

The role of light desynchronization in the development of stress-induced aging

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Abstract. The long-term change of the light mode for three months - light desynchronization, disturbs the rhythm of the signals received from the external pacemaker. As a result of the study, it was found that a long-term change in the light mode and a violation of the rhythmicity of signals received from an external pacemaker contributes to the activation of ROS formation as triggers for bioenergetic processes in the cell. At the same time, changing the light mode disrupts the balance of oxygen in the cell and this is a provoking factor for the stress of the antioxidant cell system. The resulting tissue hypoxia in chronic light desynchronization disrupts the bioenergetic potential of the cell, contributing to the development of pathophysiological processes and the death of neurons. Therefore, a violation of the balance of the pro-oxidant and anti-oxidant systems leads to destructive processes in the brain. A significant change in the concentration of the neurotrophic markers indicates destructive processes in the brain tissues. Summarizing the above, we conclude that light desynchronization is directly involved in the ROS-dependent stress-induced aging of brain cells and in that way, to the progression of processes that lead to aging of the body.

1 Introduction

According to the classical definition, aging is a multi-cause destructive process caused by a complex of regulatory and stochastic factors and determined by the genetically determined biological organization of the living system [1].

Experimental studies on various model organisms have confirmed the possibility of increasing stress resistance and prolonging active life as a result of environmental influences [2, 3]. Thus, success in the development of new climatic-geographic zones, space, world ocean is determined by the ability of people to adapt to new environmental conditions. At the same time, the expansion of the sphere of human professional activity, accelerating urbanization, chronic nervous and emotional stress, man-made accidents, catastrophes, deterioration of environmental conditions, exposure to various chemicals

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leads to desynchronization of biological rhythms – the main control of the body's functions from the cellular level to the level of behavior [4-7].

Biological rhythms have endogenous genetically determined nature. The modern multi-oscillator model of internal biorhythms organization implies the presence of the main pacemaker – suprachiasmatic nuclei of the hypothalamus that control local oscillators through the epiphysis hormone melatonin. One of the external factors that influence and transmit information about changes in the external environment is light. Photons of light have a regulating, activating effect on the consistency of internal biological systems. The endogenous component of biorhythms at the cellular level is supported by clock genes proteins, cytoplasmic molecular complexes that support the cell's redox status (redox oscillator), and membrane mechanisms of impulse activity and translocatory activity. Light activates the expression of clock genes through the generation of reactive oxygen species, mainly H₂O₂. This leads to the formation of redox oscillator molecules: oxidized glutathione, glutathione peroxidase, NADP⁺. These oscillators contribute to a rapid local change in pH in a particular cell compartment, have membrane effects on molecules - transporters, changing the impulse or translocator activity. The redox status of the cytoplasm and nucleoplasm is important for the activation of such transcription factors as CLOCK and its orthologue NPAS2, Rev-erb β , NF-kB, Nrf2, since clock-oscillator proteins in the process of posttranslational modification under the action of enzymes pass into reduced or oxidized forms, and this can affect their interactions and subsequent degradation in proteasomes at certain times of the day. It is obvious that there is a link between the oscillators of the membrane, cytosol and nucleus. Such effects can be mediated, for example, by cAMP and Ca²⁺ ions as the main buffer of acidosis. They are involved in circadian changes in membrane potential, metabolism, and energy exchange, in posttranslational modification of histones, and in the expression of *per* and *cry* genes by transcription factor CREB after its phosphorylation by calcium/calmodulin-dependent kinase (CaMKII) and cAMP [8-12].

The structure of biorhythms is not absolutely stable – it changes under the influence of external and internal factors. If possible, biorhythms should be stable and independent of random influences and the state of the body, but, on the other hand, biorhythms should adapt to specific environmental conditions, in order to give the body maximum opportunities for adaptation [13]. There are many factors that can reconfigure the mechanism of biorhythms to a variety of environmental conditions – periodic physical activity of a non-extreme nature, seasonal changes in light mode, periodic daily changes in the rhythm of life. But there are factors that do not lead to reconfiguration of biorhythms, but, on the contrary, to misalignment of rhythms. For example, unstable work schedules, emotional stress, exposure to artificial lighting all day and night long, uncontrolled use of psychoactive substances. Chronic exposure to such stress factors leads to the development of chronic desynchronization, which is the basis for the development of all pathological conditions [6, 7, 11].

