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Exposure to continuous darkness leads to atypical symptoms of seasonal affective disorder in rats

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Aim: Prolonged duration of nocturnal melatonin increase has been implicated in the pathogenesis of the seasonal affective disorder (SAD) winter depression. This study was designed to investigate behavioral and physiological consequences of constant dark exposure in male rats for its potential application to human affective disorders.

Materials and methods: A total of 12 male Wistar-Albino rats (230-290 g) were assigned to control (LD 12:12 h; n = 6) and constant darkness (DD, 10 days; n = 6) groups. Experimental protocol included comparison of behavioral (FST, open field test, and sucrose preference) and physiological (body weight, food intake, and blood glucose level) parameters.

Results: While food intake, weight gain, sucrose preference, and locomotor activity were significantly higher in the DD group, blood glucose level was lower in the same group. Depression-like behavioral despair was prominent in the DD group. As compared to control rats, total duration (s) of immobility was longer and swimming and climbing behaviors were shorter in the DD group.

Conclusion: Although continuous darkness exposure for 10 days in male rats seems to mimic atypical symptoms of SAD in terms of depression, weight gain, and the increased sucrose preference, such an extrapolation from behavioral changes in nocturnal animals to human circadian disorders needs further verification.

Key words: Seasonal affective disorder; melatonin; constant darkness; FST; sucrose preference

Sıçanlarda sürekli karanlığa maruziyete bağlı gelişen mevsimsel duygulanım bozukluğu atipik semptomları

Amaç: Uzamış nokturnal melatonin artışı mevsimsel duygulanım bozukluğu patogenezinde yer almaktadır. Bu çalışma, erkek sıçanlarda 10 günlük sürekli karanlık uygulamasının davranışsal ve fizyolojik etkilerini ve bu etkilerin mevsimsel duygulanım bozukluğu ile uyumunu araştırmak amacı ile tasarlandı.

Yöntem ve gereç: Wistar-Albino erkek sıçanlar (230-290 g); kontrol (LD 12:12 saat; n = 6) ve sürekli karanlık (DD, 10 gün; n = 6) gruplarına ayrıldı. Deney protokolünde davranışsal (FST, açık alan testi, sükroz tercihi) ve fizyolojik (vücut ağırlığı, gıda alımı, kan glukoz düzeyleri) parametreler yer aldı.

Bulgular: Sürekli karanlık grubu sıçanlarda gıda alımı ($P < 0,05$), kilo alımı ($P < 0,01$), sükroz tercihi ($P < 0,01$), ve lokomotor aktivite ($P < 0,01$) anlamlı olarak daha yüksek ancak kan glukoz düzeyi ($P < 0,05$) daha düşüktü. Sürekli karanlık grubu sıçanlarda donma toplam süresinin (saniye) daha uzun ($P < 0,001$); ancak mücadele [yüzme ($P < 0,001$) ve tırmanma ($P < 0,001$)] toplam süresinin anlamlı olarak daha kısa bulunması depresyon benzeri davranış yönünde yorumlandı.

Sonuç: Erkek sıçanlarda 10 günlük sürekli karanlık uygulamasının depresyon, kilo alımı ve artmış sükroz tercihi gibi mevsimsel depresyonun atipik bulguları ile örtüşen etkileri olmakla beraber, insanlardaki sirkadyen bozuklukların nokturnal hayvan davranışlarından yola çıkarak tahmini temkinli olmayı gerektiren bir süreçtir.

Anahtar sözcükler: Mevsimsel duygulanım bozukluğu, melatonin, sürekli karanlık, FST, sükroz tercihi

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Introduction

Arising from a failure to properly adapt to changes in the environment, seasonal affective disorder (SAD) is one of the psychiatric disorders related with an alteration of circadian secretory pattern of melatonin secretion (1). Vegetative symptoms accompanying depression, such as anergia, hypersomnia, increased appetite, carbohydrate craving, and weight gain are known as hallmark physiological signs of SAD (2).

While decreased exposure to sunlight in winter has been considered to play a significant role in triggering SAD in vulnerable individuals, the exact pathophysiological mechanisms underlying SAD remain unidentified (3)

Due to its responsiveness to environmental light, mood changes in SAD were stated to be driven with the seasonal variations in the day length (4,5). While the mechanism by which light can ameliorate depressive symptoms is not fully understood, 10 days of constant darkness exposure is known to induce endogen melatonin synthesis effectively (4-6).

