

MORPHOLOGICAL AND FUNCTIONAL CHANGES OF PITUITARY GH AND PRL CELLS FOLLOWING PROLONGED EXPOSURE OF FEMALE RATS TO CONSTANT LIGHT

Natasa Nestorovic, Natasa Ristic, Vladimir Ajdzanovic, Svetlana Trifunovic and Verica Milosevic

University of Belgrade, Institute for Biological Research "Sinisa Stankovic" - National Institute of Republic of Serbia,
Department for Cytology, Belgrade, Serbia

Received: 29.10.2019.

Accepted: 30.10.2019.

Corresponding author:

Verica Milosevic, Ph.D.

Department for Cytology, Institute for Biological
Research "Sinisa Stankovic"
National Institute of Republic of Serbia
142 Despot Stefan Blvd. Steet, 11060 Belgrade, Serbia

E-mail: dimi@ibiss.bg.ac.rs

ABSTRACT

Light regulates numerous physiological functions including secretion of different hormones. Our aim was to determine morphological and functional changes of the pituitary growth hormone (GH) and prolactin (PRL) producing cells in female rats exposed to constant light regime from the peripubertal to adult period of life. Starting from the thirtieth postnatal day, female Wistar rats were exposed to constant light (600 lx) for the following 95 days. Controls were maintained under the regular laboratory lighting conditions. The GH and PRL cells were immunohistochemically visualized. Changes in cell volumes and volume densities were evaluated by stereology. Concentrations of PRL and GH in circulation were also determined. We detected significant decrease of the GH cell volume and volume density, followed by reduced the GH blood concentration in comparison to the controls. In contrast, PRL cells were larger in size and their volume density was significantly increased when compared to the controls. Accordingly, PRL concentration was elevated. It can be concluded that exposure of female rats to constant light regime, from peripubertal to adult period of life, causes inhibition of the pituitary GH and stimulation of PRL cells.

Keywords: Female rat, pituitary, GH cells, PRL cells, immunohistochemistry, stereology, constant light.



UDK: 591.481.2.084:577.344
Eabr 2023; 24(3):219-225
DOI: 10.2478/sjocr-2019-0063

INTRODUCTION

Numerous biological activities, including energy metabolism, body temperature, feeding, locomotion, reproduction and other types of behavior are rhythmic in nature and synchronized with 24h light-dark cycle. Among the others, endocrine factors are highly rhythmical. Their rhythms are mostly endogenously generated, persist without environmental cues and are driven by intrinsic timekeeping mechanism known as the master circadian clock, which in mammals is located within the suprachiasmatic nucleus (SCN) of the hypothalamus (1). Although endogenous, circadian rhythms are synchronized, or entrained, with external environmental stimuli of which the light is most predictable synchronizing agent. The SCN receives direct retinal input through the retinohypothalamic tract that maintains entrainment of the endogenously rhythmic cells with the light-dark cycle (2). The SCN directly or *via* pineal nocturnal hormone melatonin coordinates the timing of circadian rhythms, including daily control of hormone secretion. However, some of endocrine rhythms are diurnal and are driven by alternating cycles of light and darkness, rather than being internally organized. In contrast to circadian rhythms, diurnal rhythms cease when environmental conditions are constant and no longer provide time cues (1, 2). The hypothalamo-pituitary-adrenal and -gonadal axes, prolactin (PRL) and melatonin secretion are under circadian regulation, while the growth hormone (GH) secretion is diurnal and essentially sleep-dependent, *i.e.* GH secretion is not rhythmic in the absence of sleep (3). However, it has been suggested that GH secretion relies on an interactive effect of the exogenous and endogenous components, which both drive its 24-h rhythm (4).

