

Effect of the partial solar eclipse on the behavior and activity of rats

Ronald Tarigan, Pudji Achmadi, Isdoni Bustamam, Koekoeh Santoso*

Division of Physiology, School of Veterinary Medicine and Biomedical Sciences, IPB University, Bogor, Indonesia

Received: 25 October 2022 | Revised: 12 February 2023 | Accepted: 14 February 2023

Abstract

Circadian rhythm is the adaptation response of animals against light alteration that affects various behavioral and physiological responses. Bright light is a danger signal for nocturnal animals because it exposes them more to predators. This research aimed to study the changes in the behavior of rats during the partial solar eclipse (PSE) period. The PSE period occurred across Bogor, Indonesia, on 9 March 2016. The PSE started at 06.19 AM, peaked at 07.21 AM, and ended at 08.30 AM. The light level decreased since the early solar eclipse, then dropped significantly at the peak period. The activities of six mature male Sprague Dawley rats were measured using Opto-Varimex-5 Auto Track System during the PSE period. The measurements were repeated one day after the PSE period as the non-PSE period. There were significant differences ($P < 0.05$) in stereotypic time, burst of stereotypic movement, and horizontal count in the early, peak, and end periods of the PSE period compared with the values at the same time of the non-PSE period. The activities of rats changed during the PSE period. Rats did not rest as in their normal nocturnal behavior during darkness but were more active and stressed because of the rapid change in light level during the PSE period.

Keywords: animal activity | behavior | light level | PSE | rat

Introduction

The partial solar eclipse (PSE) attracts many scientists worldwide to observe animal behavior changes during that period. The effects of the earth's cycle (day and night) on physiological and behavioral systems are reflected by the multitude of circadian endocrine and metabolic rhythms as animal strategies to adapt against environmental changes (Paredes *et al.*, 2005). As nocturnal animals, rats show increased activities such as foraging, feeding, drinking, and agonistic behavior during a dark period. These increased activities also occur when the rats are kept in an artificial light-dark environment and during blinding in the light cycle (Zucker, 1971; Spiteri, 1982).

Bright light is an ecological danger signal for nocturnal animals and is correlated with increased danger because they are more vulnerable to predators (Godsil & Fanselow, 2004). Rats tend to avoid brightly illuminated places and prefer dark places. Rats did not freeze to cope with those challenges but exhibited thigmotaxis, a tendency to remain close to the walls to reduce their detectability during dangerous situations (Grossen & Kelley, 1972). Bright light also reduces social interaction and locomotion, increases defecation, and increases the startle reflex (Godsil & Fanselow, 2004).

The PSE across Indonesia on 9 March 2016 provided a rare opportunity to understand the circadian rhythm of rats' activity. During the solar eclipse, the dramatic decrease in daylight levels is more visible when the light levels are close to their peak, such as late morning. There is anecdotal evidence during

*Corresponding author, WA: +62 857-1585-1001, Email: koekoehsa@apps.ipb.ac.id

the solar eclipse, “animals behave as they would at nightfall”. The behaviors of dragonflies and *Anopheles barbirostris* during the solar eclipse were the same as when transferred to a shady and dark place (Mitra, 1996; Chatterjee & Chandara, 1997). Alvarez-Cárdenas *et al.* (1997) found that the lizard *Uta stansburiana* showed similar behavior as they usually do at the end of the day. In contrast, Rutter *et al.* (2002) found that solar eclipse did not affect grazing and rumination activity in cattle. This study aimed to compare rats’ behavior during and after the PSE and discuss the neurohormonal mechanism that regulates rats’ activity during the PSE.

Methods

Experimental design

This study was conducted in an open space area inside the School of Veterinary Medicine and Biomedical Sciences, IPB University, Bogor, Indonesia (latitude 106.7208°E, longitude -6.5568°S) between 9 March to 10 March 2016. The PSE occurred in Bogor on 9 March 2016, with 87.14% of darkness percentage. The PSE started at 06.19 AM, peaked at 07.21 AM, and ended at 08.30 AM. The light intensity at the study site was recorded using a lux meter (range: 0–25,000 and peak response at 565 nm). The temperature and humidity averages on the PSE day (9 March) and the non-PSE day (10 March) were 26.1°C, 87% and 27°C, 86%, respectively.