Based on animal studies, seasonal rhythms have been considered to be cued to day length, or the photoperiod (7), which is transduced by the pattern of nocturnal melatonin (5-methoxy-N-acetyltryptamine) secretion by the pineal gland (8). Additionally, pineal melatonin secretion was demonstrated to occur almost solely at night with duration inversely related to day length. In this regard, long melatonin pulse was reported to signal short days leading to behavioral changes preparing the animals for the cold winter climate, (9).

Based on alikeness between symptom constellation of SAD and the seasonal physiological changes seen in animals including weight gain, hypersomnia, and lack of energy, SAD has been stated to be caused by abnormal body response to the seasonal change in day length (10). In this vein, the bright light was shown to be able to alleviate SAD symptoms as well as being capable of suppressing nocturnal pineal melatonin secretion (11). Furthermore, light therapy has been used for treating SAD, and its effectiveness has been widely demonstrated (12,13).

Previous studies revealed that a long photoperiod regime may produce an antidepressant effect in male rats (14); a single day of constant light can protect against the induction of behavioral despair in rats (15), and short photoperiod can induce a depression-like state in sand rats (16).

Based on the literature review, studies concerning current hypotheses of the etiology of SAD including photoperiod, melatonin, phase-shift, and the photon-count hypotheses have conflicting findings regarding the therapeutic efficacy of morning, midday, evening, and morning–evening light exposure in SAD (10)

Therefore the purpose of the present study was to investigate behavioral (depression, locomotor activity, anxiety, and social interaction) and physiological (food intake, weight gain, sucrose preference, and blood glucose levels) consequences of constant dark exposure in male rats for its potential application to human affective disorder.

Materials and methods

Animals

A total of 12 male Wistar-Albino rats (230-290 g) used in the present study were housed in individual polycarbonate cages, provided with standard lab chow and tap water ad libitum, and maintained in an air-conditioned room (22 ± 2 °C). Rats were divided into 2 groups and kept in 2 different light-dark regimens for 10 days. Group 1 was kept under the 12:12 h light/dark regimen and served as the control group. Rats from group 2 were maintained in constant darkness and served as the constant darkness (DD) group. Dim red light was switched on in the room only during nutrition, care, and service manipulations. Experimental protocol was approved by the Başkent University Faculty of Medicine Animal Care and Use Committee (DA07/01-2007/AP-104).

Experimental design

Experimental rats were evaluated twice for each of behavioral and physiological parameters. Open field test, sucrose preference test, and forced swim test (FST) were the behavioral tests. Body weight, food intake, and blood glucose levels were the physiological measurements. Timeline of the experiment is presented in Figure 1.

Behavioral tests

Open field test

All rats were subjected to an open field test (17,18) on the 2nd and the 7th days of the protocol. The square

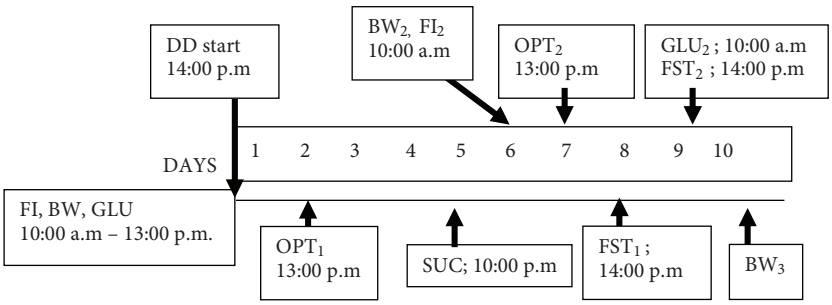

Figure 1. Timeline of the experiments. All of the measurements were made both for control (12:12 h LD cycle) and constant darkness (DD) rats. DD: constant darkness; BW: body weight; FI: food intake measurement; GLU: blood glucose measurement; OPT: open field test; FST: forced swim test; SUC: sucrose preference test.

testing arena (60 cm × 60 cm) was divided into 16 squares (15 cm × 15 cm each). The area consisting of the four-squares located in the center was defined as the center. A 40 W light bulb was positioned 90 cm above the base of the arena and provided the only source of illumination in the testing room. Rat to be tested was placed at the center of the arena and its behavior was recorded for 5 min for the ambulation scores (the number of squares crossed) and for the number of rearings and peepings.