Growth hormone and PRL are synthesized and secreted from somatotrophic (GH) and lactotrophic (PRL) cells of anterior pituitary. Somatotrophic and lactotrophic cells are considered to be “sister cells” since they are differentiated from the same, Pit1 cell lineage (5). Both hormones are controlled by stimulating and inhibitory influences from the hypothalamus, modulated by sex steroids and have important role in pubertal maturation. Growth hormone promotes growth and development of the body directly affecting muscle and bone growth, lipid metabolism, milk production, and the release of several hormones. Indirectly, it affects several processes in the body by stimulating the production and release of insulin-like growth factor from the liver (6, 7). The release of GH from anterior pituitary is regulated predominantly by two hypothalamic peptide hormones; the GH-inhibiting hormone, somatostatin and the GH-releasing hormone, (GHRH) (6). The GH release follows diurnal rhythm in humans, with the highest mean plasma GH levels and pulse frequency during sleep onset (8). Interestingly, in male rats the GH secretion patterns are independent of the time of day, but in females the GH release seems to follow a diurnal rhythm, with pulse frequency and amplitude found to be increased during the dark period (9).

Prolactin in the mammals affects reproductive, sexual, metabolic, immune, and other functions. Synthesis and secretion of the PRL is controlled by prolactin-releasing factors such as the thyrotropin-releasing-hormone, oxytocin, neurotensin, vasoactive intestinal polypeptide and vasopressin; prolactin-inhibiting factors such as dopamine, somatostatin, or gamma-aminobutyric acid (10). Its secretion has a circadian pattern that persists in constant conditions in humans and rats (10) and is abolished after lesions of the SCN (11). Experimental data strongly suggest that the daily rhythm exhibited by neuroendocrine dopaminergic neurons is endogenous in nature and entrained by light (1, 10, 12).

Modern world society is subjected to the disturbances of circadian rhythms by shift work, sleep deprivation, and environmental light pollution. Lighting during nighttime can have detrimental effect on human health (13). Cardiovascular diseases (14), metabolic syndrome (15), depression (16), and even cancer (17) are associated with disturbances of circadian rhythms.

Since the light is one of the major factors entraining circadian rhythms with the environment, valuable insight into the effects of their disturbance comes from studies of animals exposed to constant light, known as functional pinealectomy (18). It causes melatonin and serotonin deprivation associated with changes in behavior (19) including sexual behavior (20); structural and functional changes in the pituitary-thyroid (21) and -adrenal axes (22, 23); alteration in metabolism (24); promotion of aging and tumorigenesis (25) as well as changes in blood pressure (26). Additionally, continual exposure of female rats to an environment of constant illumination is used for developing of the polycystic ovary syndrome in the rodent model (27).

Having in mind that the PRL and GH secretion patterns are sex- and age-dependent, and their importance in pubertal maturation, we aimed to examine the effects of functional pinealectomy on morphofunctional features of the GH and PRL cells in female rats chronically exposed to constant light, from peripubertal to adult period of life.

MATERIAL AND METHODS

Animals and Experimental Design

Female Wistar rats were housed in the unit for experimental animals at the Institute for Biological Research “Sinisa Stankovic”, Belgrade, Serbia. The first group of rats ($n=6$) was placed into constant ambient light conditions (24h/day, light intensity of 600 lx) on the 30th day of life (CL). Animals were maintained in these conditions for the following 95 days. The second group was kept under regular laboratory lighting conditions (12h light–12h dark) during the same period and served as a control group ($n=6$; C). All animals had free access to food and water and were maintained at constant temperature conditions ($21\pm 2^\circ\text{C}$). All animal procedures were in compliance with the European

Communities Council Directive (86/609/EEC) and were approved by the Ethical Committee for the Use of Laboratory Animals of the Institute for Biological Research “Sinisa Stankovic”, the University of Belgrade (No 2–12/13).

