# The Morphological Markers of Moonphasal Changes in Rats' Organs Gerasimov A.V., Kostyuchenko V.P., Garmaeva D.K., Fedorova S.A.

## **Summary**

In the model experiment on rats in order to verify the lunasensor theory of brain aging by methods of light microscopy in the hypothalamus, responsible for the aging mechanisms, biorhythms, homeostasis and reproduction, the pineal gland, as well as in the organs related to them on the system-level, the morphological markers of moonphasal changes are studied for the first time. Concluded possible enhancement of the hypothalamic-pituitary-gonadal axis, glycogenolysis, water and electrolyte metabolism and cardiac activity on the full moon, and of the pacemakers biological rhythms - suprachiasmatic nuclei of the hypothalamus and pineal gland, the tone of the parasympathetic division of the autonomic nervous system, insulin secretion and insulin-like proteinthe on the new moon.

**Keywords**: rats, hypothalamus, endocrine glands, kidneys, testes, medulla, heart, glands of the digestive system, the lunar cycle.

#### Introduction

According to the lunasensor theory of brain aging [9, 10], under the influence of the gravitational field of the Moon the moonphasal relocation of psammoma bodies is possible in the pineal gland, which influences the secretion of the pineal hormone and activity of hypothalamic neuroendocrine cells responsible for the aging mechanisms, biorhythms, homeostasis, and reproduction.

The suprachiasmatic nuclei (SCN) of the hypothalamus are body's biological clocks. They affect the production of GnRH by small-cell nuclei of hypothalamus, the activity of gonadotropin-releasing endocrine (GnRE) of pituitary and interstitial endocrine (IE) of the testes; project the influence in paraventricular nuclei (PVN) of the hypothalamus, affect the water reabsorption in kidneys, through the "pre-autonomous" neurons they project the influence in rear nuclei of the vagus nerve (RNVN) of medulla, also in heart and pancreas, which changes the functional state of cardiac pacemaker cells, the A- and B- islet cells, and glycogen content in the cells of various tissues. Pineal hormones also affect GnRE of pituitary, glomerular zone cortical cells of the adrenal cortex, sodium reabsorption in the kidneys, that is controlled by the dense patches (DP) epithelial cells of juxtaglomerular complex. Convoluted seminiferous tubules of the testes and epithelial granular sections (GD) of striated ducts of submandibular salivary glands [4-6] are sensitive to IE testosterone.

Changes in physiological parameters of animals and humans, the ultrastructure of the HRC of rats at different moon phases are presented in a number of studies [8, 12]. The Search of morphological markers of moonphasal changes in organs, responsible for the aging mechanisms, biorhythms, homeostasis and reproduction, was never conducted before at the light-optical level. Given that previously in rats' neurosecretory nuclei of hypothalamus, pineal gland and the salivary glands light-dependent morphological changes were noted [1-3, 7, 8, 11], the role of the light factor must be excluded. For the maximum possible reduction of the diurnal and seasonal biorhythms role[8] it was important to carry out the capture of material in the same time and contain animals in a given mode of illumination environment. To exclude differences related to sex and age [12], - young adult males were used.

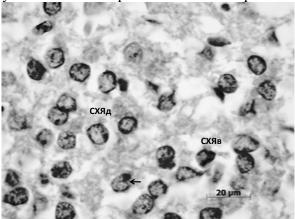
The purpose of this research – to look for markers in rats of alleged moonphasal morphological changes in organs responsible for the aging mechanisms, biorhythms, homeostasis, and reproduction.

### Material and methods

The study was performed in accordance with the "Rules of the work using experimental animals " (1977 ) on 5-month-old male Wistar rats weighing 180-200 g (n = 20), which were kept in a vivarium in the light-dark mode 12-12 h ( starting at 8:00 till 20:00, 200 lux illumination animals). The organs were elicited after the decapitation of rats at 11h daily in May and June in the various phases of the moon after a 24-hour food deprivation, fixed in 10% neutral formalin, dehydrated and embedded in paraffin. The sections were stained with hematoxylin and eosin, Mallory's method. Chromatophilic substance in nerve cells was detected by the method of Nissl. Drugs were examined in the «PrimoStar» microscope with «AxioVision 4.8.2» Software («CarlZeiss», Germany) and digital camera G- 10 ("Canon", Japan).

## **Results and Discussion**

In the SCN during the lunar cycle morphological changes occur mainly in the ventral subcore. Neurons of the nucleus of a given population, the nucleoli become larger on the new moon, tortuosity shell nuclei - more pronounced and clumps margination of condensed chromatin - less significant compared with other lunar cycle phases, which confirms the sensitivity of the circadian pacemaker to moonphasal changes (Fig. 1).