Six mature male Sprague Dawley rats were used in this study. They were obtained and managed in the laboratory animal facility unit of the School of Veterinary Medicine and Biomedical Sciences, IPB University, and under the supervision of veterinarians. All rats were maintained in a 12:12 hours light-dark cycle with free access to water and feed. On the PSE day (9 March), all rats were moved to an open space outside the laboratory animal facility one hour before the PSE started. The activities of rats were recorded at the beginning of PSE (06.19 AM), the peak of PSE (07.21 AM), and the end of PSE (08.30 AM). After PSE ended, all

rats were returned to the laboratory animal facility. The activities of rats were recorded using an animal activity meter (Opto-Varimex-5 Auto Track System, Columbus Instrument-USA) to quantify locomotor activity and trace the animal’s path for behavioral analysis. This system provided data on distance travel, resting time, stereotypic time, ambulatory time, burst of stereotypic movement, horizontal count, and ambulatory count. The measurements were repeated three times for each rat. The recording of animal activity was repeated on the next day (10 March 2016, the non-PSE day) at the same time with the same procedure. All management and treatment of rats in this study followed the principle of animal welfare for animal use in biomedical research, which is ruled by the World Medical Association in the Declaration of Helsinki (WMA, 2013).

Distance travel is the distance traveled by the animal in the indicated cage during a particular interval. Resting time is the amount of time when the animal in the indicated cage rested during a particular interval. Stereotypic activity is the amount of time when the animal in the indicated cage was engaged in a stereotypic activity (a movement that did not exceed the distance set by the box size). Ambulatory activity is the amount of time when the animal in the indicated cage was engaged in an ambulatory activity (a movement that exceeds the distance set by the box size). The box size is defined as one beam. The animal must interrupt one new beam before that movement is considered as an ambulatory movement.

Data analysis

The animal's activities (distance travel, resting time, stereotypic time, ambulatory time, burst of stereotypic movement, horizontal count, and ambulatory count) on the PSE day and the non-PSE day were analyzed using SPSS Statistics 20 (IBM, USA). Statistical differences were calculated by paired T-test with $P < 0.05$ considered significant.

Results

Light level

The changes in light intensity at the study site during the PSE day and non-PSE day are shown in **Figure 1**. There was a difference in light level between the PSE day and the non-PSE day from 06.19 AM (the early PSE period) until 08.30 AM (the end PSE period). The difference became larger during the peak PSE period (07.21 AM). The lower light level during the peak PSE decreased the stimulus for the activity of the rats. After the PSE ended (08.30 AM), the light levels returned to normal daytime levels. Besides, there was no difference in temperature and humidity between the PSE day and non-PSE day.

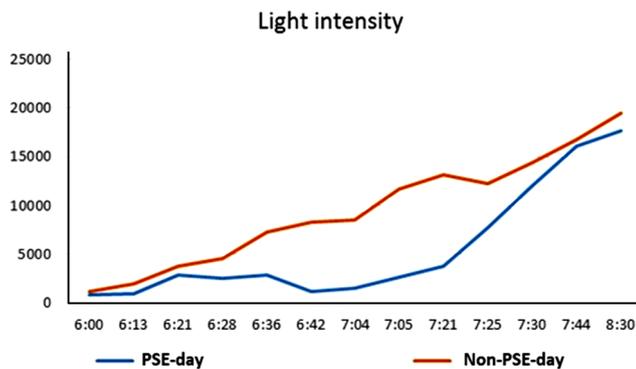


Figure 1 The lux levels recorded at the study site

Animal activity

The recorded animal activities on the PSE and the non-PSE days are shown in **Table 1**. The distance travel, resting time, stereotypic time, burst of

