Journal of Life Sciences & Biomedicine, vol. 2(75), No 1, p. 104-109 (2020)

Peculiarities of involutory processes (aging processes) on background of light desynchronization

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Accepted for publication: 12 June 2020

To identify the effect of light desynchronization on the neuroendocrine regulation of physiological processes in the development of aging, we studied the effect of light desynchronization on the degree of involutive processes (aging processes). To objectify the results of the effect of light desynchronization on the neuroendocrine regulation of physiological processes in the development of the aging organism, the parameters were evaluated before light desynchronization, on the 1st day after the light desynchronization, on the 12th and 23rd days after the light desynchronization. Since the regulatory mechanisms of the organism have pronounced biological rhythms, one of these factors is light desynchronization. Desynchronization occurs due to light pollution of environment, as well as among the people who make transmeridional flights and work on a night shift for realization of professional duties. Light desynchronization can cause not only physiological, but also psycho-emotional disorders in healthy people and the development of premature aging of the whole organism and the early development of age-associated conditions.

Keywords: *Desynchronization, involutory processes, light desynchronization, interleukin-1, interleukin-4, Ki-67 protein*

INTRODUCTION

Still exist information deficiency about neuroimmune and endocrine changes accompanying the light desynchronization - one of the signs of the dysfunction of central nervous system (Комарова, 1989; Романов, 2000; Анисимов, 2014). To the data of experimental studies, the changes of the homeostasis of a number of signal molecules, including glucocorticoids, hormones of hypothalamic-pituitary-adrenal system, serotonin, melatonin, endogenic opioids, as well as proinflammatory cytokines (Гончарова**,** 2010). Neuroendocrine status of organism usually is defined with the activities of glucocorticoid receptors regulating the functional activity of all chains of immunity.

The biological role for glucocorticoids concludes in regulation of intracellular metabolism and functions of the genetic apparatus of the cells. These hormonal-metabolic interrelations increase the organisms' resistance to stresses that occur due to light desynchronization (Костенко, 2013; Зарипов, 2015). The above-mentioned issues make grounds for the studies of the influence of light desynchronization to neuroendocrine regulation of physiological processes at the organisms aging through evaluation of the mechanisms of the changes of glucocorticoid reception in experimental studies.

MATERIAL AND METHODS

The studies were conducted on the albino nonlinear mice with mass of 17.8±0.04-21.5±0.04 g, which were culled into two groups: young (average age 10.9±0.01 week, n=78) and old (average age 19.1±0.01week, n=82) mice. Body mass of mice was determined on the laboratory scales Sartorius ED423S-RCE (Germany).

Light day lasted 12. Food and water were available to the animals ad adlibitum.

For objectification of the results the decision of investigating the influence of light desynchronization on neuroendocrine regulation of physiological processes at the course of organisms aging was

doi.org/10.29228/jlsb.47

done. We have conducted the evaluation of parameters period to conduction of light desynchronization, at the 1st day after conduction of light desynchronization, and at the 12th and 23rd days after cessation of light desynchronization. The first half of each rhythm is favorable (positive phase), the second half is unfavorable (negative phase.

The indexes to the 23rd days the sacrificed considered more objective for evaluation of full action of light desynchronization to neurohumoral regulation of physiological processes at the development of organism aging.

After 23 days mice were sacrificed and the results obtained prior to the beginning of light synchronization were accepted as a control, because the animals were kept under natural light regime.

Five minutes before conducting the manipulations (taking the blood samples) animals were anesthetized with intramuscular injection of mixture of Telasol (Zoetis Inc, USA) ata dose of 0,1 ml/kg and Xylanit (Nita-Farm, Russia) ata dose of 0,1 ml/kg.

Light desynchronization was modeled through of the changing of light regime in the laboratory. Animals have been under gone to the influence of the combination of natural and at night hours, of artificial elucidation provided by the luminescent lamp, equivalent to filament lamp of the intensity 60 Watt. For the evaluation of immune status, we measured the levels of tumor necrosis factor alpha, interleukin-1, interleukin-4 and the ratio of CD4/CD8 expressions.

The method of evaluation the levels of interleukins was based on solid phase "sandwich"-type of the ELISA -test. The specific reagents of the set for the ELISA-test are the monoclonal antibodies to the studied interleukin, adsorbed on the surface of the wells of polystyrene plate. Protein Ki-67 is the widely recognized and used marker of proliferation, expressed in all kinds of tissues. The aging process is characterized with reaching the Hayflick limit and decrease or total termination of the cells capabilities to division.

Upregulation of this marker demonstrates development of the pathological reactions in the organism. In this relation Ki-67 protein may be an important marker for evaluation of the decreasing of cell's proliferative activity and level of involutory processes in the studied organs.