Forced swimming test (FST)

The FST (19,20) consists of individually placing the rats into a cylindrical tank (28 cm × 45 cm) containing clean water at 25 °C (25 cm depth) on 2 occasions (15 min of pre-test and 5 min of test sessions) 24-h apart (day 8 and 9). The water was changed after each subject was tested and the cylinder was thoroughly rinsed in order to remove the presence of any potential alarm substances. After testing, rats were dried with a towel and returned to their home cages. Experiments were performed between 14:00 and 17:00. Subsequent evaluation of the total time spent in each one of the behaviors (swimming, climbing, and immobility) was made during the 2nd session (test session) of the experiment. Immobility was defined as the lack of motion of the whole body consisting only of the small movements necessary to keep the animal's head above the water. It was counted as "swimming" when large forepaw movements displacing the body around the cylinder, more than necessary to merely keep the head above the water, were performed. Climbing was recorded when vigorous movements with forepaws in and out of the water, usually directed against the wall of the tank, were displayed.

Sucrose preference test

During sucrose preference test performed on the 5th day of the protocol (21), rats were offered a free choice for 24 h between 2 bottles, one containing 0.8% sucrose solution and the other tap water. To prevent possible effects of side preference in drinking, the position of the bottles was switched after 12 h. The animals were not deprived of water or food before the test. The consumption of water and sucrose solution was measured by weighing the bottles. The preference for sucrose was calculated from the amount sucrose solution consumed, expressed as a percentage of the total amount of liquid drunk.

Physiological measurements

One day before the experiments (day 0) and on the 6th day of the protocol, food intake was evaluated as the intake of preweighed standard chow (g) per 100 g of body weight (bw) in 24 h. Animals were deprived of food overnight (14 h) and 50 g of preweighed rat chow was delivered. Pellets and spillage were collected and weighed at the end of the feeding periods. Blood glucose levels were determined with Accu-chek glucometer (Roche, Mannheim, Germany) from tail-bled samples (made with a needle stick) on the 1st and 9th days of the protocol under dim red light.

Statistics

The minimum number of animals and duration of the observation required to obtain consistent data

were employed. Homogeneity of group variances was controlled by Levene's test. Distributions of the variables were evaluated by Shapiro-Wilk normality test. Independent samples t-test was used for the analysis of the data concerning control and darkness groups. Paired samples t test was used for the data regarding open field test scores. The data are expressed as means ± SEM and "n" indicates the number of rats. $P < 0.05$ was considered statistically significant. The analyses were performed using SPSS 11.5 (SSPS Inc., Chicago IL, USA).

Results

Control and DD groups were identical in terms of food intake (8.21 ± 0.27 vs. 8.54 ± 0.54; $P > 0.05$), body weight (256.0 ± 8.87 vs. 248.0 ± 8.96; $P > 0.05$), blood glucose levels (124.3 ± 4.52 vs. 132.8 ± 5.58; $P > 0.05$) at the beginning of the experiment. Statistical analysis enabled the homogeneity considering group variances and distributions of the variables.

During the experimental course, food intake (9.37 ± 0.50 vs. 7.18 ± 0.48) [$t = -3.14$, $P < 0.01$] and weight gain (19.66 ± 2.57 vs. 4.0 ± 1.2) [$t = -5.5$, $P < 0.001$] were found to be significantly higher for rats in the DD group when compared to the control rats (Figure 2). Sucrose preference (51.50 ± 2.9 vs. 33.6 ± 2.8) [$t = -4.4$, $P < 0.01$] were more prominent and blood glucose levels (120.83 ± 9.24 vs. 162.83 ± 11.8) [$t = 2.79$, $P < 0.01$] were lower in the DD group when compared to the control group (Figure 3).

Open field total frequency scores were similar in the 2nd and 8th days for the control rats (24.46 ± 4.26 vs. 19.58 ± 2.78; $P > 0.05$). On the other hand, when compared to the 2nd day scores, constant darkness was found to increase total frequency (31.37 ± 2.74 vs. 47.46 ± 2.9) [$t = -3.72$, $P < 0.05$] and to decrease the time spent in the central zone (35.96 ± 2.38 vs. 19.96 ± 5.6) [$t = 2.83$, $P < 0.05$], reflecting high levels of anxiety in DD rats (Figure 4).