stereotypic movement, and horizontal count in the early PSE were statistically significantly different ($P < 0.05$) than the non-PSE day at the same time (**Table 1**). During the peak PSE period, the resting time, stereotypic time, burst of stereotypic movement, and horizontal count were statistically significantly different compared to the same time on the non-PSE-day. At the end of the PSE period, there were statistically significant differences ($P < 0.05$) in stereotypic time, burst of stereotypical movement, and horizontal count compared to the day after (non-PSE day). The ambulatory time and ambulatory count in the peak and end PSE periods were numerically lower than in normal non-PSE days but not significantly different. There was a correlation between rat activity and change in light levels during the PSE period to stimulate the activity of rats.

Discussion

Animal behavior is about what animals do and how they do it. To learn animal behavior, animals must be observed in their natural condition, and then their condition must be manipulated with internal and external stimuli to observe their response. The PSE in Bogor on 9 March 2016 might modify the circadian rhythms in animals and affect their activities. Circadian rhythm is the adaptation of animals to day-night changes and can be disrupted by manipulation of environmental conditions, such

Table 1 The activity of rats during the partial solar eclipse (PSE) period and non-eclipse (non-PSE) period

Parameter	06.19 AM (early)		07.21 AM (peak)		08.30 AM (end)	
	PSE	Non-PSE	PSE	Non-PSE	PSE	Non-PSE
DT (cm)	193.3 ± 32.4 ^a	93.9 ± 41.08 ^b	140.3 ± 61.6	126.5 ± 93.1	103.1 ± 62.3	212.1 ± 153.5
RT (sec)	14.0 ± 4.9 ^a	48.0 ± 5.2 ^b	21.5 ± 7.2 ^a	44.5 ± 10.2 ^b	36.3 ± 7.3	33.8 ± 7.9
ST (sec)	21.0 ± 4.6 ^a	0.0 ± 0.0 ^b	19.3 ± 7.7 ^a	0.0 ± 0.0 ^b	9.2 ± 6.2 ^a	0.0 ± 0.0 ^b
AT (sec)	20.0 ± 8.3	12.0 ± 5.2	4.8 ± 5.9	15.5 ± 10.2	14.6 ± 7.9	26.2 ± 7.9
BSM (x)	14.3 ± 2.4 ^a	0.0 ± 0.0 ^b	11.9 ± 1.7 ^a	0.0 ± 0.0 ^b	6.3 ± 3.6 ^a	0.0 ± 0.0 ^b
HC (x)	193.0 ± 38.6 ^a	0.0 ± 0.0 ^b	147.0 ± 50.7 ^a	0.0 ± 0.0 ^b	42.8 ± 30.5 ^a	0.6 ± 1.8 ^b
AC (x)	125.3 ± 62.2	28.9 ± 12.9	28.4 ± 31.9	39.4 ± 25.6	34.8 ± 25.0	78.8 ± 23.9

DT: Distance travel, RT: Resting time, ST: Stereotypic time, AT: Ambulatory time, BSM: Burst of stereotypic movement, HC: Horizontal counts, AC: Ambulatory counts. Different superscripts in the same row indicate significant differences between PSE and non-PSE ($P < 0.05$).

as the daily light-dark cycle (Edery, 2000).

The suprachiasmatic nucleus regulates the hypothalamus' circadian rhythm, which communicates with the pineal gland and produces melatonin, the nighttime biochemical signal. The arylalkylamine-N-acetyltransferase enzyme stimulates melatonin secretion, which is maximally secreted during the dark period (Vaughan *et al.*, 1976; Luboshitzky *et al.*, 1998; Falcón, 1999). The melatonin secretion gradually increases when lights are switched off during the light period (Urbanski, 2000). In contrast, melatonin secretion is reduced in rats that are forced to swim in darkness because of the inhibition of melatonin synthesis by corticosterone. Corticosterone is a stress hormone secreted to anticipate and initiate locomotor activity (Lowenstein *et al.* 1984, Roberts *et al.* 1985). Melatonin also affects the other physiological functions that limit endurance, such as water and electrolyte balance and energy metabolism (Mazepa *et al.*, 1999). Zucker (1971) found a decreased drinking under continuous illumination accompanied by hypertrophy of supraoptic hypothalamic neurons that indicates increased antidiuretic hormone (ADH) secretion. Increased ADH secretion will decrease urine excretion and water intake.