There are 2 known theories in the field of biorhythm research: "discrete theory" Colin Pittendrigh and "continuous theory" of Jürgen Aschoff. Discrete theory assumes that the day – night transitions at dawn and sunset the clock phases instantly reset or shift, correcting their discrepancy with the environment. Aschoff's continuous theory assumes that light acts on biorhythms throughout the circadian cycle. Currently, it is generally accepted that both theories are partially correct and further research in this area is necessary [13, 14, 15].

Stress-induced aging is a type of cellular aging that is based on damage to the DNA molecule, resulting in the triggering of intracellular signaling events in response to DNA damage and the subsequent initiation of cellular aging. Intracellular reactive oxygen species are involved in the progression of cellular aging. Changing the balance of antioxidants and overexpression of antioxidant protection enzymes contributes to the partial prevention of

cellular aging. At the same time, the long-term increased synthesis of antioxidant protection enzymes to contain the damaging effects of ROS contributes to a decrease in the energy potential of the cell necessary for protein synthesis, and the lack of cofactors, trace elements and low-molecular components for metabolic reactions. These changes lead to a change in permeability of cell membranes, which disturbs the movement of hydrogen protons, the polarity of the membrane, increasing the concentration of calcium ions in the cytoplasm of cells, a violation of the processes of bio-energy, and as a result increase the probability of apoptosis [16, 17, 18].

Thus, stress-induced cellular aging against the background of age-dependent increasing apoptosis, reduced regenerative capabilities of tissues, suppression of compensatory cell proliferation leads to degenerative disorders in tissues, which violates the functional performance of the body in general.

The aim of our work was to study the effect of changing the duration of light mode (chronic light desynchronization) on the intensification of the formation of reactive oxygen species in rat brain tissues, activating stress-induced cell aging.

2 Materials and methods

The experimental study was performed on 240 mature male rats of the Wistar breed weighing 160-200 g. from the RAMS "Rappolovo" nursery. The duration of the acclimatization period for animals was 14 days. The animals were kept in accordance with the requirements of GOST 33044-2014 dated 01.08.2015 "Principles of good laboratory practice". The animals were kept in standard vivarium conditions with 6 individuals in a cage and on a standard diet with free access to water and food. The vivarium premises a relative humidity of 50-65% and an air temperature of 20-25°C is maintained. To simulate desynchronization with a modified light mode, the animals were divided into three groups of 80 rats each. The first group of animals was placed in normal lighting conditions (control, the light was turned on at 8.30 am local time and turned off at 20.30 am local time). The average light at the level of cells with animals is 500 Lux white light. Two other groups, in the conditions of the changed light mode: constant lighting and constant darkness. The duration of the experiment was 3 months. After 1 and 3 months of the experiment, biological material – blood and brain of animals - was taken from the animals. The activity of enzymes and substrates of the antioxidant system and products of lipid peroxidation (PLP) was determined in the brain. The concentration of neurotrophic factors was determined in the blood serum.

For each time point, control groups of 6 animals and experimental groups of 10 animals were formed by randomization. Before the study, the brain was weighed, crushed and homogenized in a POLYTRON PT-1200E (Switzerland) tissue micro-shredder with a mechanical homogenizer, then centrifuged at 3000 rpm, adding a 0.1 M potassium phosphate buffer cooled to a temperature of 0 °C with a pH of 7.4 in the ratio "tissue: buffer-1: 9". The time from the moment of thawing of tissues to sampling of homogenates and deposition of protein in them by acids was no more than 1 minute. Samples were taken from the resulting homogenate to determine the concentration of reduced glutathione (RG), PLP products - malondialdehyde (MDA) and diene conjugates (DC). To determine the activity of Glutathione-S-transferase (GT), as well as the concentration of total protein, the activity of superoxide dismutase (SOD), glutathione peroxidase (GP), glutathione reductase (GR), glucose-6-phosphate dehydrogenase (GL-6PDG), a purified cytoplasmic fraction was used. The concentration of the studied substrates and the activity of enzymes were recalculated per 1 gram of tissue in the homogenate. The concentration of total protein and the activity of SOD, GP, GR, and GL-6PDG enzymes in brain tissue homogenate were