Tissue Preparation and Immunohistochemistry

Pituitary glands were excised, weighted, fixed in the Bouin's solution for 48 h and dehydrated in increasing concentrations of the ethanol and xylene. After embedding in Histowax (Histolab Product Ab, Göteborg, Sweden), each tissue block was serially sectioned at 5 μ m thickness on a rotary microtome (RM 2125RT Leica Microsystems, Wetzlar, Germany). Series of sections cut through three tissue levels (dorsal, middle and ventral portions) of the pars distalis were used for the immunohistochemical and immunofluorescence localization of the GH and PRL containing cells. After rehydration, the sections were stained immunohistochemically. Somatotrophic and PRL cells were visualized using the peroxidase enzymatic method as previously described (28). Briefly, sections were incubated with polyclonal hGH or hPRL antibodies (1:300 v/v (DAKO A/S, Glostrup, Denmark) for 24 h at 4°C. After a 5-min rinse in the PBS, sections were incubated for 45 min with secondary antibody (1:500 v/v porcine-anti-rabbit IgG (obtained from DAKO A/S, Glostrup, Denmark), rinsed for 5 min in the PBS, incubated for 45 min with rabbit-antiperoxidase serum (1:100) (DAKO A/S, Glostrup, Denmark) and again rinsed in the PBS. The antigen–antibody complex was visualized using a 0.05% DAB liquid substrate chromogen system (Dako A/S, Glostrup, Denmark). Sections were thoroughly washed under running tap water and counterstained with hematoxylin. Antibody localization was visualized using a 0.05% DAB liquid substrate chromogen system (Dako A/S, Glostrup, Denmark). Sections were thoroughly washed under running tap water and counterstained with hematoxylin.

Stereological measurements

Immunohistochemically stained 5 μ m thick sections of the pars distalis were used for morphometric examinations of the GH and LH cells that possessed visible nuclei. The cell volumes of the examined cells (V_c) and their volume densities (V_v) were estimated by light microscopy at 1000x magnification using the M_{42} multipurpose test system (29). The volumes of cells were expressed in μ m³, while their volume densities were given as percentages of total pituitary cells in mm³.

Determination of serum hormone concentrations

Serum concentrations of the GH and PRL in control and experimental rats were measured by the Delfia method (hGH and hPRL Delfia kits; LKB, Turku, Finland).

Statistical analysis

All results were expressed as means for six animals per group $\pm$ SD. The data were tested for normality of distribution by the Kolmogorov–Smirnov test, whereas the

homogeneity of variances was evaluated by the Leven's test. Student's t-test was used to compare the mean values. The minimum level of statistical significance was set at $p < 0.05$.

RESULTS

Body mass and pituitary weights

Results on bodymass, absolute and relative pituitary weights are summarized in Table 1. Exposure of female rats to CL, from peripubertal period of life to adulthood, resulted in significant decrease of the weight by 22.9% ($p < 0.05$) comparing to the control group. In absolute and relative pituitary weights, no significant changes were detected (Table 1).

Table 1. Effects of prolonged exposure to constant light (CL) on body mass and pituitary weight in adult female rats

(g)	Body mass	Absolute pituitary weight (mg)	Relative pituitary weight (mg x 100)/body mass
C	304.2 $\pm$ 19.6	15.3 $\pm$ 0.8	5.1 $\pm$ 0.5
CL	234.4 $\pm$ 25.6*	14.3 $\pm$ 1.6	5.3 $\pm$ 0.9

All values are given as mean $\pm$ SD (n=6); * $p < 0.05$ CL vs. Control

Immunohistochemical analysis

Somatotropic cells (GH) in the pituitary pars distalis of control females were stained intensely and homogeneously. They were ovoid to pyramidal in shape, usually present in groups and in close contact with blood capillaries (Fig. 1a). After chronic exposure to constant light, the immunolabeled GH cells were ovoid to polyhedral in shape, but smaller in size and more frequently present alone than in groups (Fig. 1b) comparing to the controls.

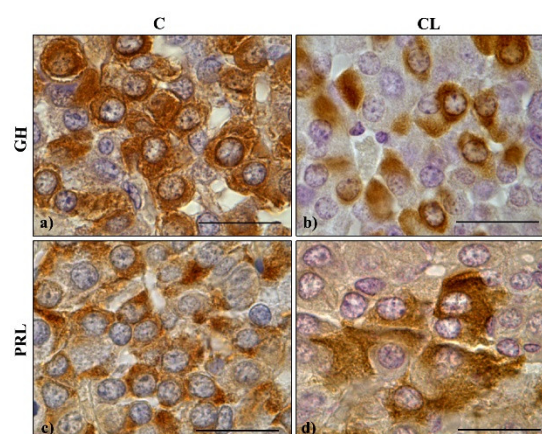


Figure 1. Representative micrographs of the GH (a, b) and PRL cells (c, d) in the pituitary of control (C; a, c) and females exposed to constant light (CL; b, d).