The detailed mechanism of the rats' response during the PSE period is shown in **Figure 2**. During PSE, rats became stressed because of the rapid change in the light level (**Figure 1**). They still typically reacted as if they were in the dark cycle, although it was in the morning. They should respond by secreting more melatonin, but it might not have occurred. The melatonin level might not be increased because of the rapid increase in glucocorticoid level as the stress response. They traveled a longer distance, rested less time, had more stereotypic activity, and had a lower ambulatory activity; which were characterized by a longer distance travel (DT), a shorter resting time (RT), more stereotypic movements (BSM), and a longer stereotypic time (ST) in PSE period compared

with non-PSE period (**Table 1**). Stereotypies are a kind of psychotic behavior characterized by a lack of locomotion and repetitive purposeless movements such as body-rocking and repetitive jumping (Mason, 1991; Fowler *et al.*, 2003). Stereotypies have typically occurred in the condition of stress and frustration induced by the dull, restricted captive environment, rapid change of environment in wild and farm animals, and after the administration of psychoactive drugs (Carlstead *et al.*, 1993). Stereotypies have been considered pathological conditions that indicate distress and poor welfare in captive animals (Broom, 1983).

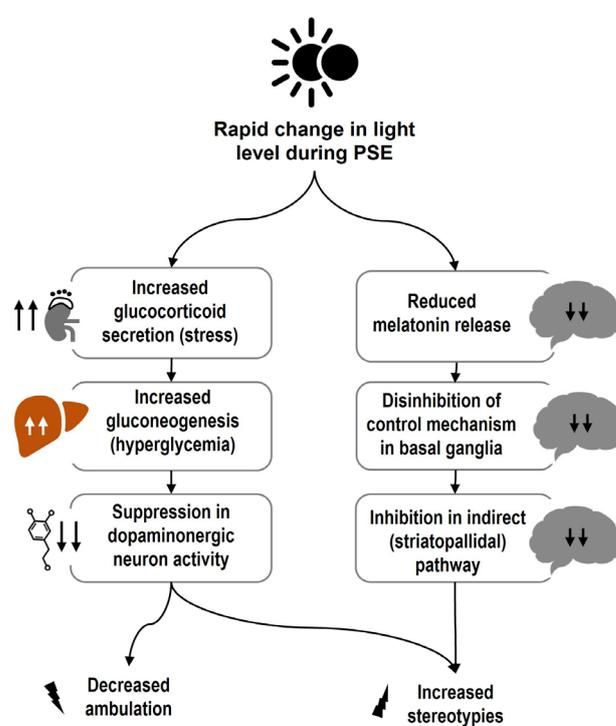


Figure 2 Pathway of rat's response and regulation against partial solar eclipse (PSE)

Caged stereotypy can be developed by moving wild animals from their natural habitat to a small captive environment (Dantzer & Mormede, 1983; Rushen, 1984). Stereotypies are animals' coping strategies against the adverse environment. The inability to cope with a stressor has a poor effect on animals, such as cardiovascular pathology, ulcer formation, and infectious diseases (Koolhaas *et*

al., 1999). The stereotypies (ST and BSM) raised during the PSE period were thought to be caused by the increased glucocorticoid secretion during the stress response. Several studies suggested that environmental enrichment can prevent stereotypies by inducing alterations in neuronal metabolic activity and dendritic morphology that are mediated by neurotrophins (Pham *et al.*, 1999; Ickes *et al.*, 2000).

