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# Morpho-functional State Of Hippocampus Of Rats Of Young And Senile Age At Fixed Light Regime And Its Inversion.

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**ABSTRACT**

The study was carried out on 80 Wistar rats at the age of 3 and 18 months. The morphofunctional state of the hippocampus was studied under a fixed light regime (L:D) and under constant illumination. It was found that under constant illumination in the CA1 and CA3 regions of the hippocampus a number of morphofunctional changes occur, more pronounced in rats at the age of 18 months.

**Keywords**. Hippocampus, light regime, constant illumination.

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## INTRODUCTION

Circadian (24 hour) clocks are fundamentally important for coordinated physiological functions in organisms as diverse as bacteria and humans.

The circadian system of mammals includes three key components: endogenous "clock" that generates a circadian rhythm; afferent pathway, which determines the circadian rhythm in accordance with the astrophysical day; efferent pathway that distributes signals from the central generator to peripheral organs.

All the biological rhythms of the organism are strictly subordinate to the main pacemaker located in the suprachiasmatic nuclei of the hypothalamus, where through a retinohypothalamic path information on illumination from a retina of eyes arrives (Meijer J., Schwartz W., 2003; Morin L.P., 2007). The molecular mechanism of SCN is formed by "clock-genes" (*Per1, Per2, Per3, Cry-1, Cry-2, Clock, Bmal1/Mop3, Tim,* etc.). It is shown that light directly affects the work of those of them that provide a circadian rhythm. These genes regulate the activity of the genes of the key cell-division cycle and the genes of apoptosis (Fu L. et al., 2002; Ko C. H., Takahashi J. S., 2006).

Human circadian rhythms are synchronized to light/dark cycles. Over time, organisms adapts to diurnal variations in their physiology and metabolism. These rhythms are regulated by molecular circadian clocks. However, in modern time formation of human's life regimes have shifted away from the naturally occurring solar light cycle to artificial, irregular light schedules produced by electrical lighting.

Long illumination at nighttime promotes oppression of melatonin-forming function of the pineal gland and entails acceleration of aging processes of this organ (Pierpaoli W., Bulian D., 2005; Vinogradova I.A. et al., 2009). Violations of the light status in general, and especially the presence in conditions of continuous illumination, lead to significant violations; the modifying effect of continuous illumination on the physiological processes of an organism is described by many researchers. The impact of continuous lighting on an organism of rats at a young age leads to disruption of homeostasis, an increase in age pathology and the risk of developing of a metabolic syndrome, contributes to a decrease in life expectancy and development of oncogenesis (Maitra S.K., Arun K.R., 2000; Otálora B.B. et al., 2008; Bukalev A.V., 2012). Constant exposure to light also induces alterations in melatonin levels and circadian rhythms in rats, decrease of food intake, visceral adiposity. Exposure of adult female rats to continuous light leads to the gradual development of chronic anovulation. It was established that changes in the light-dark cycle *in vivo* entrain the phase of islet clock transcriptional oscillations, whereas prolonged exposure (10 weeks) to LL disrupts islet circadian clock function through impairment in the amplitude, phase, and interislet synchrony of clock transcriptional oscillations. It is also reported that exposure to LL light regime leads to decrease in glucose-stimulated insulin secretion due to a decrease in insulin secretory pulse mass (Rakshit K. et al., 2015).

An important brain structure of mammals that has secondary oscillatory properties is the hippocampus. The influence of hippocampal activity on the dynamics of a number of biological rhythms of various frequencies has been experimentally proved – from slow long-period oscillations (diurnal, monthly, seasonal) to high-frequency fluctuations (minute and second ranges) of behavior. On the other hand, the state of this structure itself varies rhythmically under the influence of periodic external processes. It is very important to note that the hippocampus is able to make a significant contribution to the organization of a vital circadian biorhythm. In natural conditions, the functional significance of this brain formation comes down to providing more flexibility (plasticity) of rhythmic processes, which should facilitate their response to external influences. At present, the destabilizing influence of the hippocampus on the dynamics of various-period oscillatory processes due to its emotiogenic properties, close interaction with HPA axis, and also functional contention with brain structures participating in the synchronization of biological rhythms (SCN, pineal gland) has been established.

There is evidence that with aging a number of circadian rhythms are smoothed or distorted, both under normal conditions and under the influence of stressors (Cutolo M., et al., 2005; Zisapel N. et al., 2005; Hardeland R. 2017). This kind of age-related changes can be caused by ontogenetic disorders in the functioning of the pineal gland (Margi F. et al, 2004; Zawilska, J. B. et al, 2009).