determined using a biochemical analyzer "A-25" using sets from BioSystems S.A. (Spain) (total protein) and RanDox (the Great Britain).

The concentration of DC in brain tissue homogenate was determined using the method of I.D. Stalnya (1977) [19]. The concentration of MDA in brain tissue homogenate was determined by the method of M. Uchiyama (1978) [20]. The activity of Glutathione-S-transferase in brain tissue homogenate was determined using the method of W.H. Habig and W.B. Jakoby (1981) [21]. The concentration of reduced glutathione in brain tissue homogenate was determined using 5.5 dithio-bis (-2-nitrobenzoic) acid (DTNB) using method of G.L. Ellman (1959) [22].

Biochemical markers of neurotoxicity in the blood serum of laboratory animals were determined by enzyme immunoassay (EIA) using Cloud-Clone Corp.'s ELISA Kit. (USA) for Enolase, Neuron Specific – neuron – specific enolase (NSE), for Myelin Basic Protein – the main protein of myelin (MBP).

Statistical data processing is performed using AtteStat software, version 13. The Mann-Whitney criterion is used as a nonparametric criterion. The conclusion about the statistical reliability of differences between the groups was accepted at $p < 0.05$ and lower.

3 Results and Discussion

After 1 month of stay of animals under constant lighting, a significant increase in diene conjugates was detected in the brain tissue homogenate by 7.3% compared to the value of this indicator in the brain homogenate of animals from the group with normal light. The DC concentration in the brain homogenate of animals from the constant dark group, on the contrary, decreased by 9.9% ($p < 0.05$) compared to normal light. The activity of glutathione transferase (GT) in the homogenate of both groups with a changed light mode significantly decreases by 16.1% – in the group with constant light, and by 18.8 % – in the group with constant darkness compared to the values of indicators from the group with normal light. In addition, the activity of the synergistic enzyme – glutathione peroxidase - is significantly reduced in the constant light group by 8.3 % and by 9.6% in the constant dark group compared to the indicators from the normal light group. The activity of the superoxide dismutase reaction (SOD) increases in the brain tissue homogenate of animals under conditions with a changed light mode, in the group with constant light by 35.8%, and in the group with constant darkness by 65.5%, respectively, compared with the values of indicators from the group with normal light ($p < 0.05$). Respectively, the activity of the enzyme glucose-6-phosphate dehydrogenase (G-6PDG) decreases, namely, in the group – constant light by 34.5% ($p < 0.05$) and 27.1 % ($p < 0.05$) in the group constant darkness compared to the activity of the enzyme in the normal light group.

The results of studies of indicators of the AOS system and PLP processes in the brain tissue homogenate of animals under normal light conditions and changed light after 1 month of the experiment are shown in table 1.

Table 1. Values of indicators of the antioxidant system and PLP processes in normal light, constant light, constant darkness after 1 month of the experiment

Indicators (M ± m)	Experimental groups		
	Normal light	Constant light	Constant darkness
RG, mmol/gP	1.87±0.15	2.08±0.09	2.00±0.09
DC, nmol/gP	108.2±1.0	116.1±2.6*	97.4±0.8*

MDA, nmol/gP	194.5±4.1	225.2±6	185.1±8.4
GT, U/gP	356.1±5.9	298.6±9.0*	289.0±10.4*
SOD, U/gP	73.1±4.3	99.3±5.9*	121.0±4.7*
GP, U/gP	2.03±0.07	1.86±0.03*	1.83±0.05*
GR, U/gP	3.1±0.8	3.0±0.6	2.8±0.4
G-6-PDG, U/gP	48.9±3	32.0±2.6*	35.6±2.5*
Note – * – p<0.05, Mann-Whitney criterion			

