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# Chronic Stress and Pathological Aggression as Premise for Killer Rats Formation

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**ABSTRACT. It is detected that disturbance of the biological rhythm with light deprivation and social isolation, together with low temperatures, results in stress and pathological aggression, leading to the formation of killer rats. That is the result of the disruption in the metabolism of biogenic amines.** *© 2007 Bull. Georg. Natl. Acad. Sci.*

***Key words:*** *light deprivation, stress, aggression, killer rats.*

Study of the neurochemical basis of chronic stress, aggression and formation of killer rats is one of the actual problems of modern neurobiology. This problem is of great importance regarding former Soviet republics, where the number of criminal actions and murders rose 2-3 times within the last 10 years. There is no doubt that one of the main reasons of increased aggressive attacks is of social nature: unemployment, business failures, bankruptcy, alcoholism, drug abuse, etc.

With the use of the motivation-emotional analysis method, a strong link between stress, aggression and murder has been determined. Each form of emotional outburst is integrated by its own psychophysiological state and is realised by the CNS using specific neurotransmitters and neurochemical reactions. In particular, electrical stimulation of the central and centromedial amygdaloid nuclei increases fight and fear reactions. Aggressive outbursts may be elicited by stimulation of central grey matter, the dorsal and lateral hypothalamus. The increased aggression evoked by stimulation of amygdala is associated with a decrease in the concentration of norepinephrine and serotonin in brain.

A similar phenomenon has been observed in cats with decerebrating lesion. Bilateral lesions of the septal nuclei induce aggressiveness that is not always goal-directed. Septal lesions increase aggressive behaviour in the mouse-killing rats as they attack mice and frogs [1].

Unfortunately, despite serious achievements in functional neurochemistry, there are no significant data that would allow us to determine cause-and-result interaction between psychological and neurochemical processes. In our opinion, these particular gaps could be filled out by psychological attitude to this particular problem, using the model of "killer-rats". As shown previously, formation of the killer rats is the result of chronic stress and development of pathological aggressiveness [2].

Bearing in mind the above mentioned, we have conducted research of rats' behaviour and neurochemical correlations, during natural and pilocarpine stress, leading to pathological aggression and formation of killer rats.

## Materials and Methods

Experiments were performed on outbred male albino rats weighing 100-250g. Rats with initially aggressive nature were selected by Miczek method [3]. Behaviour of the animals was assessed in the "open field", and tested with the computer [4].

Quantitative assessment of biogenic amines was implemented, using HPLC with electrochemical detector ("WATERS"). Biogenic amines from the brain extract were isolated using aluminate reaction [5]. Protein concentration was detected by Lowry et al. [6].

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Stress was modelled for 4 weeks using Miczek method with modification [3]. The rats were maintained in individual cages at the 1:23 light/dark regimen and 15-18$^0$C. Control animals were kept 5 per cage at the 14:10 light/dark regimen (normal circadian rhythm). The animals were decapitated after 1 month of exposition.

In order to investigate changes in the number of lectinbinding receptor during chronic stress, the erythrocyte mass was obtained by blood centrifugation at 700g for 10 min and 3-fold washout in physiological saline. The erythrocyte membrane surface was examined using lectins with different specificity to carbohydrates: Val, mistletoe lectin from *Viscum album* L. (galactose, sialic acid); PNA, peanut lectin from *Arachis hypogaea* L. (β-D-galactose, lactose); PSA, pea lectin from *Pisum sativum* L. (mannose, glucose); WGA, wheat lectin from *Triticum aestivum* L. (N-acetyl-D-glucosamine, N-acetyl-D-glucosamine oligomers, sialic acid oligomers); and SNA, elder lectin from *Sambucus nigra* L. (sialic acid - NANA).

Binding of lectins to erythrocytes was measured using the method of Lutsik et al. [7]. The specific activity (SA) of lectins was calculated as follows:

$$SA=T/C,$$

where SA is the limit dilution of 1 mg protein causing hemagglutination; T is hemagglutination titer or protein dilution in the last well with agglutination; and C is the protein concentration (mg/ml).