Depression-like behavioral despair was prominent in the DD group. Total duration (s) of immobility was longer (101.0 ± 11.4 vs. 213.6 ± 12.8) [$t = -6.53$, $P < 0.001$] while swimming (94.33 ± 16.3 vs. 39.8 ± 4.15); [$t = 3.23$, $P < 0.001$] and climbing behaviors were shorter (64.33 ± 6.05 vs. 31.00 ± 3.4); [$t = 4.79$, $P <$

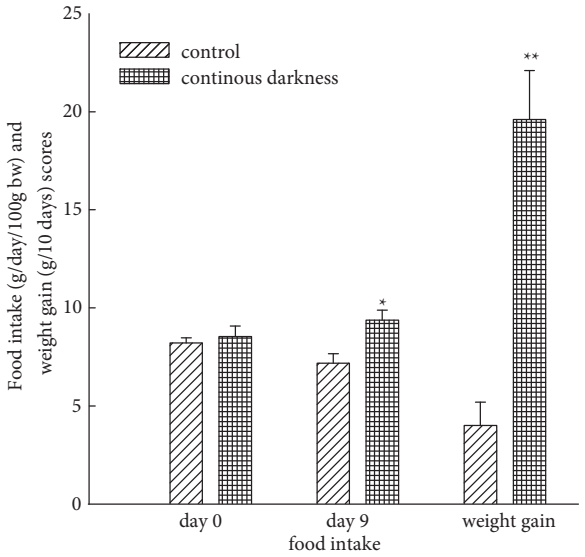

Figure 2. Food intake (g/100 g body weight, day 9) and weight gain (g/10 days) values for control (n = 6) and continuous darkness (n = 6) rats. Food intake was evaluated before the application of the experimental protocol and on the 9th day of the experiment. Rats from both groups were homogenous initially considering body weights and food intake. Data are shown as mean ± SEM.
* $P < 0.01$ and ** $P < 0.001$; compared to corresponding scores of control rats

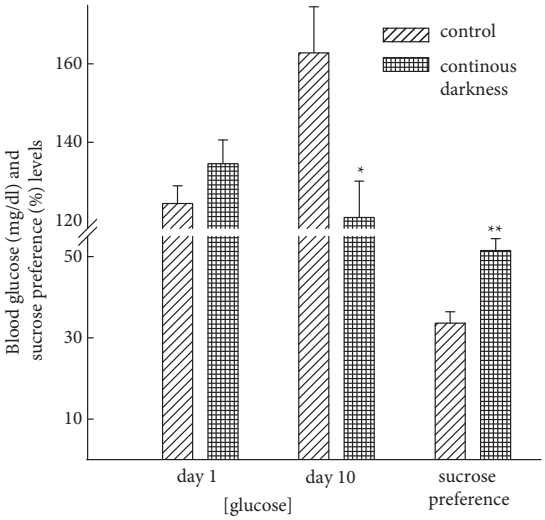

Figure 3. Blood glucose levels (mg/dL, day 10) and sucrose preference (%, day 6) scores for control (n = 6) and continuous darkness (n = 6) groups. Rats from both groups were homogenous considering initial blood glucose levels. Data are shown as mean ± SEM.
* $P < 0.05$ and ** $P < 0.01$; compared to corresponding scores of control rats

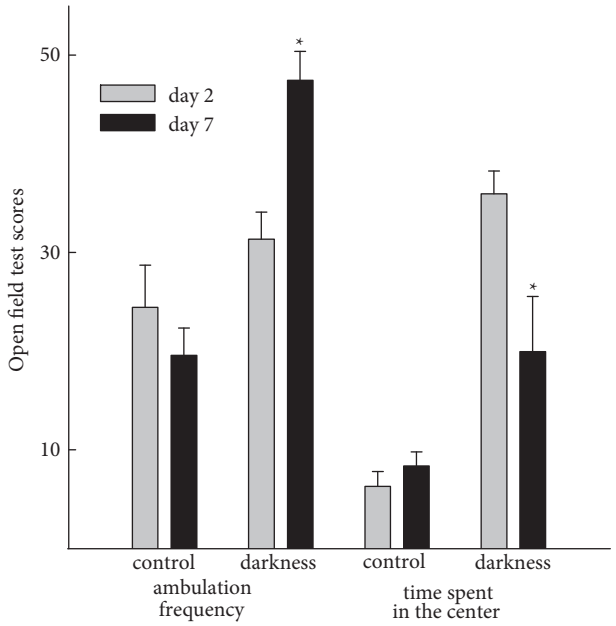

Figure 4. Locomotor activity and anxiety scores of control (n = 6) and continuous darkness (n = 6) groups obtained by open field test on the 2nd and 7th days. Locomotor activity was represented by the total ambulation frequency. Anxiety was negatively correlated with the time spent in the center zone. Rats from both groups were homogenous considering initial locomotor activity. Data were shown as mean ± SEM.
* P < 0.05; compared to corresponding frequency and time scores of the same rats obtained on the 2nd day.