Based on the above, we found it relevant to study the morphofunctional state of the hippocampus of rats at the age of 3 months and 18 months and of the nature of their circadian rhythmicity at a fixed light regime (L:D 12:12) and constant illumination (L:L).

## MATERIALS AND METHODS

### Animals

The study was carried out on 80 Wistar rats at the age of 3 and 18 months. Animals were taken from the Stolbovaya nursery (the "Stolbovaya" affiliate of the Federal State Budgetary Institution of Science "Scientific Center for Biomedical Technologies of the Federal Medical and Biological Agency).

### Treatment design

For the first group of animals, 20 male Wistar rats of each age were used. During the whole experiment, the rats were housed under a fixed illumination, L:D 12:12 (±180 lux, respectively; 8:00 AM lights on) (unless mentioned otherwise) in a temperature-controlled environment with ad libitum access to tap water and food (rat chow). 20 rats of second group were studied under the same experimental conditions except for the light regime, representing constant light (L:L ±180 lux). Both the first and second groups of animals were kept at the specified light regime for three weeks.

After three weeks, euthanasia of the animals in the carbon chamber was performed at 9.00, 15.00, 21.00 and 3.00 hours, the brains were removed.

All animal experiments were performed in according to the compliance with EC Directive 86/609/EEC and with the Russian law regulating experiments on animals.

### Histological studies

Brain tissues previously fixed in formalin buffer (10%) for 24 h were washed under tap water for 20 min. Then, the serial dilutions of alcohol (methyl, ethyl and absolute ethyl) were used for dehydration. Specimens were cleared in xylene and embedded in paraffin at 56°C in hot air oven for 24 h. Paraffin-beeswax tissue blocks were prepared for sectioning at 4-mm-thick using sledge microtome. The obtained tissue sections were collected on glass slides, deparaffinized and stained with hematoxylin and eosin stains for histological examination through the light microscope.

The sections were successively rehydrated with 100% alcohol, 95% alcohol, and distilled water. Subsequently, the sections were stained in 0.1% Cresyl violet (Sigma-Aldrich) solution. The sections were then differentiated in 95% ethyl alcohol, dehydrated in 100% alcohol, and rinsed in xylene. Finally, the sections were mounted and observed under a light microscope Nikon ECLIPSE 80i (Japan). The average quantity of neurons was calculated by randomly selecting five Nissl-stained sections at the same site from each rat.

We determined the relative number of neurons in multiple fields of view on the total area of the pyramidal layer of CA1 and CA3 regions (further recalculated per 10.000 μm), areas of neuron's bodies, areas of nuclei, nuclear-cytoplasmic ratio, number of apoptotic cells. All measurements were taken with use of image analyzer "AxioVision" (ZEISS, Germany).

### Statistical Analysis

The obtained data, analyzed using Graph Pad Prism 6.0, were expressed as Mean±SD. The statistical difference determined using Student t-tests. A p value of $<0.05$ was considered statistically significant.

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## RESULTS

### Morphological parameters of neurons of hippocampus of rats

Neurons of the CA1 region of the hippocampus of rats at the age of 3 months are characterized by a nuclear area equal to 46.39±2.85 μm$^2$, the area of the neuron made 146.20±4.80 μm$^2$, NCR, accordingly, made 0.32±0.08 (Fig.1).

The shift of the light regime leads to an increase in the area of the nuclei of the neurons of this region to 51.31±3.16 μm$^2$, but the area of the neurons themselves is reduced to 138.35±7.11 μm$^2$, and NCR is equal to 0.37±0,08.

For neurons of the same region in rats at the age of 18 months at a fixed light regime the area of neuron nuclei, equal to 49.14±3.71 μm$^2$ was characteristic, and area of nuclei made 131.47±5.20 μm$^2$ at NCR equal to 0.43±0.08. When the light regime is shifted, the area of the nuclei increases to 56.14±3.85 μm$^2$, area of cells decreases to 131.47±5.20 μm$^2$ at NCR 0.42±0.1.