The lectin-binding activity of glycoconjugates of erythrocyte ghosts was studied by the hapten inhibitory method [7] and was estimated as the ratio between lectin and lectin-binding protein. Lectin-binding glycoconjugates of erythrocyte ghosts were extracted with Triton X-100 in phosphate buffered saline [8]. Erythrocyte ghosts were homogenized with 0.1% Triton X-100 on ice. The suspension was centrifuged at 16,000g for 20 min. The supernatant was dialyzed against agglutination buffer (40 Mm $K^+$-phosphate and 0.9% NaCI, pH7.4) for 24 h. The pellet was treated with 0.5% Triton X-100, after centrifugation and dialysis, the pellet was treated with 1% Triton X-100.

Analytical electrophoresis was performed in the presence of 0.1% SDS under dissociation conditions. The study was conducted in a PAAG gradient (10-25%) on a Hoefer Scientific Instruments SE-200 device. The gels were stained with 0.2% Coomassie blue G-250. Carbohydrate concentration in proteins was estimated in the Schiff reaction. The results were analysed by Student's t-test.

## Results

During the first series of the experiments, quantitative distribution of biogenic amines in various parts of the brain was estimated during natural and pilocarpine aggression. Intravenal injection of 12.5 mg/kg pilocarpine significantly changed the behaviour of the rats.

Exploratory and stereotypical activity differed in naturally aggressive rats against the control group: the number of grooming increased.

Bearing in mind that the aggressiveness increases with the stimulation of lateral hypothalamus, midbrain and hippocampus, quantitative distribution of biogenic

Table 1

Quantitative distribution of biogenic amines (mg/g) in various regions of the rat's brain during natural and pilocarpine aggression

| Brain region | Control | Aggression | |
|---|---|---|---|
| | | Natural | Pilocarpine-induced |
| Midbrain<br>Dopamine<br>Norepinephrine<br>Serotonin | 0.32±0.04<br>0.48±0.02<br>0.50±0.05 | 0.80±0.13<br>0.68±0.21$^{••}$<br>0.35±0.06$^{••}$ | 0.52±0.03$^{••}$<br>1.20±0.18$^{••}$<br>0.25±0.02$^{•}$ |
| Hypothalamus<br>Dopamine<br>Norepinephrine<br>Serotonin | 0.58±0.05<br>1.45±0.08<br>1.12±0.09 | 0.95±0.07$^{•}$<br>2.24±0.13$^{••}$<br>0.62±0.02$^{•}$ | 0.73±0.04$^{•}$<br>2.83±0.20$^{•}$<br>0.45±0.06$^{•}$ |
| Hippocampus<br>Dopamine<br>Norepinephrine<br>Serotonin | 0.61±0.09<br>0.75±0.06<br>0.48±0.04 | 0.85±0.02$^{••}$<br>1.12±0.17$^{••}$<br>0.24±0.04$^{••}$ | 0.74±0.04$^{•}$<br>1.18±0.12$^{••}$<br>0.28±0.06$^{••}$ |

$^{•}P<0.01$, $^{••}P<0.05$ in comparison to the control.

amines, namely dopamine, norepinephrine and serotonin, was measured in particular regions during natural and pilocarpine induced aggression (Table 1).

As shown in Table 1, during natural and pilocarpine aggression in comparison with the rats in the control group, in the midbrain with a large quantity of dopaminergic and norepinenephrinergic neurons, concentration of dopamine and norepinephrine increases approximately 1.5-2.5 times. At the same time the concentration of serotonin decreases more than by 70%.

Similar changes can be found in hippocampus and hypothalamus. As usual, more effective changes in the distribution of biogenic amines are found in naturally aggressive rats.

Assuming that the quantitative relativity between norepinephrine and serotonin plays an important role in the brain activity [5], these data have been calculated on purpose in rats with pilocarpine-induced aggression. It has been determined that relation between norepinephrine and serotonin in the midbrain increases 5 times upon the decrease of the serotonin. Within lateral hypothalamus as well as hippocampus, playing an important role in the formation of motivation and emotional state, the amount of neurotransmitters was increased by 5.3 and 2.7 times.

Similar displacement within quantitative relation between norepinephrine and serotonin was found in naturally aggressive rats, but at a lower degree.

Bearing in mind the problems with electrical power in Georgia, light deprivation, combined with low temperature was used as the stress-factors in further experiments [9]. Quantitative distribution of serotonin was studied within the above-mentioned regions of the rat's brain (Table 2).