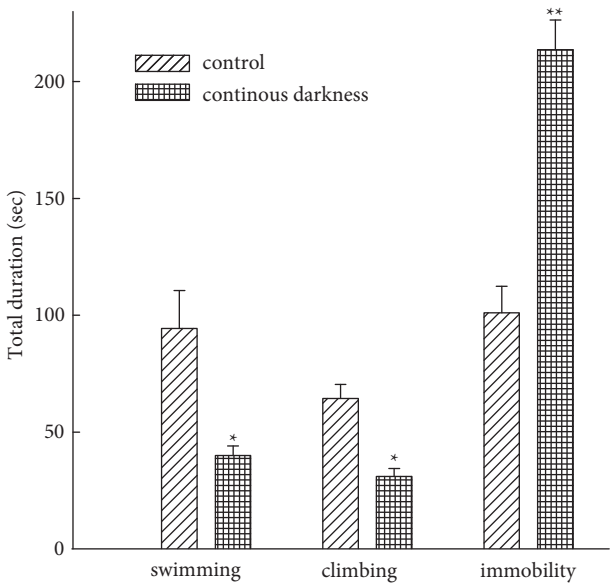

Figure 5. Average duration (s) of swimming, climbing and immobility behaviors related with depression-like state in control (n = 6) and continous darkness (n = 6) groups obtained on 8th and 9th days. Longer duration of immobility was confirmed in favor of depression-like behavioral despair. Decreased swimming time was indicative of underlying serotonergic mechanism. Data are shown as mean ± SEM.
* P < 0.01 ve ** P < 0.001; compared to corresponding scores of control rats

0.001] in the DD group when compared to the control rats (Figure 5).

Discussion

The physiological hallmarks of SAD, such as reduced energy, hypersomnia, increased appetite, carbohydrate craving, and weight gain, correlate with seasonal acclimation evolving to enable seasonal energy savings in mammals (22). SAD is accepted as an exaggerated form of this situation (23), including clinical features of atypical depression. According to our results, weight gain, increased food intake, and sucrose preference accompanying depression observed in the DD rats resemble the winter depression.

Lengthened duration of elevated nocturnal melatonin secretion during the winter nights has been implicated in the pathogenesis of winter depression (24). Long photoperiod regimen was reported to produce antidepressant action in the male rat (16) and short photoperiod was stated to induce a depression-like state in diurnal rodents (14).

Besides the known interaction with the induction of endogenous melatonin synthesis (6,25), the time course selected as 10 days in our study seems appropriate since maintenance of the animals in darkness for several weeks was reported to increase the intensity of phase shifts evoked by possible light pulses (26,27). In that sense, the light pulses encountered unavoidably by the rats during the subsequent behavioral tests in the present study seems to have a minor effect on the rhythms of the animals.

Serotonergic and noradrenergic antidepressants were shown to increase the duration of swimming and climbing behaviors respectively, in both male and

female rats (28). In that sense, the finding of increased duration of immobility accompanied by decreased duration of swimming and climbing behaviors in our DD rats was consistent with the previously hypothesized central monoaminergic pathway in the pathogenesis of SAD (29).

Melatonin-treated rats were shown to display an increase in total activity under DD conditions (30). Locomotor activities in the open-field and open arm exploration in the elevated plus-maze were reported to be increased after sudden darkness (31). Continuous darkness was found to be related with increased locomotor activity in our DD rats, too.

Atypical symptoms of SAD (5) have been considered as energy-conserving behaviors resulting from patho-physiological changes in energy-regulating systems (32). Finding of increased food intake and weight gain in spite of increased locomotor activity in our DD rats seems compatible with the lesser energy expenditure reported in SAD patients with atypical depressive symptoms (32).