The disinhibition of behavioral control mechanisms in the basal ganglia produced the stereotypies (Garner & Mason, 2002, Garner *et al.*, 2003). The basal ganglia consist of subcortical nuclei located in the midbrain, around the thalamus. The basal ganglia are associated with various functions, such as voluntary motor control, procedural learning relating to routine behaviors, eye movement, and cognitive and emotional functions (Stocco *et al.*, 2010). Basal ganglia have multiple parallel and segregated circuits targeting different cortical areas by direct (striatonigral) and indirect (striatopallidal) pathways. The activation of the striatonigral pathway will stimulate the thalamus, whereas the striatopallidal pathway will inhibit the thalamus. The inhibition of the striatopallidal pathway will induce stereotypic behavior. In contrast, activating the striatonigral pathway leads to hyperactivity, not stereotypy (Garner, 2006). The increased stereotypic behaviors in this study show the inhibition of the striatopallidal pathway.

Locomotor activity is related to the activity of dopaminergic neurons. Acute administration of glucose suppressed the secretion of dopamine (DA) from substantia nigra neurons. The decreased locomotor activity was reported in diabetic rats. Saller (1984) reported that hyperglycemia reduced striatal DA metabolism 3 and 6 weeks after alloxan administration. Ambulatory activity gradually decreases in diabetic rats due to increased blood glucose, especially in the dark cycle (Shimomura *et al.*, 1988). In addition to ambulatory activity, stereotypic activity is also associated with dopaminergic nerve

activity. Dopamine is produced during the day and inhibits the production of melatonin. Injection of a dopamine neurotoxin (6-OHDA) and dopamine D1 receptor antagonists into the striatum region of the basal ganglia showed a significant reduction in stereotypy in rats (Antelman & Szechtman, 1975). The suppression of dopaminergic neurons alters the balance of the direct and indirect basal ganglia pathways to an overall suppression of the striato-cortical output (Presti *et al.*, 2003).

The same thing occurred during the PSE period. The rats were stress because of the rapid change in the light level. They secreted more glucocorticoids to mobilize more glucose from the body storage to balance the increased metabolic rate. The increased glucocorticoid level also inhibited melatonin secretion and failed to make the rats enter a resting period, and reduced their activity level. The increased glucose blood level inhibits the dopaminergic neuron activity to decrease the ambulatory activity of rats, characterized by a longer ambulatory time (AT) and more ambulatory counts (AC) in the PSE period compared with the non-PSE period.

Under normal circumstances, the authors would repeated this study by themselves, with more groups and kinds of animals, and measured for a longer period. However, the next solar eclipse will not occur in Indonesia until 2023, thus a similar study could not be performed in Indonesia at least until 2023. Therefore, the authors encourage other research groups outside Indonesia to repeat the study when total and partial solar eclipses happen in their country.

Conclusion

The rapid light level change during the PSE period changed the rats' behavior as nocturnal animals. The rats became more active in response to stress during the PSE period. The increased stereotypic activities, reduced resting activities, and reduced ambulatory activities characterized stress response in rats during the PSE period.

Funding: None

Competing Interests: The authors have declared that there was no conflict of interest in this research.

Author contributions: RT analyzed the data; RT, PA, IB, and KS designed and performed the experiments, and RT wrote the paper.