It was found that during light deprivation the quantitative concentration of serotonin is decreased within the brain of non-aggressive, as well as aggressive rats. It seems that the relation of serotonin concentrations in the brains of non-aggressive and aggressive rats, in dependence on light deprivation, combined with low temperature, points to the following fact: formation of aggressiveness takes place in accordance with the increase of the above-mentioned numbers, that is the decrease of serotonin concentration in the brain (non-aggressive/non-aggressive in dark, non-aggressive/aggressive in dark): midbrain: 1.08, 2.13 (aggressive in dark); hippocampus - 1.08, 2.38 (aggressive in dark); lateral hypothalamus - 1.06, 4.00 (aggressive in dark); visual core - 1.03; 4.18 (aggressive in dark).

Thus, chronic light depression is one of the main reasons leading to pathological aggression and formation of killer-rats [9].

Similar influence on the development of aggression and formation of killer-rats was evinced by light deprivation in combination with social isolation: together with the quantitative changes in biogenic amines concentration, serious disturbances have been revealed in monoaminoxidase (MAO) activities. As is known, this enzyme participates in the metabolism of biogenic amines. Enzyme activity was investigated using the method [10].

In fact, mitochondrial MAO activity, during stress, within the studied brain regions decreased about 2 times. After chronic stress, the number of pathologically aggressive animals was measured as 60% of all, and killer-rats estimated 20% [2, 11].

Interesting results were obtained regarding the influence of chronic stress on the structural organization of the erythrocyte membrane. These data can be used in further research and for the diagnosis of the emotional state.

Changes in the structure of erythrocyte membrane under chronic stress were investigated by the estimation of quantitative binding of carbohydrate-specific lectins on surfaces of erythrocyte membrane.

Experiments with binding of lectins with different specificity to carbohydrates showed that chronic stress is accompanied by changes in the chemical composition of the erythrocyte membrane surface (Table 3).

In stressed rats β-D-galactose-binding lectin PNA caused lesser agglutination of erythrocytes in comparison with control animals. Specific activity of PNA de-

Table 2

Influence of light deprivation, in combination with low temperature on the quantitative distribution of serotonin in various regions of the brain in naturally aggressive and non-aggressive rats (M/g)

| Brain region | Non-aggressive | Non-aggressive in the dark | Aggressive in the dark |
|---|---|---|---|
| Mid-brain | 0.889±0.039 | 0.823±0.083• | 0.418±0.066• |
| Hippocampus | 0.465±0.041 | 0.432±0.068• | 0.195±0.049• |
| Lateral hippocampus | 1.212±0.081 | 1.140±0.115•• | 0.302±0.088•• |
| Visual core | 0.598±0.028 | 0.581±0.089• | 0.143±0.056• |

•$P<0.01$, ••$P<0.05$ in comparison with the non-aggressive.

Table 3

Binding ability of lectins to rat erythrocytes under normal conditions and after chronic stress

| Lectin source | Lectin | Carbohydrate specificity | Specific activity | |
|---|---|---|---|---|
| | | | control | stress |
| *Viscum album* L. | VAL | Gal, NANA | 533 | 33 |
| *Arachis hypogaea* L. | PNA | βDGal,Galβ(1,4), Glc | 1024 | 8 |
| *Pisum sativum* L. | PSA | Man, Glc | 64 | 64 |
| *Triticum aestivum* L. | WGA | GlcNac, GlcNac-oligomers, NANA-oligomers | 128 | 64 |
| *Sambucus nigra* L. | | NANA | 256 | 128 |

Table 4

D-galactose-specific lectin-binding capacity of erythrocyte ghosts protein fractions obtained with Triton X-100 of various concentrations

| Rat | Lectin μg/Lectin-binding protein μg | | | |
|---|---|---|---|---|
| | Erythrocyte ghosts | 0.1% Triton X-100 | 0.5% Triton X-100 | 1% Triton X-100 |
| Norm | 0.011±0.005 | 0.132±0.082 | - | - |
| Stress | 0.0011±0.0002• | 1.539±1.126• | - | - |

•P<0.05

creased significantly more than 128 times, SNA and WGA 2 times. No changes were found in the experiments with PSA.