Bar scale – 20 μ m.

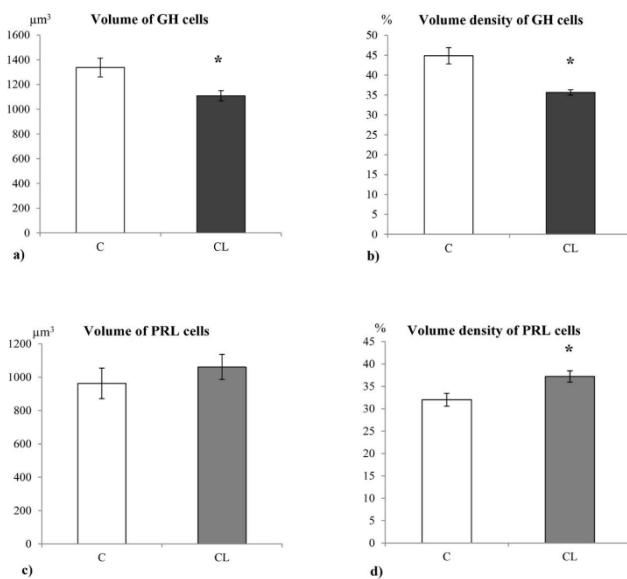


Figure 2. Stereological parameters of the GH (a, b) and PRL cells (c, d) in the pituitaries of control (C) and females exposed to constant light (CL). All values are provided as the mean $\pm$ SD; n = 6. *p < 0.05, CL vs. C.

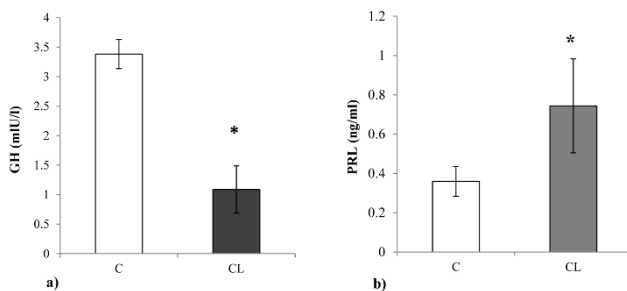


Figure 3. Serum levels of the GH (a) and PRL (b) in control (C) and females exposed to constant light (CL). All values are presented as the mean $\pm$ SD; n = 6. *p < 0.05, CL vs. C.

Lactotropic (PRL) cells of control females were polygonal in shape with the spherical nuclei, surrounded by relatively small portion of the pronounced immunolabeled cytoplasm (Fig. 1c). In the pituitaries of females exposed to constant light, PRL cells were larger; their shape was irregular, with more pronounced prolactin content within their cytoplasm (Fig. 1d).

Stereological analysis

Stereological analysis confirmed findings of the histological examination. Namely, volume of the GH cells was significantly decreased after constant light exposure, by 17.1% (p < 0.05), comparing to the control values (Fig. 2a). Consequently, the percentage of somatotrophic cells within the pituitaries of females exposed to constant light, i.e. their volume

density, was decreased by 20.5% (p < 0.05) (Fig. 2b). Volume of the PRL cells was increased by 10.0% compared to the control values (Fig. 2c), however, this increase did not reach statistical significance. On the other hand, volume density of the immunoreactive PRL cells was increased by 16.3% (p < 0.05), comparing to the controls (Fig. 2d).

Serum GH and PRL concentrations

After exposure to the constant light, serum GH concentration decreased by 67.0% (p < 0.05) (Fig. 3a). On the other hand, the PRL concentration increased (p < 0.05) two-fold (Fig. 3b) compared to the controls.

DISCUSSION

In an effort to elucidate whether the functional pinealectomy influence pituitary GH and PRL cells, their immunohistochemical and morphofunctional properties were assessed in adult female rats exposed to the constant light regime from peripubertal period of life. Our results provide evidence that constant light conditions induce structural and functional changes of pituitary GH and PRL cells.

