ISSN 0006-3509, Biophysics, 2013, Vol. 58, No. 6, pp. 837–840. © Pleiades Publishing, Inc., 2013.
Original Russian Text © A.V. Kulikov, L.V. Arkhipova, D.A. Kulikov, G.N. Smirnova, P.A. Kulikova, 2013, published in Biofizika, 2013, Vol. 58, No. 6, pp. 1065–1068.

COMPLEX SYSTEMS BIOPHYSICS

In memoriam teacher and friend L.M. Chailakhyan

Study of the Accidental Thymus Involution during the Formation of Hierarchic Communities by a Novel Physical Method for Recording the Social Stress

A. V. Kulikov[a], L. V. Arkhipova[a], D. A. Kulikov[a, b], G. N. Smirnova[a], and P. A. Kulikova[c]

[a] *Institute of Theoretical and Experimental Biophysics, Russian Academy of Sciences, Pushchino, Moscow Region, 142290 Russia*

[b] *Vladimirskii Moscow Regional Research and Clinical Institute, Moscow, 129110 Russia*

[c] *Faculty of Basic Medicine, Moscow State University, Moscow, 117192 Russia*

Received September 19, 2013

Abstract—A novel method for recording the aggressive behavior in newly formed hierarchic communities has been developed. A temporal and age-related dynamics of the accidental thymus involution in mammals has been studied.

Keywords: social stress, thymus, thymus involution

DOI: 10.1134/S0006350913060109

INTRODUCTION

The organism of a human is regularly subjected to stressful impacts of various etiology. Dynamically changing economical condition in the country, sharp separation of the population by the degree of financial provision, unsatisfaction of groups of the population with their social status demand detailed investigation of such a phenomenon as social stress. To date the fact that stress of any etiology (both acute and chronic) bears a potential of morphofunctional destructurization of the main systems of maintenance of homeostasis of the organism, in particular the immune system, causes no doubt [1, 2]. At that there remains not finally elucidated a question of the influence of chronic professional stresses on the mechanisms of immunological ageing connected with acceleration of age-related involution of the thymus [3–5]. A significant decrease of the quantity of thymocytes upon age-related irreversible involution of the thymus gland is caused by weakening of the ability of the epithelial cells of the thymus to recruit bone-marrow precursor cells, provide their differentiation, proliferation and support survivability [6]. Debatable heretofore remains the problem of evaluation of the stress stability of the organism, especially from the standpoint of personal morphofunctional state of the immune system.

Laboratory investigations of stress have been executed in the main on a model of two mice upon their regular short-term contacts [7]. In these works it was established that the results of conflicting interaction are not identical for the victor and the defeated. However this artificial model does not reflect all social interactions emerging in large groups of animals. It is noted that the psychophysiological structure of interactions in a large group of animals is studied poorly because of the absence of a model of group behavior with definite indices of evaluation. A proposed communicational apparatus has allowed conducting an investigation of behavior in groups by means of direct observation and disclosing a series of regularities upon formation of groups. However this method is very laborious and carries in itself elements of subjectivity, for example, depends on qualification and concentration of the observer. Some authors for reducing the laboriousness of the method restrict themselves to three observations per day each of 15–20 min [8, 9]. To our regret, it is obvious that restriction of the duration of the observation reduces the precision of the method, because a conflict may occur not in those 15–20 min in the course of which registration is carried out.

Complex investigation of peculiarities of the passage of various processes in the organism in conditions of social stress is possible only upon availability of a

Editor's Note: I certify that this is the closest possible equivalent of the original publication with all its practical details, statements and terminology, phrasing and style, so the reader can make sound judgment; English title and Abstract provided by authors. *A.G.*

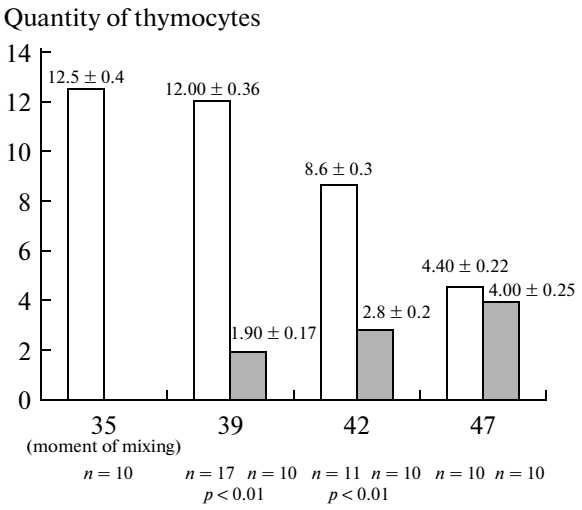

Fig. 1. Quantity of thymocytes in mice in control and in 4, 7 and 12 d from the beginning of social conflict (stress). Light bars – control, dark – experimental group.

convenient experimental model on animals. However in the accessible literature we have failed to disclose data about such a model. Therefore we have decided to create a system modeling conflicting stressful situations in experiment, and in the quality of supplement thereto, a new method of long-term registration of social stress, which upon necessity would have allowed automatically registering conflicts in large groups of animals round the clock.