References

- Alvarez-Cárdenas S, Ortega-Rubio A, Galina-Tessaro P, Vega-Villasante F. 1997. Observations on behavior of the lizard *Uta stansburiana* during a total solar eclipse. *The Southwestern Naturalist* 42(1): 108–112.
- Antelman SM, Szechtman H. 1975. Tail pinch induces eating in sated rats which appears to depend on nigrostriatal dopamine. *Science* 189(4204): 731–733. DOI: 10.1126/science.1154024.
- Broom DM. 1983. Stereotypies as animal welfare indicators. In: Smidt D (ed). Indicators relevant to farm animal welfare. *Current Topics in Veterinary Medicine and Animal Science*, 23: 81–87. Dordrecht (NL): Springer Netherlands. DOI: 10.1007/978-94-009-6738-0_11.
- Carlstead K, Brown JL, Seidensticker J. 1993. Behavioral and adrenocortical responses to environmental changes in leopard cats (*Felis bengalensis*). *Zoo Biology* 12(4): 321–331. DOI: 10.1002/zoo.1430120403.
- Chatterjee S, Chandra G. 1997. Behaviour of *Anopheles barbirostris* before, during and after total solar-eclipse. *Entomon* 22(1997): 157–158.
- Dantzer R, Mormede P. 1983. De-arousal properties of stereotyped behaviour: Evidence from pituitary-Adrenal correlates in pigs. *Applied Animal Ethology* 10(3): 233–244. DOI: 10.1016/0304-3762(83)90144-X.
- Ederly I. 2000. Circadian rhythms in a nutshell. *Physiological Genomics* 3(2): 59–74. DOI: 10.1152/physiolgenomics.2000.3.2.59.
- Falcón J. 1999. Cellular circadian clocks in the pineal. *Progress in Neurobiology* 58(2): 121–162. DOI: 10.1016/S0301-0082(98)00078-1.
- Fowler SC., Birkestrand B, Chen R, Vorontsova E, Zarcone T. 2003. Behavioral sensitization to amphetamine in rats: changes in the rhythm of head movements during focused stereotypies. *Psychopharmacology* 170: 167–177. DOI: 10.1007/s00213-003-1528-5.
- Garner J. 2006. Perseveration and stereotypy: systems-level insights from clinical psychology. In: Stereotypic animal behaviour: Fundamentals and applications to welfare. London (UK): Cromwell Press.
- Garner JP, Mason GJ. 2002. Evidence for a relationship between cage stereotypies and behavioural disinhibition in laboratory rodents. *Behavioural Brain Research* 136(1): 83–92. DOI: 10.1016/S0166-4328(02)00111-0.
- Garner JP, Mason GJ, Smith R. 2003. Stereotypic route-tracing in experimentally caged songbirds correlates with general behavioural disinhibition. *Animal Behaviour* 66(4): 711–727. DOI: 10.1006/anbe.2002.2254.
- Godsil BP, Fanselow MS. 2004. Light stimulus change evokes an activity response in the rat. *Animal Learning & Behaviour* 32: 299–310. DOI: 10.3758/BF03196029.
- Grossen NE, Kelley MJ. 1972. Species-specific behavior and acquisition of avoidance behavior in rats. *Journal of Comparative Physiological Psychology* 81(2): 307. DOI: 10.1037/h0033536.
- Ickes BR, Pham TM, Sanders LA, Albeck DS, Mohammed AH, Granholm AC. 2000. Long-term environmental enrichment leads to regional increases in neurotrophin levels in rat brain. *Experimental Neurology* 164(1): 45–52. DOI: 10.1006/exnr.2000.7415.
- Koolhaas J, Korte S, De Boer S, Van Der Vegt B, Van Reenen C, Hopster H, De Jong I, Ruis M, Blokhuis H, 1999. Coping styles in animals: current status in behavior and stress-physiology. *Neuroscience & Biobehavioral Reviews* 23(7): 925–935. DOI: 10.1016/S0149-7634(99)00026-3.