After 1 month of staying in the changed light mode, the activity in the constant dark group increases significantly more than in the constant light group. Under normal light conditions and daily cell activity, reactive oxygen species (ROS) are generated continuously and removed by mitochondrial and cytoplasmic superoxide dismutase to form H₂O₂. In case of violation of the frequency of the light signal, ROS are used as intracellular local oscillators. Also, reactive oxygen species contribute to the induction of synthesis of various regulatory metabolites, ensuring the adaptation of the cell to the changed light mode [23, 24]. In its turn, hydrogen peroxide is decomposed by catalase or reduced by glutathione or thioredoxin to form water and disulfides. These reactions are catalyzed by the corresponding peroxidases: glutathione peroxidase or thyroperoxidase. Regeneration of thiol-containing compounds occurs as a result of the reduction of disulfides due to NADPH⁺, the reaction is catalyzed by glutathione reductase. The NADPH⁺ level provides the pentose phosphate cycle. The pentose cycle functions at a sufficient concentration of oxygen in the cell. In previous studies, we have shown that changing the light mode leads to tissue hypoxia, respectively, the inhibition of the pentose cycle in hypoxia conditions leads to a decrease in the regeneration of NADPH⁺, which is necessary for the activity of glutathione reductase. Under conditions of hypoxia, anaerobic glycolysis and lactate formation are enhanced. An increase in lactic acid activates enzymes that catalyze the breakdown of purine nucleotides. Glucose deficiency inhibits the hexose monophosphate path, which leads to a decrease in the production of pentose-5 phosphates necessary for the reutilization of purine bases.

Glutathione-S-transferase catalyzes the conjugation of glutathione with non-polar compounds of exogenous and endogenous origin, which is especially important in protecting the cell from the toxic effects of these compounds. Increased ROS production leads to s-glutathionylation of proteins, which plays an important role in cellular signaling mechanisms due to the sensitivity of cysteine residues to redox modification. This modification involves proteins participating in the formation of the cytoskeleton, signaling proteins, heat shock proteins, ras-proteins, and transcription factors. S-glutathionylation inhibits glycolysis enzymes, creatine kinase, protein kinase A, tyrosine hydroxylase, complex I of the mitochondrial respiratory chain, transcription factor NF-κB, activates microsomal glutathiontransferase, sarcoplasmic calcium adenosine triphosphatase, complex II of the mitochondrial respiratory chain. The s-glutathionylation process proceeds both enzymatically and non-enzymatically. GT takes an active part in these reactions. GT is involved in the regulation of serine/threonine AMP-activated protein kinase (AMPK), which plays a key role in controlling the energy balance of the cell. Activated AMPK activates the transcription factor FOXO3, which through the activation of the PI3K/AKT

signaling pathway affects the processes of cell proliferation, gluconeogenesis, and protection from oxidative stress. The expression of SOD, catalase, thioredoxin, metallothioneins, mitochondrial disconnecting protein, GP, and GT genes increases [25].

In the group of constant light, the concentration of DC significantly increases, followed by their transformation into MDA. This indicates that the ROS are formed more than necessary. Thus, the permeability of membranes in the cell and the plasma membrane is disrupted, increasing the damage probability to both the cell and the processes of cellular metabolism.

The resulting hypoxia when changing the light mode contributes to an increase in glucose consumption, a decrease in the speed of the pentose phosphate cycle and a corresponding decrease in the activity of all glutathione-dependent enzymes.

The results of studies of indicators of the AOS system and PLP processes in the brain tissue homogenate of animals under normal light conditions and changed light after 3 months of the experiment are shown in table 2.