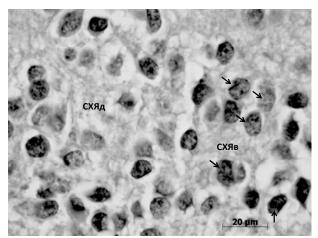


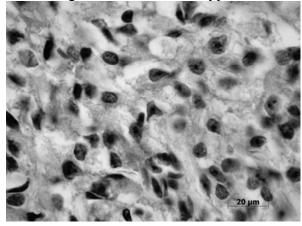
Fig. 1. Suprachiasmatic nucleus on the full moon (a) and the new moon (b): SCNv ventral subcore, SCNs - spinal subcore, arrows indicate intussusception of nuclear envelope. Hematoxylin and eosin stain. Obj. 100, ocular lens 10.

It is possible that a special genetic program is produced in pacemakers with aroundmonth period, which launches on the new moon on the back of revitalization of the pineal gland. On the other hand, in the ventral SCN subcore the cells are found in a state of recovery on the new moon more often than on other lunar cycle phases. They have large nucleoli, and dark colored perikaryonic with evenly distributed clumps of chromatophilic substance. Apparently, pacemakers, along with around-month cycle of functional activity, show a more pronounced reduction ability in the daytime and on the new moon.

The presence of large HRC with light-colored perikaryonic, large nucleus and nucleoli, moderately or low condensed chromatin, deep and narrow intussusception of nuclear envelope ("cariosoma") in the organ on this lunar cycle phase indicates the activation of the pineal gland on the new moon. In opposed phase similar cells are less characteristic to the pineal gland. The light HRC on the full moon often have a small spherical or ovoid nucleus with central nucleolus, moderately condensed chromatin and shallow shell invaginations, and also its protrusion into the HRC with a small pear-shaped nucleus (Fig. 2).

GnRE of the anterior adenohypophysis show signs of increased functional activity, on the contrary, on the full moon. In this phase they acquire larger sizes, the macula, that pushes the core to the periphery, increases, the cytoplasm contains endocrine granules (Fig. 3).

Obviously, that the production of luteinizing hormone activates in GnRE on the full moon against the background of the pineal gland HRC activity inhibition, since on a similar phase IE also acquire larger sizes in the testes. Their nuclei and nucleoli markably increase comparing with the new moon phase, the number of clumps of condensed chromatin in the nucleus reduces, light vacuoles in the oxyphilous stained cytoplasm increase.



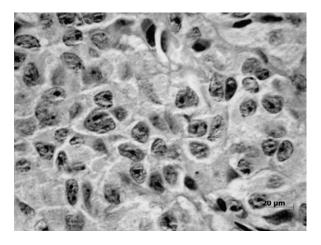


Fig. 2. The pineal gland on the full moon (a) and on the new moon (b). Hematoxylin and eosin stain. Obj. 100 oc.lens 10.

Respectively, the thickness of the spermatogenic epithelium of the convoluted seminiferous tubules in areas with the same spermatogenesis phases appearance is the most pronounced on the full moon compared with other lunar cycle phases, and GD epithelial secretory activity in submandibular salivary glands as well. GD lumen diameter expands, nuclei in epithelial cells are shifted to the central part of the cytoplasm, the size and number of endocrine granules reduce. Apparently on the new moon against the background of GnRE pituitary activity oppression and IE testis in GD, on the contrary, depositing of endocrine granules in the epithelial cells cytoplasm is observed (Fig. 4).

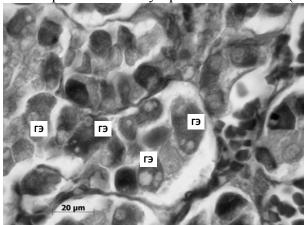
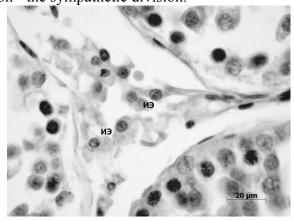
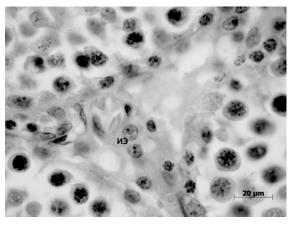
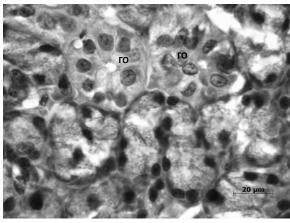


Fig. 3. Prehypophysis on the full moon: GnRE - gonadotropic endocrinocytes. Mallory's stain. Obj. 100. oc.lens 10.

Hypothalamic neuroendocrine cells of the small cell PVN ("pre-autonomous"), autonomous preganglionic neurons RNVN of medulla and postganglionic neurons in the heart show morphological signs of functional activity in the waning moon phase and on the new moon. Nucleus neurons are larger, in perikaryonic parts of the nerve cells focal (mainly central) chromolysis is marked. In the waxing moon phase and on the full moon, on the contrary, their nuclei are smaller, nucleoli are large, hyperchromatic neurons without wrinkling of the nucleus are found more often. Perhaps that not only during the day and seasons of the year, but also the lunar cycle the functional state of the sympathetic and parasympathetic divisions of the autonomic nervous system changes with a shift in the waning moon phase and on the new moon in the direction of enhancing the parasympathetic division, in the waxing moon phase and on the full moon - the sympathetic division.