At the same time, detection of lower blood glucose levels in our DD rats seems to correlate with the previous data concerning low blood glucose concentration in DD conditions as a result of critically depleted glycogen stores (33). The increase in carbohydrate cravings and ingestion was suggested to have a role in the regulation of brain serotonin levels (34) and also in the inability to lose weight in SAD patients (35). Therefore, sucrose preference may be increased due to homeostatic compensation (36) of lower blood glucose levels detected in our DD rats. In addition, increased locomotor activity may also lead to increased calorie consumption (30,36), correlating with the presence of higher sucrose and food intake in the DD rats.

The main limitation of the present study is the documented difference between nocturnal and diurnal mammals in a wide variety of physiological and behavioral responses to light/dark conditions including entrainment process that restricts the extrapolation from behavioral alterations in nocturnal animals to human disorders associated with circadian rhythm manipulations (37). Nevertheless, a causal link shown between the proposed model and SAD seems to entail the reversal of the behavioral and physiological alterations with appropriate treatments known to ameliorate SAD related symptoms.

In conclusion, the sensitivity of the nocturnal rat to constant darkness may have an adaptive value considering the interaction between the psycho-physiological roots of SAD and the circadian system. Further studies addressing the effects of constant light and dark exposure in nocturnal rodents, including determination of the circadian phase and use of antidepressant drugs, are mandatory.

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References

1. Pacchierotti C, Iapichino S, Bossini L, Pieraccini F, Castro-Giovanni P. Melatonin in psychiatric disorders: a review on the melatonin involvement in psychiatry. Front Neuroendocrinol 2001; 22(1): 18-32. Review.
2. Rohan KJ, Sigmon ST, Dorhofer DM. Cognitive-behavioral factors in seasonal affective disorder. J Consult Clin Psychol 2003; 71: 22-30.
3. Eastman CI. Natural summer and winter sunlight exposure patterns in seasonal affective disorder. Physiol Behav 1990; 48: 611-616.
4. Lewy AJ, Kern HA, Rosenthal NE, Wehr TA. Bright artificial light treatment of a manic–depressive patient with a seasonal mood cycle. Am J Psychiatry 1982; 9: 1496-1498.
5. Rosenthal NE, Sack DA, Gillin JC, Lewy AJ, Goodwin FK, Dakahashi K et al. Seasonal affective disorder: a description of the syndrome and preliminary findings with light therapy. Arch Gen Psychiatry 1984; 4l: 72-80.
6. Lopez-Gonzalez MA, Guerrero JM, Delgado F. Presence of the pineal hormone melatonin in rat cochlea: its variations with lighting conditions. Neurosci Lett 1997; 238: 81-3.
7. Aschoff J. Circadian timing. Ann N Y Acad Sci 1984; 423: 442-468.
8. Goldman B, Darrow J. 1983. The pineal gland and mammalian photoperiodism. Neuroendocrinol 1983; 37:386-396.
9. Wade GN. Seasonal variations in body weight and metabolism in hamsters. In: Rosenthal NE, Blehar MC. (Eds.), Seasonal Affective Disorders. The Guildford Press, NY, 1989; pp. 102-126.

10. Lee TM, Blashko CA, Janzen HL, Paterson JG, Chan CC. Pathophysiological mechanism of seasonal affective disorder. J Affect Disord. 1997; 46(1): 25-38.

11. Lewy AJ, Wehr TA, Goodwin FK, Newsome DA, Markey SP. Light suppresses melatonin secretion in humans. Science. 1980; 12; 210(4475): 1267-9.

12. Rosenthal NE, Wehr TA. Seasonal affective disorders. Psychiatry Ann 1987; 17: 670-674.

13. Rosenthal NE, Jacobsen FM, Sack DA, Arendt J, James SP, Parry BL et al. Atenolol in seasonal affective disorder: a test of the melatonin hypothesis. Am J Psychiatry 1988; 145(1): 52-6.

14. Molina-Hernandez M, Tellez-Alcantara P. Long photoperiod regimen may produce antidepressant action in the male rat. Prog Neuro Psychopharmacol Biol Psychiat 2000; 24: 105-16.

15. Yılmaz A, Aksoy A, Canbeyli R. A single day of constant light (L/L) provides immunity to behavioral despair in female rats maintained on an L/D cycle. Prog Neuro-Psychopharmacol Biol Psychiat 2004; 28: 1261-5.