Proceeding from the foregoing, the binding ability of PNA to erythrocyte glycoconjugates isolated from normal and stressed rat blood was studied. In order to identify glycoconjugate receptors, the protein fractions were used, separated by the treatment of ghosts with 0.1%, 0.5% and 1% Triton X-100 (fraction 1) from normal and stressed rats (Table 4).

Agglutinating activity was revealed only in the protein fraction isolated with 0.1% Triton X-100. This points to its important role as a lectin-binding receptor [8,9]. It is clear that in stress-experienced rats erythrocyte membranes are modified, strongly decreasing the binding ability of D-galactose-specific lectin PNA.

After electrophoresis protein fraction-1, 3 major subfractions with molecular weight 24, 37 and 50 kDa were separated. They consisted of glycoproteins (Schiff reaction). By molecular weight, protein fractions were similar to erythrocyte membrane glycophorines. It is necessary to underline that chronic stress is not accompanied by qualitative changes in these compounds but leads to quantitative changes in lectin-binding proteins.

Thus, it was found that disturbance of the biological rhythm with light deprivation and social isolation, together with low temperatures, results in stress and pathological aggression, leading to the formation of killer rats. This is the result of the disruption in the metabolism of biogenic amines.

A simple method is proposed with the use of lectins for the diagnosis of stress and aggression.

*ბიოქიმია*

# ქრონიკული სტრესი და პათოლოგიური აგრესია როგორც მკვლელი ორგანიზმების ფორმირების წინაპირობა

**ნ. ალექსიძე**

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**შესწავლილია ქრონიკული სტრესისა და პათოლოგიური აგრესიის ნეიროქიმიური საფუძვლები, რაც მკვლელი ორგანიზმის ფსიქოემოციურ სუბსტრატად არის მიჩნეული და თავის ტვინში ბიოგენური ამინების მეტაბოლიზმის დარღვევასთანაა დაკავშირებული. აგრესიისა და მკვლელობის პრევენციის მიზნით, ავტორი გვთავაზობს: 1. შეიქმნას სკოლებში ფსიქოლოგების ინსტიტუტი და კომპიუტერული ბანკი თითოეული მოსწავლის მიერ ფსიქოემოციური ქცევითი რეაქციების დარღვევის შესახებ; 2. ყველა მოსწავლეში ციტოგენეტიკურად იქნეს შესწავლილი ქრომოსომების თვისობრივი განაწილება გენეტიკურად განსაზღვრული აგრესორის დასადგენად. 3. ერითროციტების დონეზე დადგინდეს დარღვევები მემბრანების სტრუქტურულ ორგანიზაციაში ქრონიკულად სტრესული და პათოლოგიურად აგრესიული ახალგაზრდების გამოსავლენად. მიღებულ იქნეს გადამწყვეტი ზომები ფსიქოემოციური დარღვევების მქონე ბავშვების მედიკამენტოზური თუ კოგნიტური მკურნალობისათვის.**

## REFERENCES

1. *W. Kostowski*. (1978), Mod. Probl. Pharmacopsych., **13**: 1-12.
2. *R. Goguadze et al*. (2004), Izv. AN Gruzii, Ser. Biol. A., **30**, 6: 781-784.
3. *K.A. Miczek.* (1976), Psychopharmacology, **47**: 59-64.
4. *I. Maisuradze, B. Maisuradze.* (1994), Radiatsionnoe issledovanie. Tbilisi (Russian).
5. *N.G. Aleksidze et al*. (2001), Bull. Eksp. Biol. i Med. **132**, 8: 170-173.
6. *O.H. Lowry et al.* (1951), J. Biol. Chem., **193**: 265-275.
7. *M.D. Lutcik et al*. (1981), Lektiny, L'vov (Russian).
8. *M. Chachua et al.* (2004), Bull. Georg. Acad. Sci. **169**, 2: 364-366.
9. *R. Goguadze et al*. (2006), Bull. Eksp. Biol. i Med. **141**, 5: 519-524.
10. *V.Z. Gorkin, S.D. Davidov*. (1970), Voprosy Meditsinskoi Khimii, 143-146 (Russian).
11. *N. Mikiashvili et al*. (2001), Nauka i Tekhnologii, N. 4-6: 91-93 (Russian).
12. *R. Goguadze et al*. (2006), Exp. Biol. Med. **141**, 5: 581-583.

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