In the pituitary *pars distalis* of light-treated females, the GH cells were smaller in size than in control females which was confirmed by stereological analysis, i.e. their volume and volume density were decreased. On the other hand PRL cells were enlarged, with more intensely stained cytoplasm and increased volume density. Francou et al. (30), however, did not observe changes in average percentage of the GH or PRL cells in females that were exposed to continuous illumination in adulthood. This discrepancy may originate from the different experimental settings. Namely, in our study females were exposed to constant light conditions throughout sensitive pubertal maturation, which elicit more stronger changes in PRL and GH cells, since it is also a period of maturation of Gh1 and Prl genes expression (31).

Smaller GH cell volume and decreased overall percentage in *pars distalis* resulted in decreased GH blood concentration in our study. Changes of environmental lighting conditions cause lower amplitudes of GH peaks in adult male rats (32) and decreased growth rates, judged by the decreased body weight and tail length (33) exposed to continuous light in adult or juvenile period of life, respectively. We have also observed the reduced body weight in light-treated females in the present and in our previous study (21).

Alternation of circadian PRL rhythms and elevated PRL blood level in females exposed to long periods of constant light (60-120 days) (34) or from birth till adulthood (35) were previously reported. Accordingly, in our study we observed increased volume density of PRL cells in light-treated females that resulted in the increased PRL concentration. Rhythmic urine PRL, but with elevated average value, was also observed in males under longer (three weeks) (36) or shorter (one week) (37) continuous light regime.

The effects of melatonin on the GH and PRL cells, *i.e.* its lowered concentration in animals exposed to the constant light (36) must be taken into account. It has been reported that the GH rhythm was suppressed in rats with removed pineal gland, while after melatonin administration a distinct increase of GH concentration during the day was observed (38). Melatonin plays facilitatory role in the GH secretion at the hypothalamic level by inhibiting endogenous somatostatin release in men (39). Additionally, there is evidence that melatonin treatment decreases rat levels of ghrelin (40), an endogenous ligand for the growth hormone secretagogue receptors (41).

It is generally accepted that melatonin modulates the PRL secretion in the rat. Its inhibitory effect on the hypothalamic dopamin release, thus decreasing inhibitory influence of dopamine on the secretion of PRL (42) has been shown *in vitro*. However, there are reports that melatonin reduced the PRL production from rat pituitary cells in culture (43). Presence of melatonin receptors was not confirmed in the PRL cells of pituitary *pars distalis* (44), in contrast to pituitary *pars tuberalis*, which contains a high density of receptors for the melatonin and it is implicated in the expression of both circadian and seasonal endocrine cycles (45). This structure also expresses clock genes (46). Hence, in melatonin mt1 receptor knockout mice amounts of circulating prolactin are significantly higher than in wild-type littermates (47).

Alterations in light cycles are typically considered as stressful. Continuous light exposure caused a significant increase in serum adrenocorticotrophic hormone and serum corticosterone concentration in female rats (22, 23). Stimulated pituitary-adrenal axis and elevated glucocorticoid levels may affect the GH and PRL cells since exposure to different stressors induces the decrease of GH and increase of PRL levels (48). Growth hormone concentration can be modulated by elevated glucocorticoids by different actions on the hypothalamus, pituitary gland and liver (49). There is also evidence that corticotropin-releasing hormone serves as the mediator of the stress-induced suppression of GH *via* direct synaptic connections between somatostatin neurons (6). Many hypothalamic substances are implicated in the prolactin-secretory stress response, and besides dopamine they include serotonin, histamine, N-methyl-D,L-aspartic acid, atrial natriuretic peptide, b-endorphin and dynorphin, oxytocin, vasopressin (10) and melanocortins (50).

Chronic exposure of female rats to constant light regime is used for developing of the polycystic ovary syndrome in the rodent model, in which levels of estradiol and estrone are elevated (27). It has been shown that estradiol inhibits the GH (51) and stimulates the PRL secretion (10).

In conclusion we have demonstrated that exposure of female rats to constant light regime from peripubertal to adult period of life, cause inhibition of pituitary GH and stimulation of PRL cells, judged by their immunohistochemical and morphometric features, and physiological endpoint, *i.e.* concentration of these hormones in circulation.