The study revealed no significant changes in the concentration of RG, but in both groups, with a changed light mode, a decrease in the activity of G-6PDG in the constant light group by 35.8% ($p < 0.05$) and 28.6% ($p < 0.05$) in constant darkness group, respectively, compared to indicators from the normal light group.

The concentration of diene conjugates in the homogenate of samples from the constant light group is 11.7% higher compared to the value of the indicator from the control animals group ($p < 0.05$), and in the constant darkness group, the DC concentration increases by 9.2% ($p < 0.05$) compared to the indicators of the normal light group.

The concentration of MDA in both groups with a changed light mode significantly increases. So in the constant darkness group by 9.9%, and in the group constant darkness group by 18.4% compared to the indicators from the normal light group.

The GT activity in the animal tissue homogenate of both groups with changed light mode significantly decreases by 25.8% – in the group with constant light, and by 19.4% – in the group with constant darkness compared to the values of indicators from the normal light group.

Table 2. Values of indicators of the antioxidant system and PLP processes in normal light, constant light, constant darkness after 3 months of the experiment

Indicators (M ± m)	Experimental groups		
	Normal light	Constant light	Constant darkness
RG, U/gP	1.98±0.13	2.07±0.09	2.05±0.15
DC, U/gP	111.5±2.4	124.6±2.7*	121.8±2.4*
MDA, U/gP	159.4±4.8	175.2±3.5*	188.8±6.3*
GT, U/gP	339.4±5.5	251.7±9.1*	273.4±10.7*
SOD, U/gP	62.7±7.3	42.4±3.6*	58.6±4.7
GP, U/gP	2.33±0.12	2.63±0.05	2.56±0.07*
GR, U/gP	2.7±1.2	3.5±0.3	4.0±0.9
G-6-PDG, U/gP	49.9±1.8	32.0±3.0*	35.6±2.6*

Note – * – $p < 0.05$, Mann-Whitney criterion

After 3 months of the experiment, the activity of the synergistic GT enzyme of glutathione peroxidase significantly increases in the constant dark group by 9.8% compared to the indicators from the normal light group. The activity of SOD in the animal tissue homogenate under constant light conditions significantly decreases by 32.3% compared to the indicators of the normal light group.

Thus, after 3 months of animal presence in changed light mode in the tissues of the brain, an increase DC in both groups and at the same time significantly increases the concentration of MDA in the constant light group and constant darkness group. SOD activity significantly decreases in the constant light group. A long-term violation of photo periodization led to the depletion of these reserves due to tissue hypoxia and lack of energy for the full maintenance of cellular metabolism, a violation of the balance of reactive oxygen species and, as a result, the development of destruction processes in the brain tissues of rats.

Table 3 shows the results of studies of neurotrophic markers in the blood serum of rats under normal light conditions and changed light mode after 1 month of the experiment.

Table 3. Changes in the concentration of neurotrophic markers indications in the blood serum of laboratory animals in normal light, constant light, constant darkness after 1 month of the experiment

Indicators (M ± m)	Experimental groups		
	Normal light	Constant light	Constant darkness
NSE, ng/ml	17038.2±927.5	14999.1±612.2	15212.1±630.2
MBP, ng/ml	5.50±0.47	7.71±0.52*	8.28±1.26*
* - reliable in comparison with the normal light group (at $p \leq 0.05$; Mann-Whitney criterion)			

1 month after changing the light mode, the rats in the constant light group showed a significant increase in the concentration of MBP by 40.1%, and in the constant darkness group - by 50.5% compared to the normal light group. There was a decrease in the concentration of neuron-specific enolase by 11.9 % in the constant light group - by 10.7 % in the constant darkness group compared to the control (normal light).

3 months after the change, the concentration of neuron-specific enolase in the blood serum in the groups with the changed light mode significantly decreased compared to the indicators of the normal light group. In the constant light group, the NSE concentration decreased by 17.4%, and in the constant darkness group - by 13.6 % (table 4).

A significant change in the concentration of MBP indicates destructive processes in the brain tissues.