Decreasing the increased expression of Ki-67 protein, which is initiated at the background of stress stimuli of any origin, indicates to the existence of the physiological abilities of the organism to adaptation, to the normal physiological status of the antioxidant system, to the higher regenerative ability, as well as to the capabilities to decrease physiologically the level of involutory processes (aging processes) in the organism (Buondonno, 2019).

23 hours later the mice were sacrificed. As control additionally 10 young mice prior to conducting the light desynchronization were taken additionally, 10 young mice at the 1st day after conducting of light the cuts of the brain prepared. The desynchronization and 10 young mice at the 12th day after conducting the light desynchronization; 10 old mice till conducting the light desynchronization, 10 old mice at the 1st day after the light desynchronization and 10 old mice at the 12th day after the light desynchronization.

Then we have prepared cuts of the brain. Parts of the brain was placed in the 10% solution of paraformaldehyde at phosphate buffer (PBS pH=7.3) for 24 at a temperature 4ºC. We have made cuts of thickness of 20 mcm with the application of manufactured by Leica model of CM 1510S, (Germany). Then cuts were placed on the slides and were stained with hematoxylin and eosin.

We have determined the changes of the expressions of nuclear antigen Ki-67, on the brain cuts of young and old mice after the influence of light desynchronization. The antigens Ki-67, was chosen for analyses, because it is present at all stages of cell cycle, besides G0, and is a marker of the cell proliferation.

Characteristics of the used antigens Cell Marque Ki-67 (Cell Marque, США, Positive control slides: 275S):

- Clone: SP6, rabbit
- 0.1 ml Conc.: 275R-14
- 0.5 ml Conc.: 275R-15
- 1.0 ml Conc.: 275R-16
- 1.0 ml Predilute: 275R-17
- 7.0 ml Predilute: 275R-18

For our studies we have used microscope Olympus IX81. Microscope was provided with the digital camera Olympus DP72 (Japan), which is joined to personal computer. Measuring of the depth of outer nuclear layer (ONL) was conducted at microphotographs of stained cuts.

RESULTS AND DISCUSSION

The results of immune histochemical reactions were expressed as of percent ratios of the stained cells to the total amount of cells at preparation. Protein Ki-67 is a generally recognized and widely used marker of proliferation, expressed at all kinds of tissues.

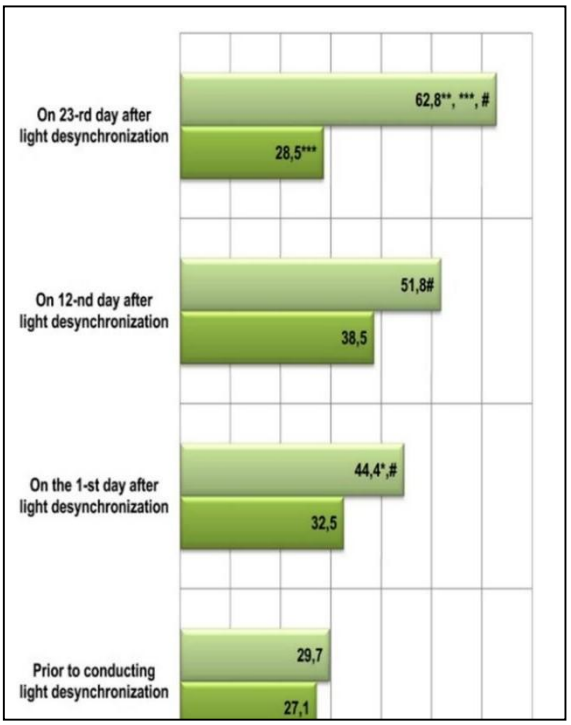

Figure 1. The dynamics of expression of Ki67.

* p<0.05 between indexes before conducting of light desynchronization and at the 1st day after light desynchronization;

** p<0.05 between indexes at the 12th day after conducting of light desynchronization and at the 23rd days after light desynchronization;

*** p<0.05 between the indexes before conducting light desynchronization and at the 23rd day after light desynchronization;

# p<0.05 between indexes of the young and old mice.

Process of aging is characterized with reaching to the Hayflick limit and decreasing or totally cessation of cell capabilities to division. Increasing of its level effects advent of the pathological reactions in the organism.

In this relation protein Ki-67 may be an important marker for evaluation of decreasing of the proliferating activity of the cells degree of involutory processes of the studied organism (picture 1, 2).

At the figure 2 the brain cuts, stained with hematoxylin and eosin at the 23rd days after conducting of light desynchronization at young and old mice are presented. Much mice predominance of the expression area of the nuclear antigen Ki-67 on the old mice than on the young ones, is observed.

We have determined the changes of the expression of the nuclear antigen Ki-67, which is present at the all stages of cell cycle, besides G0, at the brain cuts in the young and old mice after the effect of light desynchronization.