ACKNOWLEDGMENTS

This work was supported by the Ministry of Education, Science and Technological Development of the Republic of Serbia, contract number 451-03-68/2020-14/200007.

REFERENCES

1. Butler MP, Kriegsfeld LJ, Silver R. Circadian regulation of endocrine functions. In DW Pfaff, AP Arnold, AM Etgen, SE Fahrbach, RT Rubin (Eds.), *Hormones, Brain and Behavior Online* (pp. 473-507). San Diego: Academic Press, 2010.
2. Lee TM, Smale L. Neuroendocrinology of behavioral rhythms. In A Lajtha, JD Blaustein (Eds.), *Handbook of Neurochemistry and Molecular Neurobiology: Behavioral Neurochemistry, Neuroendocrinology and Molecular Neurobiology* (pp. 835-67). Boston MA: Springer, 2007.
3. Hastings M, O'Neill JS, Maywood ES. Circadian clocks: regulators of endocrine and metabolic rhythms. *J Endocrinol.* 2007; 195(2):187-98.
4. Weibel L, Follenius M, Spiegel K, Gronfier C, Brandenberger G. Growth hormone secretion in night workers. *Chronobiol Int.* 1997; 14(1):49-60.
5. Zhu X, Gleiberman AS, Rosenfeld MG. Molecular physiology of pituitary development: signaling and transcriptional networks. *Physiol Rev.* 2007; 87(3):933-63.
6. Muller EE, Locatelli V, Cocchi D. Neuroendocrine control of growth hormone secretion. *Physiol Rev.* 1999; 79(2):511-607.
7. Hull KL, Harvey S. Growth hormone: roles in female reproduction. *J Endocrinol.* 2001; 168(1):1-23.
8. Veldhuis JD, Roemmich JN, Rogol AD. Gender and sexual maturation-dependent contrasts in the neuroregulation of growth hormone secretion in prepubertal and late adolescent males and females - a general clinical research center-based study. *J Clin Endocrinol Metab.* 2000; 85(7):2385-94.
9. Pincus SM, Gevers E F, Robinson IC, van den Berg G, Roelfsema F, Hartman ML, Veldhuis JD. Females secrete growth hormone with more process irregularity than males in both humans and rats. *Am J Physiol.* 1996; 270(1 Pt 1):E107-15.
10. Freeman ME, Kanyicska B, Lerant A, Nagy G. Prolactin: structure, function, and regulation of secretion. *Physiol Rev.* 2000; 80(4):1523-631.
11. Bethea CL, Neill JD. Lesions of the suprachiasmatic nuclei abolish the cervically stimulated prolactin surges in the rat. *Endocrinology.* 1980; 107(1):1-5.
12. Sellix MT, Freeman ME. Circadian rhythms of neuroendocrine dopaminergic neuronal activity in ovariectomized rats. *Neuroendocrinology.* 2003; 77(1):59-70.
13. Navara KJ, Nelson RJ. The dark side of light at night: physiological, epidemiological, and ecological consequences. *J Pineal Res.* 2007; 43(3):215-24.
14. Knutsson A. Health disorders of shift workers. *Occup Med (Lond).* 2003; 53(2):103-8.