MBP is a proteolipid protein fraction that interacts with cytoskeleton proteins, affecting their configuration and polymerization. When nerve tissue is damaged, the destruction of myelin is a universal mechanism for the reaction of nerve tissue. When nerve tissue is damaged, the function of the blood-brain barrier is damaged, which is accompanied by an increase in the concentration of MBP in blood plasma [26].

Neuron-specific enolase (NSE) is defined in neurons, neuroendocrine cells of the nervous system, red blood cells, platelets, and is a common marker of all differentiated neurons and belongs to the intracellular enzymes of the CNS. The possibility of using NSE

as a clinical and diagnostic criterion for assessing the degree of damage to brain neurons in destructive diseases of the central nervous system has been proved [27, 28].

Table 4. Changes in the concentration of neurotrophic markers indications in the blood serum of laboratory animals in normal light, constant light, constant darkness after 3 months of the experiment

Indicators (M ± m)	Experimental groups		
	Normal light	Constant light	Constant darkness
NSE, ng/ml	13970.93±813.14	11530.6±374.4*	12063.06±238.66*
MBP, ng/ml	11.41±1.44	9.11±0.57	8.82±0.49
* - reliable in comparison with the normal light group (at $p \leq 0.05$; Mann-Whitney criterion)			

4 Conclusion

In conclusion, we would like to note that the experiment included 60-day-old sexually mature rats that were exposed to changes in light mode for 1 and 3 months. That is, at the analyzed points, the animals were 90 and 150 days old from their birth. According to a number of authors, it is believed that one day of a rat's life is equal to 30 days of a human's life. According to Robert Quinn, to estimate the age of a rat and extrapolate to the age of a human, it is necessary to specify the analyzed factors. In this regard, Quinn offers a more detailed system for calculating the age of the rat. Rats become sexually mature in 40-50 days after birth. For human, this age corresponds to 12-14 years. Further, the calculation of the age of the rat is based on the calculation of 3.3 rat days equal to 1 year of human life. It turns out that the age of the rats subjected to a change in light mode corresponded to 12-14 years of age of the human and the first analyzed time point corresponds to 21-23 years of age of the human. The second analyzed point, in another 60 days of rat life, corresponds to 18 years of human life, that is, a human aged 39-41 years from the date of birth [29]. Based on this scheme, the interpretation of the data obtained by us corresponds to the forecast of the long-term consequences of changes in the light mode over the course of 20 years of human life.

As a result of the study, it was found that a long-term change in the light mode and a violation of the rhythmicity of signals received from an external pacemaker contributes to the activation of ROS formation as triggers for bioenergetic processes in the cell [23, 24]. At the same time, changing the light mode disrupts the balance of oxygen in the cell and this is a provoking factor for the stress of the antioxidant cell system. Changes in the duration of the light period cause a reaction of the photosensitive PAS domain of clock genes. The PAS domain is activated by light, it can bind O₂, CO or NO, and respond to the effects of steroids, peptide hormones. Among the proteins important for maintaining energy homeostasis, the photosensitive PAS domain has Bmal1, Cry, Per1, Per2, Clock, HIF1 α , HIF1 β , controlling the energy metabolism of the cell, which, in turn, is associated with the concentration of oxygen in the cell and the presence of reducing equivalents [23, 24, 30]. The resulting tissue hypoxia in chronic light desynchronization disrupts the bioenergetic potential of the cell, contributing to the development of pathophysiological processes and the death of neurons [30]. Due to the rich structural diversity of lipids, the brain shows poor resistance to oxidative damage. Therefore, a violation of the balance of the pro-oxidant and anti-oxidant systems leads to destructive processes in the brain. Also, a violation of the rhythm of melatonin synthesis due to impaired light periodization, deprives the brain of full

participation in the processes of antioxidant protection of one of the most powerful antioxidants – melatonin. The use of melatonin's antioxidant effect in the treatment of neurodegenerative diseases is known in clinical practice [31].

Summarizing the above, we can conclude that light desynchronization is directly involved in the stress-induced aging of brain cells and in that way, to the progression of processes that lead to aging of the body.

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