So, the expression area of the nuclear antigen Ki-67 at the young mice prior to conducting light desynchronization made of 27,1±0,3%, that is related to physiological expression of given marker. It means, that the degree of involutory processes (aging processes) was with in at the physiological limits and was accepted for the point control on the young mice.

After conducting light desynchronization the expression area of Ki-67 antigen at the young mice on the first day slightly increased to 32.5+1.1 %, p>0,05 between indexes prior to conducting light desynchronization and on the 1st day after conducting light desynchronization; but it does not reach to reliable differences, demonstrates of induction of normal physiological reactions of the organism ward adaption in response to the effect of light desynchronization and of degree of involutory processes (aging processes) with in the physiological limits in the young ages.

Furthermore at the 12th day the expression area of the nuclear antigen Ki-67 on the young mice slightly increased up to till 38.5±0.7 %, by 1.2 times compared to the index on the 1st day and by 1.4 times in comparison to the control level the, p>0,05 between indexes at the 1st day after conducting light desynchronization and at the 12 days after conducting the light desynchronization, p<0,05 between indexes till conducting light desynchronization and on the 12th day after conducting light desynchronization, reflects the induction of the normal physiological reaction of the organism to adaptation in response to the effect of light desynchronization and the degree of involutory processes (aging processes) with in the physiological limits at the young ages.

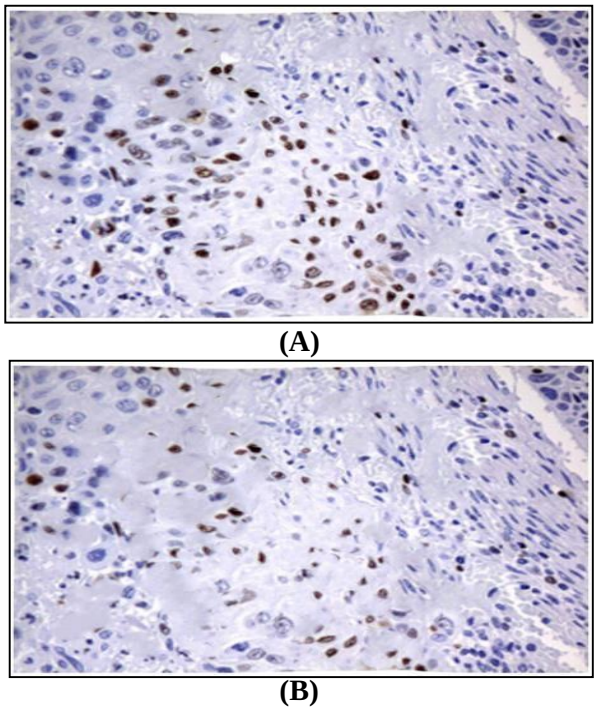

Figure 2. Expression of Ki-67 in the brain. Stained with hematoxylin and eosin X 400.
A- Young mice on the 23rd - days after light desynchronization; B- Old mice on the 23rd - days after light desynchronization

To the 23rd day the expression area of the nuclear antigen Ki-67 at the young mice was recovered to 28.5±0.3 %, p<0.05 between the indexes on the 12th day after conducting the light desynchronization and on the 23rd day after the of light desynchronization, p<0.05 between indexes prior to conducting the light desynchronization and at 23rd day after conducting the light desynchronization, that proves existing of adequate physiological capabilities on the young ages to adaptation of organism on the 23rd day to stressful stimuli in the form of a light desynchronization and the degree of involutory processes (aging processes) with in the physiological limits in the young ages.

The expression area of the nuclear antigen Ki-67 on the old mice prior conducting the light desynchronization made 29.7±0.3%, that really does not differ from the expression area of the nuclear antigen Ki-67 on the young mice and refers to the physiological expression of the given marker, that means the degree of the involutory processes (aging processes) was within the physiological limits and was accepted as a control point on the old mice.

After conducting the light desynchronization the expression area of nuclear antigen Ki-67 in the old mice at the first days was significantly increased up to 44.4+1.5 %, p<0,05 between the indexes conducting of light prior to desynchronization and on the 1st days after the light desynchronization, indicating to induction of pathological insufficient physiological reaction for the organism to adaptation as response to the effects of light desynchronization and to the increased degree of involutory processes at the old ages after the effects of light desynchronization.

Furthermore on the 12th day the expression area of the nuclear antigen Ki-67 at the old mice obviously slightly up to 51.8±1.7 %, by 1.2 times compared to the index on the 1st day and by 1.7 times compared to the control point, p>0,05 between the indexes on the 1st day after light desynchronization and on the 12th day after the light desynchronization, p<0,05 between the indexes prior to conducting light desynchronization and on the 12th day after light desynchronization, p<0,05 between the indexes on the young and old mice that reflects induction of the pathological in sufficient physiological reaction for the organism toward adaptation in response to the effect of the light desynchronization and increasing the level of involutory processes (aging processes) after the effect of the light desynchronization at the old ages.