15. Bellet MM, Sassone-Corsi P. Mammalian circadian clock and metabolism-the epigenetic link. *J Cell Sci.* 2010; 123(Pt 22):3837-48.
16. Salgado-Delgado R, Tapia Osorio A, Sadari N, Escobar C. Disruption of circadian rhythms: a crucial factor in the etiology of depression. *Depress Res Treat.* 2011; 2011:839743.
17. Hansen J. Increased breast cancer risk among women who work predominantly at night. *Epidemiology.* 2001; 12(1):74-7.
18. Delibas N, Tuzmen N, Yonden Z, Altuntas I. Effect of functional pinealectomy on hippocampal lipid peroxidation, antioxidant enzymes and N-methyl-D-aspartate receptor subunits 2A and 2B in young and old rats. *Neuro Endocrinol Lett.* 2002; 23(4):345-50.
19. Voiculescu SE, Le Duc D, Rosca AE, Zeca V, Chitimus DM, Arsene AL, Dragoi CM, Nicolae AC, Zagrean L, Schoneberg T, Zagrean AM. Behavioral and molecular effects of prenatal continuous light exposure in the adult rat. *Brain Res.* 2016; 1650:51-9.
20. Hardy DF. The effect of constant light on the estrous cycle and behaviour of the female rat. *Physiol Behav.* 1970; 5(4):421-5.
21. Miler M, Sošić-Jurjević B, Nestorović N, Ristić N, Medigović I, Savin S, Milosević V. Morphological and functional changes in pituitary-thyroid axis following prolonged exposure of female rats to constant light. *J Morphol.* 2014; 275(10):1161-72.
22. Milosević V, Nestorović N, Negić N, Filipović B, Brkić B, Starčević V. Characteristics of the pituitary immunopositive ACTH cells in rat females after chronic exposure to constant light. *Jugoslav Med Biochem.* 2003; 22(1):27-32.
23. Milosević V, Trifunović S, Sekulić M, Sosić-Jurjević B, Filipović B, Negić N, Nestorović N, Manojlović-Stojanoski M, Starčević V. Chronic exposure to constant light affects morphology and secretion of adrenal zona fasciculata cells in female rats. *Gen Physiol Biophys.* 2005; 24(3):299-309.
24. Wideman CH, Murphy HM. Constant light induces alterations in melatonin levels, food intake, feed efficiency, visceral adiposity, and circadian rhythms in rats. *Nutr Neurosci.* 2009; 12(5):233-40.
25. Vinogradova IA, Anisimov VN, Bukalev AV, Semchenko AV, Zabezhinski MA. Circadian disruption induced by light-at-night accelerates aging and promotes tumorigenesis in rats. *Aging (Albany NY).* 2009; 1(10): 855-65.
26. Briaud SA, Zhang BL, Sannajust F. Continuous light exposure and sympathectomy suppress circadian rhythm of blood pressure in rats. *J Cardiovasc Pharmacol Ther.* 2004; 9(2):97-105.
27. Walters KA, Allan CM, Handelsman DJ. Rodent models for human polycystic ovary syndrome. *Biol Reprod.* 2012; 86(5):149, 1-12.
28. Milosević V, Brkić B, Velkovski S, Sekulić M, Lovren M, Starčević V, Severs WB. Morphometric and functional changes of rat pituitary somatotropes and lactotropes after central administration of somatostatin. *Pharmacology.* 1998; 57(1):28-34.
29. Weibel ER. *Stereological Methods. 1. Practical Methods for Biological Morphometry.* New York: Academic Press.; pp 1-415, 1979.
30. Francou M, Durdos M, Salvetti NR, Baravalle C, Rey F, Ortega HH. Characterization of pituitary cell populations in rats with induced polycystic ovaries. *Cells Tissues Organs.* 2008; 188(3):310-9.
31. Bjelobaba I, Janjić MM, Kucka M, Stojilković SS. Cell type-Specific sexual dimorphism in rat pituitary gene expression during maturation. *Biol Reprod.* 2015; 93(1): 21, 1-9.
32. Tannenbaum GS, Martin JB. Evidence for an endogenous ultradian rhythm governing growth hormone secretion in the rat. *Endocrinology.* 1976; 98(3):562-70.
33. Relkin R. Effects of pinealectomy, constant light and darkness on growth hormone levels in the pituitary and plasma of the rat. *J Endocrinol* 1972; 53(2):289-93.
34. Vaticon MD, Fernandez-Galaz C, Esquifino A, Tejero A, Aguilar E. Effects of constant light on prolactin secretion in adult female rats. *Horm Res.* 1980; 12(5): 277-88.
35. Mhatre MC, Shah PN, Juneja HS. Effect of varying photoperiods on mammary morphology, DNA synthesis, and hormone profile in female rats. *J Natl Cancer Inst.* 1984; 72(6):1411-6.
36. Claustrat B, Valatx JL, Harthe C, Brun J. Effect of constant light on prolactin and corticosterone rhythms evaluated using a noninvasive urine sampling protocol in the rat. *Horm Metab Res.* 2008; 40(6):398-403.
37. Kizer JS, Zivin JA, Jacobowitz DM, Kopin IJ. The nyctohemeral rhythm of plasma prolactin: effects of ganglionectomy, pinealectomy, constant light, constant darkness or 6-OH-dopamine administration. *Endocrinology.* 1975; 96(5):1230-40.
38. Ostrowska Z, Kos-Kudła B, Swietochowska E, Marek B, Kajdaniuk D, Ciesielska-Kopacz N. Influence of pinealectomy and long-term melatonin administration on GH-IGF-I axis function in male rats. *Neuro Endocrinol Lett.* 2001; 22(4):255-62.
39. Valcavi R, Zini M, Maestroni GJ, Conti A, Portoli I. Melatonin stimulates growth hormone secretion through pathways other than the growth hormone-releasing hormone. *Clin Endocrinol. (Oxf)* 1993; 39(2):193-9.
40. Mustonen AM, Nieminen P, Hyvarinen H. Preliminary evidence that pharmacologic melatonin treatment decreases rat ghrelin levels. *Endocrine.* 2001; 16(1): 43-6.
41. Howard AD, Feighner SD, Cully DF, Arena JP, Liberato PA, Rosenblum CI, Hamelin M, Hreniuk DL, Palyha OC, Anderson J, Paress PS, Diaz C, Chou M, Liu KK, McKee KK, Pong SS, Chaung LY, Elbrecht A, Dashkevich M, Heavens R, Rigby M, Sirinathsinghji DJ, Dean DC, Melillo DG, Patchett AA, Nargund R, Griffin PR, DeMartino JA, Gupta SK, Schaeffer JM, Smith RG, Van der Ploeg LH. A receptor in pituitary and hypothalamus that functions in growth hormone release. *Science.* 1996; 273(5277):974-7.