It is known that the impact of stressful factors causes accidental thymic involution [10]. A change in the cellular composition of thymus gland, as in the central organ of hemopoiesis and immune defense, cannot but influence the functioning of the organism in whole. In the present work apart from biophysical approaches for evaluation of the influence of stressful factors we also investigated physiological parameters, in particular the state of thymus at different terms of observation.

EXPERIMENTAL

Experiments were conducted on 360 SHK mice kept in standard conditions of vivarium. As a result of experiments we have developed original biophysical methodics of modeling social stress and long-term automated registration of episodes of aggressive behavior of animals. The laboratory installation consisted of a plastic reflector covering the whole area of a cage with animals and a therein inbuilt dictaphone with automatic switch-on upon appearance of a sound signal and switch-off after its cessation. The installation was switched on at 16 h and taken off at 10 h of the next day—total 18 h of registration. The offered temporal scheme of registration is connected with that mice present as twilight animals and a peak of their activity falls on the evening and night time. Preliminarily conducted experiments confirmed that in the period from 10 to 16 h they are essentially less active. The microphone was tuned to switching-on upon intensive motor activity in the time of fights, and also the fight-accompanying chases and squeaks. In the morning the record on the dictaphone was listened to and the number of short-term and prolonged conflicts counted. The number of conflicts was divided by the quantity of hours of registration, the quantity of animals in the cage and, in this way, we obtained the number of conflicts per mouse per hour.

For evaluation of the state of the T-cellular link of immunological reactions of the organism we determined the number of thymocytes of the thymus gland of experimental animals.

Determination of the quantity of thymocytes. After decapitation of animals we opened the chest cavity, extracted the thymus and placed it into a salt Hanks solution without phenol red with glucose (5 mmol/L) and antibiotic (gentamycin, 10 μg/mL), containing 20 mmol/L HEPES (Sigma, USA) pH 7.2, at a temperature of 37°C. The tissue of the thymus under mechanical pressure was filtered through a capron filter, singly washed-off after centrifugation (700 *g*, 5 min) and resuspended in Hanks solution or in medium RPMI-1640 (Sigma, USA) to a concentration in an interval 10^5–10^8 cells in 1 mL. The concentration of cells was determined by counting in a Goryaev chamber. Cells were incubated either in plastic Petri dishes or in cells of a 96-well flat-bottom plate [11].

RESULTS AND DISCUSSION

During intensive usage of the territory animals form groups, the members of which are tied by relations of dominance–subordinance. This system of interrelationships is customarily called hierarchical [11]. Formation of such a group and change in its structure takes place at the expense of conflicts establishing or changing the structure of dominance–subordinance. Therewith animals experience significant stress conditioned by interaction with each other.

In an experimental cage we mixed 5 mice each of identical mass from two cages with established hierarchy (dominant, subdominant and subordinants). In the course of several days there established itself a new hierarchical system, this process was accompanied by an increase of the quantity of fights for leadership.

Evaluation of dynamics of thymic involution after mixing animals from different hierarchical groups. We investigated the dynamics of age-related irreversible thymic involution in experimental animals. The obtained data presented as control during evaluation of the influence of stress on this process (Fig. 1).

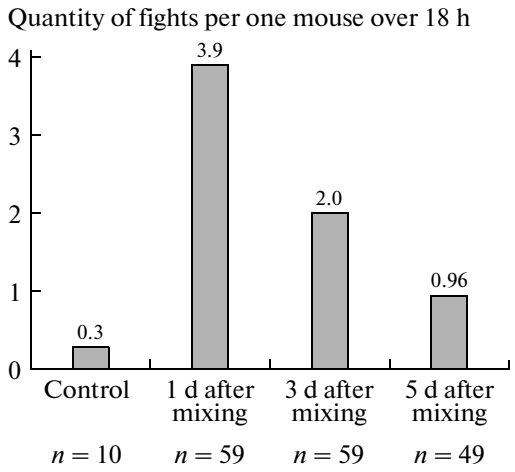

Fig. 2. Quantity of fights per one animal at an initial age of mice of experimental and control groups 35 d (mass 21 g).

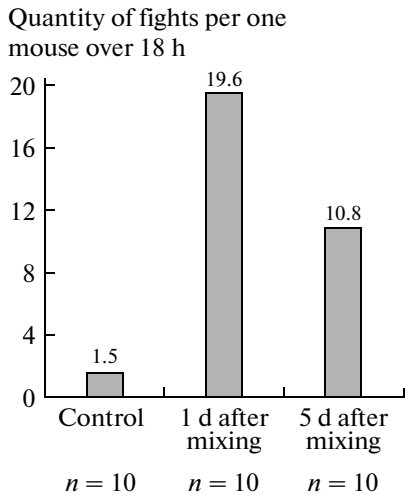

Fig. 3. Quantity of fights per one animal at an initial age of mice of experimental and control groups 47 d (mass 32 g).

