz B. "*Effects of mistletoes (Viscum album L.) extracts on electrocardiogram and respiratory parameters of the Syrian hamsters (Mesocricetus auratus).*" **4th International Congress of Ethnobotany (ICEB 2005). 21-26 August 2005** Istanbul/TURKEY
- 14) **Karakaş A.**; Uçar T.A.; Gündüz B. "*Mistletoes (Viscum album L.) extracts tests on Activity rhythms of the Syrian hamsters.*" **4th International Congress of Ethnobotany (ICEB 2005). 21-26 August 2005** Istanbul/TURKEY
- 15) **Karakaş A.**; Çamsarı Ç.; Gündüz B. "*Leptin hormone phase advances the locomotor activity rhythm of Syrian hamsters (Mesocricetus auratus).*" **10th Congress of the EPBRS. 1-5 September 2005** Frankfurt/GERMANY
- 16) **Karakaş A.**; Gündüz B. "*Suprachiasmatic nuclei directly regulate the rhythm of leptin hormone release in Syrian hamsters (Mesocricetus auratus).*" **10th Congress of the EPBRS. 1-5 September 2005** Frankfurt/GERMANY
- 17) **Karakaş A.**; Hacıoğlu E.; Gündüz B. "*The effect of maternal leptin, insulin and melatonin injections on prepubertal development of the Syrian hamster.*" **31. National Physiology Congress 27-30 September 2005** Gaziantep/TURKEY
- 18) **Karakaş A.**; Gündüz B. "*The arrhythmic locomotor activity rhythm of the Syrian hamsters can be reentrained by the leptin infusions.*" **31. National Physiology Congress 27-30 September 2005** Gaziantep/TURKEY

20- Karakaş A. ;Aya Z.; Gunduz B. *The effects of formaldehyde and xylene on the respiratory parameters of the Syrian hamsters.* **31. National Physiology Congress 27-30 September 2005** Gaziantep/TURKEY

21- Karakaş A. ; Kümbet Y.; Gunduz B. *The effects of Plantago lanseolata extracts on the formaldehyde induced lung deficiency in Syrian hamsters.* **31. National Physiology Congress 27-30 September 2005** Gaziantep/TURKEY

22- Karakaş A. ; Andaç Ş.; Gunduz B. *The effects of maternal leptin administration on the fetal brain development.* **31. National Physiology Congress 27-30 September 2005** Gaziantep/TURKEY

Abroad Experience

1) Martin-Luther University, Zoologie Institute, Halle-Wittenberg, GERMANY 15 August-15 September 2002.

2) Nottingham University, Queen Medical Centre, Nottingham, UK 15-25 May 2004

Courses

1) Ibro Course in Neuroscience, Ege University Center for Brain Research, IZMİR 10-18 September 2003

2) PowerLab Training Course, Istanbul University, ISTANBUL, 21-25 June 2004

3) Basic and Clinical Electrophysiological Course From Cell to System Level, Mersin University, Faculty of Medicine, MERSİN, 29 March 2005

Memberships

1) Turkish Physiological Sciences Society (2004-)

2) EPBRS (2004-)