42. Zisapel N, Egozi Y, Laudon M. Inhibition by melatonin of dopamine release from rat hypothalamus in vitro: variations with sex and the estrous cycle. *Neuroendocrinology*. 1983; 37(1):41-7.
43. Griffiths D, Bjoro T, Gautvik K, Haug E. Melatonin reduces the production and secretion of prolactin and growth hormone from rat pituitary cells in culture. *Acta Physiol Scand*. 1987; 131(1):43-9.
44. Wittkowski W, Bockmann J, Kreutz MR, Bockers TM. Cell and molecular biology of the pars tuberalis of the pituitary. *Int Rev Cytol*. 1999; 185:157-94.
45. Morgan PJ. The pars tuberalis: the missing link in the photoperiodic regulation of prolactin secretion? *J Neuroendocrinol*. 2000; 12(4):287-95.
46. Messager S, Ross AW, Barrett P, Morgan PJ. Decoding photoperiodic time through Per1 and ICER gene amplitude. *Proc Natl Acad Sci U S A*. 1999; 96(17): 9938-43.
47. von Gall C, Garabette ML, Kell CA, Frenzel S, Dehghani F, Schumm-Draeger PM, Weaver DR, Korf HW, Hastings MH, Stehle JH. Rhythmic gene expression in pituitary depends on heterologous sensitization by the neurohormone melatonin. *Nat Neurosci*. 2002; 5(3):234-8.
48. Seggie JA, Brown GM. Stress response patterns of plasma corticosterone, prolactin, and growth hormone in the rat, following handling or exposure to novel environment. *Can J Physiol Pharmacol*. 1975; 53(4):629-37.
49. Mazziotti G, Giustina A. Glucocorticoids and the regulation of growth hormone secretion. *Nat Rev Endocrinol*. 2013; 9(5):265-76.
50. Dutia R, Kim AJ, Mosharov E, Savontaus E, Chua SC Jr, Wardlaw SL. (). Regulation of prolactin in mice with altered hypothalamic melanocortin activity. *Peptides*. 2012; 37(1):6-12.
51. Meinhardt UJ, Ho KK. Modulation of growth hormone action by sex steroids. *Clin Endocrinol (Oxf)*. 2006; 65(4):413-22.

EABR Experimental and Applied
EABR Biomedical Research
 sciencendo