To the 23rd day the expression area of the nuclear antigen Ki-67 on the old mice increased up to 62.8±1.8 %, p<0,05 between the indexes on the 12th day after the light desynchronization and on the 23rd day after the of light desynchronization, p<0.05 between the indexes prior to conducting the light desynchronization and on the 23rd day after the of light desynchronization, p<0.05 between the indexes on the young and old mice, that proves the existence of the pathological (insufficient) physiological reaction of the organism toward adaptation as an on organisms response to the effect of light desynchronization and of the increasing level of involutory processes (aging processes) at the old years after the effect influence of light desynchronization (Hoffman, 2006).

Downregulation of the increased expression of Ki-67 protein which occurs at the background of stress stimuli of any origin, indicates to the about presence of physiological potential of the organism toward adaptation, to the normal physiological status of antioxidant system, high regenerative abilities, as well to the abilities to decrease physiologically the degree of involutory processes (aging processes) in the organism.

CONCLUSION

We have determined the changes of the expression of the nuclear antigen Ki-67, which is presented at the all stages of cell cycle besides G0, at the brain cuts on the young and old mice after the effects of light desynchronization.

Analysis of the dynamics of the expression areas dynamics of nuclear antigen Ki-67 at young mice informed about normal physiological reaction of the organism to ward adaptation as response to influence of light desynchronization and the degree of the involutory processes (aging processes) with in the physiological limits on the young ages.

Analysis of the dynamics of the expression areas of the nuclear antigen Ki-67 on the old mice showed pathological (insufficient) physiological reaction of the organism forward adaptation as response to the effects of light desynchronization and up regulation of the degree of involuntary processes (aging process) in old ages after the effect of light desynchronization.

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İşıq desinxronlaşması zamanı involyutiv proseslərin (qocalma proseslərinin) xüsusiyyətləri

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Orqanizmin qocalmasının inkişafında fizioloji proseslərinin neyroendokrin tənzimlənməsinə işıq desinxronlaşmasının təsiri öyrənilmişdir. Alınan nəticələrinin obyektivliyi üçün biz orqanizmin qocalmasının inkişafında fizioloji proseslərinin neyroendokrin tənziminə işıq desinxronlaşmanın təsirini öyrənməkdən ötrü nəzərə aldığımız parametrlər işıq desinxronlaşmasından qabaq , işıq desinxronozu tətbiq edildiyindən 1 həftə sonra, 12-ci və 23-cü həftələrdə təyin edilmiş və qiymətləndirilmişdir. Orqnizmin tənzimləyici mexanizmləri aydın ifadə olunan bioloji ritmlərə malikdir,ritmləri pozan faktorlardan biri də işıq desinxronlaşmasıdır. Desinxronlaşma ətraf mühitdə işıqlılıq şəraitinin dəyişilməsi, həmçinin transmeridial təyyarə uçuşlarını həyata keçirən şəxslərdə və peşə zəruriyyəti ilə əlaqədar gecə növbəsində işləyən işçilərdə baş verir. İşıq desinxronlaşması sağlam adamlarda tək emosional pozuntuları doğura bilər və bütün orqanizmin erkən qocalmasının inkişafının və yaş-assosiyalaşmış vəziyyətlərinin erkən inkişafının səbəbi ola bilər.

Açar sözlər: *Desinxronoz, involyutiv proses, işıq desinxronozu, interleykin-1, interleykin-4, Ki-67 zülalı*

Особенности инволютивных процессов (процессов старения) при световой десинхронизации

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Для выявления влияния световой десинхронизации на нейроэндокринную регуляцию физиологических процессов в развитии старения организма нами были проведены исследования, цель которых заключалась в изучении влияния световой десинхронизации на степень инволютивных процессов (процессов старения). Для объективизации результатов влияния световой десинхронизации на нейроэндокринную регуляцию физиологических процессов в развитии старения организма оценка параметров была проведена до световой десинхронизации, в 1–е сутки после проведения световой десинхронизации, на 12-е и 23-е сутки после проведения световой десинхронизации. Поскольку регуляторные механизмы организма имеют выраженные биологические ритмы, одним из таких факторов является световая десинхронизация. Десинхронизация происходит вследствие светового загрязнения окружающей среды, а также у лиц, совершающих трансмеридиональные перелеты и, в силу профессиональной необходимости, работающих в ночную смену. Световая десинхронизация может вызывать не только физиологические, но и психоэмоциональные расстройства у здоровых людей и стать причиной развития преждевременного старения всего организма и раннего развития возраст-ассоциированных состояний.

Ключевые слова: *Десинхронизация, инволютивные процессы, световая десинхронизация, интерлейкин -1, интерлейкин -4, белок Ki-67*