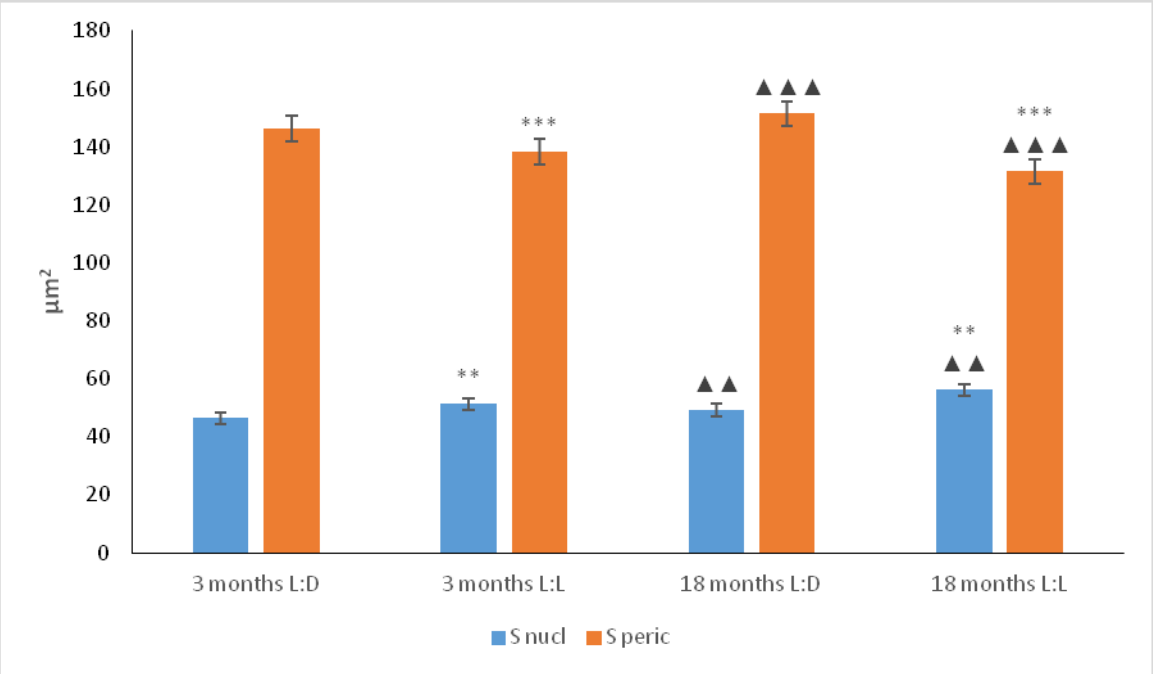


**Fig.1. Some morphometric parameters of neurons of the CA1 region of the hippocampus of rats. (hereinafter: * - significant differences between the parameters at a fixed light mode and constant light mode, ▲ - significant differences between different ages with the same lighting mode).**

At the morphometric analysis of the CA3 region of the hippocampus of rats at the age of 3 months, it was established that the area of the nuclei was 70.10±3.62 μm$^2$, the area of the cell reaches 201.90±8.40 μm$^2$, and NCR – 0.35±0.06. Under conditions of constant illumination, the area of the nuclei decreases to 64.10±3.51 μm$^2$, area of cells – to 181.90±8.40, NCR is equal to 0.35±0.06 (Fig.2).

At the age of 18 months at a fixed light regime the area of the nuclei was 74.10±3.51 μm$^2$, cell area made 217.33±4.20 μm$^2$, and NCR, accordingly, made 0.34±0.09. Transition to constant illumination leads to a decrease in the area of the nucleus to 60.10±4.21 μm$^2$, area of cell decreases to 169.35±5.85 μm$^2$, and NCR is equal to 0.35±0.08

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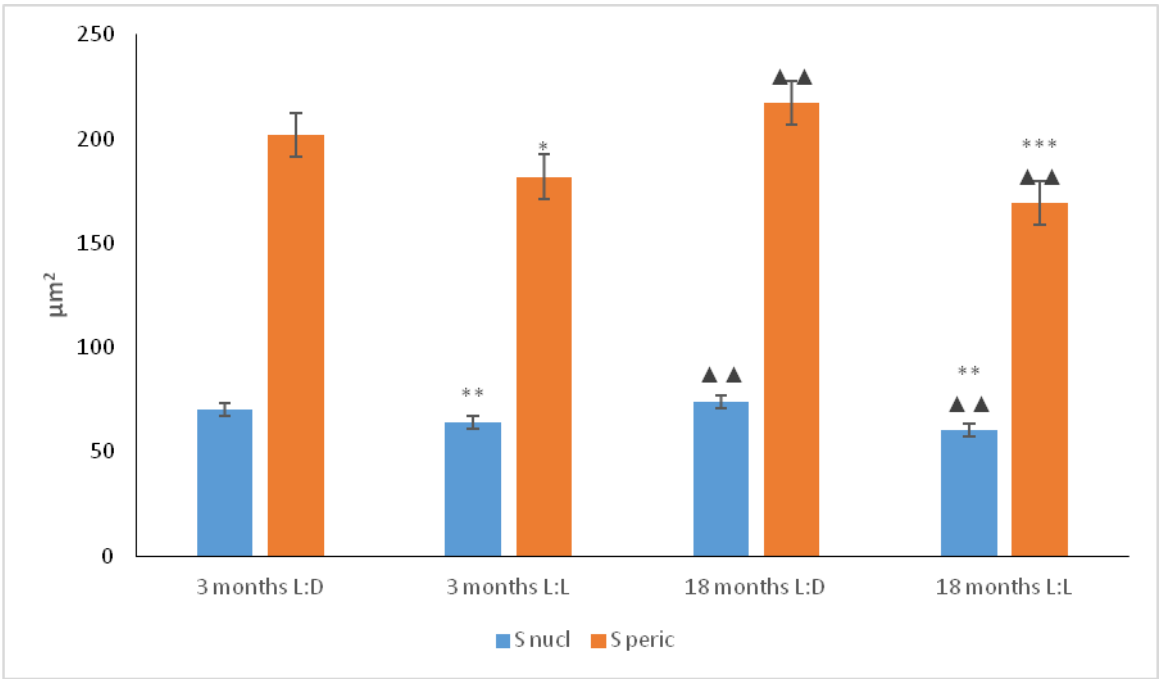