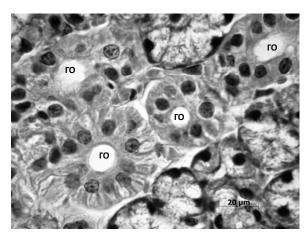
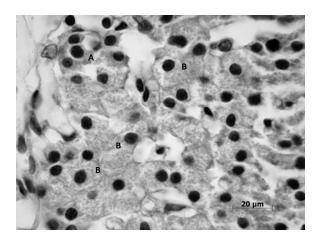


Fig. 4. Testes and submandibular glands: a, b - new moon; c, d - full Moon. IE interstitial endocrinocytes, GD - granular sections. Hematoxylin and eosin stain. Obj. 100 oc.lens10.

B-cells of pancreatic islets have larger nuclei and unevenly dispersed endocrine granules in the cytoplasm on the new moon, and smaller nuclei with more condensed chromatin on the full moon. A- cells, which are located on the periphery of the islets, on the contrary, are larger on the full moon. The size of their nuclei on the new moon is smaller thanon the full moon (Fig. 5).

On the new moon the secretion activization of not only insulin-like protein in granular striated ducts sections of submandibular salivary glands is possible, but also the secretion activization of insulin by pancreas islets B-cells, which favors the absorption of glucose by cells of different tissues, glycogen synthesis and deposition in the " parasympathetic " phase of reduced reproductive activity of rats. On the full moon, apparently, not only sympathetic-adrenal system and reproductive activity of the animals becomes more active, but also the secretion of pancreas islets A-cells of glucagon into blood, and the glycogen decomposition.



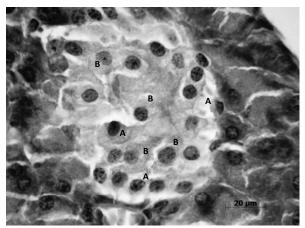
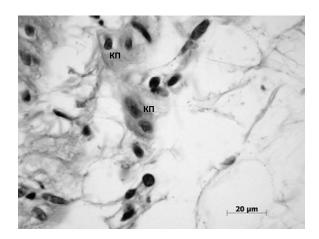


Fig. 5. Pancreas Islet on the full moon (a) and on the new moon (b). A and B - A-and Bcells. Hematoxylin and eosin stain. Obj. 100 oc.lens 10.

Pacemakers cells of the sinoatrial node in the heart on the new moon, in comparison with the full moon, have smaller nuclei with more condensed chromatin and winding nuclear envelope, which indicates their functional activity oppression, and, obviously, heart rhythm changes over the lunar cycle. It is probable that on the new moon not only the heart rhythm slows down, but also the blood pressure decreases. Thus, epithelial cells of DP juxtaglomerular kidney complex on the new moon and in the waxing moon phase are less densed (wider), have larger nuclei with less condensed chromatin. It is possible that during this period of lunar cycle against the background of increasing level in blood plasma of adrenoglomerulotropic hormone and aldosterone of sodium reabsorption in kidneys, the inhibitory activity of DP epithelial cells towards the juxtaglomerulocytes, that secrets renin, becomes more active (Fig. 6).



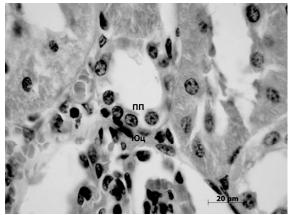


Fig. 6. Atrionector on the new moon (a) and renal cortex on the full moon: PC - cardiac pacemakers cells, DP - dense patch, Jx - juxtaglomerulocyte. Hematoxylin and eosin stain. Obj.100 oc.lens10.

### Conclusion

Thus, during the experiment on rats in different lunar cycle phases, organs responsible for the aging mechanisms, biorhythms, homeostasis, and reproduction are analysed for the first time. By the criteria of structural analysis the alleged morphological markers of moonphasal changes are highlightes and possible cell and tissue mechanisms of functional changes in the body. Morphological signs of increasing secretion in the pituitary lutropin, testosterone and spermatogenesis in the testes, glucagon in the pancreas, heart pacemaker cells appear on the full moon. On the new moon morphological signs of functional activity of suprachiasmatic and paraventricular nuclei of the hypothalamus, the pineal gland, the parasympathetic section of the autonomic nervous system, the insulocytes of pancreatic islets, granular epithelial sections of submandibular glands producing insulin protein, dense patch of juxtaglomerular kidney complex, the cardiac pacemaker cells activity oppression and renin secretion by juxtaglomerular myocytes in kidneys. Moonphasal changes, verified on the light-optical level, confirm the fundamentals of the lunasensor theory of brain aging and require more detailed study by methods of quantitative light and electron microscopy.



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