16. Einat H, Kronfeld-Schor N, Eilam D. Sand rats see the light: short photoperiod induces a depression-like response in a diurnal rodent. Behav Brain Res 2006; 173(1): 153-7.

17. Kameyama T, Suzuki M, Nabeshima T. Effects of 5-hydroxytryptamine on defecation in open-field behavior in rats. Pharmacol Biochem Behav 1980; 12: 872-5.

18. Wang D, Noda Y, Tsunekawa H, Zhou Y, Miyazaki M, Senzaki K et al. Behavioral and neurochemical features of olfactory bulbectomized rats resembling depression with comorbid anxiety. Behav Brain Res 2007; 178(2): 262-73.

19. Porsolt RD, Anton G, Blavet N, Jalfre M. Behavioral despair in rats: a new model sensitive to antidepressant treatments. Eur J Pharmacol 1978; 47: 379-91.

20. Lino-De-Oliveria C, De Lima TC, De Padua Carobrez A. Structure of the rat behaviour in the forced swimming test. Behav Brain Res 2005; 158(2): 243-50.

21. Rygula R, Abumaria N, Flügge G, Fuchs E, Rüther E, Havemann-Reinecke U. Anhedonia and motivational deficits in rats: impact of chronic social stress. Behav Brain Res 2005; 162(1): 127-34.

22. Prendergast BJ, Nelson RJ. Affective responses to changes in day length in Siberian hamsters (Phodopus sungorus). Psychoneuroendocrinology 2005; 30: 438-52.

23. Nelson RJ, Badura LL, Goldman BD. Mechanisms of seasonal cycles of behavior. Ann Rev Psychol 1990; 41: 81-108.

24. Wehr TA. Photoperiodism in humans and other primates: evidence and implications. J Biol Rhythms 2001; 16(4): 348-64. Review.

25. Cevik H, Erkanli G, Ercan F, Isman CA, Yegen B. Exposure to continuous darkness ameliorates gastric and colonic inflammation in the rat: both receptor and non-receptor-mediated processes. J Gastroenterol Hepatol 2005; 20(2): 294-303.

26. Refinetti R. Effects of prolonged exposure to darkness on circadian photic responsiveness in the mouse. Chronobiol Int 2003; 20: 417-40.

27. Refinetti R. Enhanced circadian photo-responsiveness after prolonged dark adaptation in seven species of diurnal and nocturnal rodents. Physiol Behav 2007; 90(2-3): 431-7

28. Cryan JF, Valentino RJ, Lucki I. Assessing substrates underlying the behavioral effects of antidepressants using the modified rat forced swimming test. Neurosci Biobehav Rev 2005; 29(4-5): 547-69.

29. Neumeister A, Konstantinidis A, Praschak-Rieder N, Willeit M, Hilger E, Stastny J, et al. Monoaminergic function in the pathogenesis of seasonal affective disorder. Int J Neuropsychopharmacol 2001; 4: 409-420

30. Resuehr D, Olcese J. Caloric restriction and melatonin substitution: effects on murine circadian parameters. Brain Res 2005; 1048(1-2): 146-52.

31. Nasselo AG, Machado C, Bastos JF, Felicio L. Sudden darkness induces a high activity-low anxiety state in male and female rats. Physiol Behav 1998; 63 (3): 451-454.

32. Pinchasov BB, Shurgaja AM, Grischin OV, Putilov AA. Mood and energy regulation in seasonal and non-seasonal depression before and after midday treatment with physical exercise or bright light. Psychiatry Res 2000; 94(1): 29-42.

33. Zhang J, Kaasik K, Blackburn MR, Lee CC. Constant darkness is a circadian metabolic signal in mammals. Nature 2006; 439(7074): 340-3.

34. Christensen L. The effect of food intake on mood. Clin Nutr 2001; 20(1): 161-166.

35. Wurtman J, Wurtman R, Reynolds S, Tsay R, Chew B. Fenfluramine suppresses snack intake among carbohydrate cravers but not among noncarbohydrate cravers. Int J Eat Disord 1987; 6: 687-699.

36. Vanitalie TB. Sleep and energy balance: Interactive homeostatic systems. Metabolism 2006; 55(10; Suppl 2): 30-5.

37. Yannielli P, Harrington ME (2004). Let there be more light: enhancement of light actions on the circadian system through non-photic pathways. Prog Neurobiol; 74(1): 59-76.