In Fig. 1 the white bars denote the average quantity of thymocytes in the thymus gland of intact animals, i.e. in mice with established hierarchical structure without planting individuals from other cages. Investigated at different terms of observation, this index reflects the dynamics of natural age-related thymic involution. Upon mixing in one cage of 10 mice (5 + 5) from different hierarchically established communities as a result of struggle for the place of dominant and subdominant individuals in animals of the entire population there take place a sharp reduction of the quantity of thymocytes in the thymus gland. Maximal differences are observed in four days after the beginning of social conflict: the quantity of thymocytes in animals of the experimental group decreases 6.3 times as compared with control. In 7 d these indices differ three times, while in 12 d, just as should have been expected, differences become unreliable. This may speak only of that there is establishment of a new hierarchical structure, where discerned is a dominant, a subdominant, while the rest of animals – subordinants.

Reduction of the quantity of thymocytes as a result of initiation of conflicts (fights) shows the significance of impact of such a kind of stressful factors for the organism and allows using the proposed model of stress for experimental usage.

Long-term registration of conflicts in groups of animals. Described in Experimental, the new means of registration of social conflicts has allowed showing the following regularities (Figs. 2–4).

Even if we analyze data obtained upon studying only control animals of different age not subjected to experimental stress, it is seen that with age there is a reliable increase in the quantity of fights. Mice of age of 35 d (body mass 21 g) fought five times less than 47-day (32 g) and 12.3 times less than 51-day (36 g) animals. It should be noted that into the experiment we selected mice groups very close in mass: the initial mass of animals in group differed not more than by 1 g.

From Figs. 2–4 it is seen that the maximal number of episodes of aggressive behavior took place in the 1st day after mixing. At that in 51-day mice there were 3.4 times more fights than in 45-day and 10.2 times more than in 35-day animals. By the 5th day the quantity of fights relative to maximal declined in the denoted age groups 4, 1.8 and 3.3 times respectively and approached natural control values.

CONCLUSIONS

We have investigated the dynamics of age-related thymic involution of experimental animals and the change of the quantity of thymocytes in the thymus

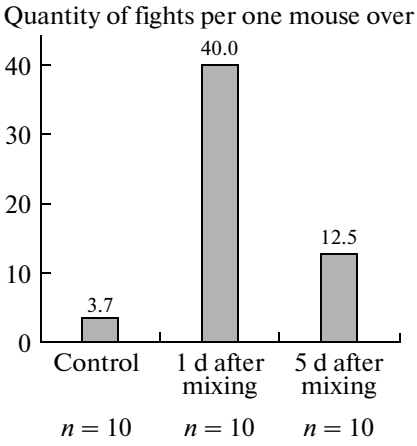

Fig. 4. Quantity of fights per one animal at an initial age of mice of experimental and control groups 51 d (mass 36 g).

gland at different terms from the beginning of social conflict.

The proposed methodic of initiation of aggressive behavior by means of creation of new hierarchical groups of animals may be used in investigation of various indices in a situation modeling social and martial stress.

We have developed an automated method of registration of episodes of aggressive behavior of animals in conditions of social stress.

We have shown that with increasing age of experimental animals the number of fights increases during formation of new hierarchical communities.

ACKNOWLEDGMENTS

The work was supported by the RAS Presidium programs "Support of Innovations and Developments" and "Basic Sciences to Medicine."

REFERENCES

1. S. A. Vorontsov, Zarubezh. Voen. Obozr., No. 5, 87 (2005).
2. V. A. Ponomarenko, Psikhol. Zh. **25** (3), 98 (2004).
3. E. S. Fedorova, Candidate's Dissertation in Biology (St. Petersburg, 2009).
4. E. M. Six, D. Bonhomme, M. Monteiro, et al., J. Experim. Medicine **204** (13), 3085 (2007).
5. D. M. Nance and V. M. Sanders, Brain Behav. Immun. **21** (6), 736 (2007).
6. D. Aw and A. B. Silva, Aging (Albany N. Y.) **17** (1–2), 146 (2009).
7. N. N. Kudryavtseva, Ross. Fiziol. Zh. **85**, 67 (1999).
8. A. V. Osadchuk, Dokl. AN SSSR **261**, 1238 (1981).
9. V. P. Poshivalov, *Experimental Psychopharmacology of Agressive Behavior* (Nauka, Leningrad, 1986) [in Russian].
10. M. Yu. Kapitonova and A. I. Krayushkin, Fundam. Issled., No. 2, 25 (2005).
11. A. V. Kulikov, L. V. Arkhipova, E. G. Novoselova, et al., Biophysics **53** (6), 659 (2008).