- Lowenstein PR, Pereyra EN, Solveyra CG, Cardinali DP. 1984. Effect of naloxone on the nocturnal rise of rat pineal melatonin content. *European Journal of Pharmacology* 98(2): 261–264. DOI: 10.1016/0014-2999(84)90598-3.
- Luboshitzky R, Yanai D, Shen-Orr Z, Israeli E, Herer P, Lavie P. 1998. Daily and seasonal variations in the concentration of melatonin in the human pineal gland. *Brain Research Bulletin* 47(3): 271–276. DOI: 10.1016/S0361-9230(98)00105-1.
- Mason GJ. 1991. Stereotypies: a critical review. *Animal Behaviour* 41(6): 1015–1037. DOI: 10.1016/S0003-3472(05)80640-2.
- Mazepa R, Cuevas M, Collado P, Gonzalez-Gallego J. 1999. Melatonin increases muscle and liver glycogen content in nonexercised and exercised rats. *Life Sciences* 66(2): 153–160. DOI: 10.1016/S0024-3205(99)00573-1.
- Mitra T. 1996. A note on dragonfly behaviour during the 1995 total solar eclipse near Calcutta, India. *Notulae Odontologicae* 4(8): 133–134.
- Paredes SD, Sánchez S, Rial RV, Rodríguez AB, Barriga C. 2005. Changes in behaviour and in the circadian rhythms of melatonin and corticosterone in rats subjected to a forced-swimming test. *Journal of Applied Biomedicine* 3(1): 47–57.
- Pham T, Ickes B, Albeck D, Söderström S, Granholm A-C, Mohammed A. 1999. Changes in brain nerve growth factor levels and nerve growth factor receptors in rats exposed to environmental enrichment for one year. *Neuroscience* 94(1): 279–286. DOI: 10.1016/S0306-4522(99)00316-4.
- Presti MF, Mikes HM, Lewis MH. 2003. Selective blockade of spontaneous motor stereotypy via intrastriatal pharmacological manipulation. *Pharmacology Biochemistry and Behaviour* 74(4): 833–839. DOI: 10.1016/S0091-3057(02)01081-X.
- Roberts AC, Martensz ND, Hastings MH, Herbert J. 1985. Changes in photoperiod alter the daily rhythms of pineal melatonin content and hypothalamic β -endorphin content and the luteinizing hormone response to naloxone in the male syrian hamster. *Endocrinology* 117(1): 141–148. DOI: 10.1210/endo-117-1-141.
- Rushen J. 1984. Stereotyped behaviour, adjunctive drinking and the feeding periods of tethered sows. *Animal Behaviour* 32(4): 1059–1067. DOI: 10.1016/S0003-3472(84)80222-5.
- Rutter S, Tainton V, Champion R, Le Grice P. 2002. The effect of a total solar eclipse on the grazing behaviour of dairy cattle. *Applied Animal Behaviour Science* 79(4): 273–283. DOI: 10.1016/S0168-1591(02)00150-8.
- Saller C. 1984. Dopaminergic activity is reduced in diabetic rats. *Neuroscience Letters* 49(3): 301–306. DOI: 10.1016/0304-3940(84)90306-9.
- Shimomura Y, Shimizu H, Takahashi M, Sato N, Uehara Y, Suwa K, Kobayashi I, Tadokoro S, Kobayashi S. 1988. Changes in ambulatory activity and dopamine turnover in streptozotocin-induced diabetic rats. *Endocrinology* 123(6): 2621–2625. DOI: 10.1210/endo-123-6-2621.
- Spiteri NJ. 1982. Circadian patterning of feeding, drinking and activity during diurnal food access in rats. *Physiology & Behaviour* 28(1): 139–147. DOI: 10.1016/0031-9384(82)90115-9.
- Stocco A, Lebiere C, Anderson JR. 2010. Conditional routing of information to the cortex: A model of the basal ganglia's role in cognitive coordination. *Psychological Review* 117(2): 541.
- Urbanski HF. 2000. Influence of light and the pineal gland on biological rhythms. In: *Neuroendocrinology in Physiology and Medicine*. New York (US): Humana Press.
- Vaughan GM, Pelham RW, Pang SF, Loughlin LL, Wilson KM, Sandock KL, Vaughan MK, Koslow SH, Reiter RJ. 1976. Nocturnal elevation of plasma melatonin and urinary 5-hydroxyindoleacetic acid in young men: attempts at modification by brief changes in environmental lighting and sleep and by autonomic drugs. *Journal of Clinical Endocrinology & Metabolism* 42(4): 752–764.

DOI: 10.1210/jcem-42-4-752.

[WMA] World Medical Association. 2013. World Medical Association Declaration of Helsinki: ethical principles for medical research involving human subjects. *JAMA* 310(20): 2191–2194.

DOI: 10.1001/jama.2013.281053.

Zucker I. 1971. Light-dark rhythms in rat eating and drinking behavior. *Physiology & Behaviour* 6(2): 115–126. DOI: 10.1016/0031-9384(71)90078-3.