**Fig.2. Some morphometric parameters of neurons of the CA3 region of the hippocampus of rats.**

**Characteristic of population of neurons of a hippocampus of rats**

At analysis of the neuronal population of the hippocampus CA1 region, it was found that changing of the illumination regime to constant light leads to decrease in the number of unchanged cells in both studied ages, and also to increase in the number chromatolyzed neurons, hyperchromatic neurons, wrinkled cells (Table 1). Changes at the age of 18 months are more pronounced than at age of 3 months.

**Table 1. Characteristic of population of neurons of a hippocampus of rats**

| Region CA1 | | | | | | |
|---|---|---|---|---|---|---|
| **Group** | **Unaltered neurons, %** | **Chromatolyzed neurons, %** | **Hyperchromatic neurons, %** | **Ghost neurons, %** | **Wrinkled cells, %** | **Apoptotic cells, %** |
| **L:D 3 months (n=20)** | 71.12±6.41 | 8.41±1.15 | 12.14±1.68 | 4.72±0.81 | 2.51±0.84 | 1.10±0.39 |
| **L:L 3 moths (n=20)** | 47.51±7.14 ** | 18.41±4.51 *** | 23.51±3.92 *** | 5.11±0.91 | 4.58±0.35 * | 1.15±0.44 |
| **L:D 18 moths (n=20)** | 59.81±5.11 ▲▲ | 10.10±1.21 | 18.17±2.41 ▲ | 6.82±1.10 | 3.68±0.71 ▲ | 1.43±0.39 |
| **L:L 18 moths (n=20)** | 34.33±4.55 *** ▲ | 22.75±5.81 ** | 28.16±4.15 * ▲ | 8.14±1.15 ▲▲ | 5.0±0.41 * | 1.62±0.44 |
| **Region CA3** | | | | | | |
| **Group** | **Unaltered neurons, %** | **Chromatolyzed neurons, %** | **Hyperchromatic neurons, %** | **Ghost neurons,%** | **Wrinkled cells, %** | **Apoptotic cells, %** |
| **L:D 3 months (n=20)** | 74.28±4,25 | 7.80±1.10 | 9.51±0.88 | 5.11±0.51 | 2.15±0.22 | 1.15±1.3 |

| | | | | | | |
|---|---|---|---|---|---|---|
| **L:L 3 moths (n=20)** | 59.04±4.88 *** | 12.88±1.14 ** | 15.22±1.61 ** | 5.88±1.12 | 5.10±0.5 ** | 1.88±0.05 |
| **L:D 18 moths (n=20)** | 73.03±8.80 | 6.15±1.25 | 11.15±1.88 | 5.52±0.44 | 2.95±0.35 | 1.20±0.5 |
| **L:L 18 moths (n=20)** | 56.19±5.85 *** | 14.20±2.81 *** | 16.0±2.55 * | 5.88±0.27 | 6.28±0.40 ** | 1.45±0.35 |

In the CA3 region, the change in the illumination regime causes similar changes, but no inter-age differences are noted.

## DISCUSSION AND CONCLUSION

As a result of the study, it was found that under conditions of constant illumination, a number of morphofunctional changes in the hippocampus occur in animals of both studied age groups, which manifest in the CA1 region as an increase in the area of neuronal nuclei and a decrease in the size of the cells themselves. Under the same conditions, the both studied morphometric parameters in the CA3 region decrease. It is worth noting that the changes found in 18-month-old rats are more pronounced.

In animals both in the CA1 region and in the CA3 region of the hippocampus there is a significant change in the number of unchanged neurons, herewith for the CA1 region, but not for CA3 region, we found inter-age differences.

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