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# Dynamics of Neuronal Activity in the Limbic System under Emotional Stress

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#### **Abstract**

**Objectives:** Under longstanding segregation the rats demonstrate changes in behavioral responses, birth rate, weight gain, levels of Alpha tocopherol in blood, number of eosinophils and lipid per oxidation that show developing stress conditions. **Methods:** Statistical processing was assisted by software package Statistica 6.0; absolute values were compared applying Student's t-test. **Findings:** It is a well-known fact that the emotional status of an organism is predetermined by the limbic structures of the brain. The study presents the data on the microelectrode investigation of neuronal activity under normal conditions and under stress in the limbic system of the outbred rats that were put under the conditions of the prolonged sexual conflict situation. The multidirectional changes have been observed in hypothalamus impulse activity, in septum, in hippocampus and in amygdale of the brain. **Applications/Improvements:** The study puts forward the assumption that hypothalamus and amygdale nuclei complex are the most labile in the limbic system under conditions of developing emotional stress.

**Keywords:** Emotional Stress, Limbic System, Neuronal Activity

### 1. Introduction

It is a well-known fact that the limbic structures of the brain predetermine the emotional status of an organism and are responsible for behavior, including sexual behavior. In<sup>1,2</sup> consequently, the discovered changes in behavioral and endocrine status of rats under the conditions of the prolonged sexual deprivation are predetermined by the activity of their limbic systems<sup>3,4</sup>.

Studying different aspects of physical and chemical changes in the brain is of current importance, as it can make it possible to identify the mechanisms of functional disturbances of the entire body under stress.

It has been established<sup>5</sup> that different pathological processes in the brain are stimulated by the changes in the functionality of neuronal membranes and by their increased ion permeability and their depolarization.

6.7 However, the dynamics of excitability in different

structures of the limbic systems of the animals (rats) under stress have not been investigated in sufficient detail.

Studying the functions of separate neurons in the limbic system is of paramount importance to understand the mechanisms of maintaining hemostasis. <sup>8-12</sup>This fact dictates the objective of this study that has been set as carrying out statistical analysis of the spontaneous impulse activity of separate cells in the limbic system under normal conditions and under the developing emotional stress in rats in the prolonged sexual conflict situation.

## 2. Method

The dynamics of the background impulse activity of the neurons under the conditions of developing stress have been investigated with 90 rats (42 in Control group + 48 in Experimental group).

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The experiments have been undertaken with the adult white outbred female rats of mass 180-220 g that have been anesthetized (ether + Nembutal calculated as 25-40 mg/kg) in the environment of acute experiment.

Extracellular recording of impulse activity of the neurons of hippocampus, amygdale, septum and hypothalamus was implemented by the glass microelectrodes according to the standardized methodology. 11Background Activity (BA) was being registered for up to 40 min. The absolute values of neuronal inter-impulse periods were calculated in automated mode and evaluated afterwards. Emotional stress was attained through the prolonged sexual deprivation of the rats under the conditions of zoo-social conflict<sup>13,15</sup> i.e., the specially equipped cage divided in three sections by the mesh partitions was used to accommodate three separate groups of rats: Male group, female group and the family colony.

The tips of the electrodes were identified in line with the atlas14 upon processing the brain slices according to the Nissl method.

The control was represented by similar investigations on the intact rats.

## 3. Results

As a result, studies of neuronal activity with microelectrode in the limbic system in outbred rats found that during the formation of the state of "stress" in the limbic system increases the number of cells with a multimodal distribution of interpulse intervals of values and had place changes the frequency of neuronal discharges (Table 1).

In conditions of prolonged sexual conflict revealed changes in the background activity of neurons in the limbic system of the rats. Different structures of the limbic system are characterized by the same degree of changes in individual values. Thus, compared to the control, under stress discharges frequency in limbic areas such as the hippocampus, amygdala and septum was increased, but significantly decreased in the anterior (-8.9%) and posterior (-14.9%) hypothalamus. The maximum frequency of neuronal discharges increase as compared with the control observed in the hippocampus (26.9%); in the septum it was 10.8%, and the least marked changes in the amygdala (8.8%).

The number of neurons with a multimodal distribution of interpulse intervals in stressed animals in all parts of the limbic system were increased, including both the areas of the hypothalamus. It is shown that in a conflict situation in rats the maximum changes of amount of neurons (in % to control) with polymodal distribution of interpulse intervals were reached in amygdala nuclei (21.7%) and hypothalamus (15.7%).

The differences in the parameters of the background activity of neurons in various limbic system structures point to the differences in their levels exciter bridge both in normal and in the formation of the stress reaction.

#### 4. Discussion

The obtained data represented in Table 1 show that developing the conditions of "stress" leads to the increased number of cells with multimodal distribution of inter-impulse periods in the limbic systems of rats and to changes in the frequency of the neuronal discharges.

It has been generally accepted that the electric impulse of a neuron emerges as a consequence of translocations of some certain ions through the cytoplasmic membrane; thereat, the proteins of the membrane under go conformational transformation<sup>3,5,7,16,18</sup>. In other words: the property of conformational transformation is one of the attributes of the electronic (electronically-reactive) membranes. Consequently, identifying the change in BA of neurons in the limbic system in the course of developing stress in rats makes it reasonable to assume that the foundations for generating the emotional stress are represented by the continuous and stable changes in the properties of the conformational transformations of neuronal membranes in the limbic system. As a result, the ion equilibrium on different sides of the cytoplasmic membrane can change and the new integration processes of agitation and suppression in the brain can be established. Thereat, changes in other properties of the membrane can occur, for example, in the properties of reception, selectivity, etc. 4,16,19In representing the above mentioned phenomenon such terms as "excitability and agitation", etc., it would be plausible to think that the differences in the parameters of the background activity of neurons in different structures of the limbic system should identify the differences in the levels of their excitability. Moreover, this parameter can characterize the dynamics of excitability in one and the same structure of the limbic system in different groups of rats (the control group and the group under stress). The analysis shows that under the

conditions of the prolonged sexual conflict situation the excitability of the structures of the limbic system of rats changes. However, the narrowness of such assumptions is evident, insofar as the neural networks possess wide opportunities for altering their states<sup>17,20</sup>.

Spontaneous rhythm reflects the periodic changes in the activities of the cells and it also testifies of the tonic phenomena. This change of activity can be stimulated either by the differences in metabolism of neurons of different structures or by the differences in the system of neuron – glia. 8,17,18 It cannot be excluded that there are some specific attributes of impulsion of cells in separate areas of the limbic system together with the specific transformation processes that occur in those areas and that are characteristic for that or another type of developing the condition of stress. 21For instance, the increased number of neurons with multimodal distribution of the values of periods testifies of the regular nature of their discharge activities, it as much as the truly random, i.e. Gaussian processes, are approximated by the normal distribution. <sup>22,23</sup>Hence, the changes in the character of impulsions of neurons under stress can show that they dispose of the redundant degrees of freedom. <sup>24</sup>At the same time, the possibility of approximating the parent populations of the values of the inter-impulse periods by the normal distribution among the control group of animals and among the animals under stress indicates some certain stability in the functionality of separate structures of their limbic system, their equilibrium, or, more precisely, quasi-equilibrium state under different conditions. For the avoidance of doubt, it should be noted that the term "limbic system", given its official status, would be used below in its anatomic meaning only. The name "system" shall be applied to each of the structures under investigation.

Thereby, biological systems, being the open type systems, are subject to the laws of thermodynamics where the second law can use the ideas of boundary (the initial and the final) conditions of systems which makes it feasible to apply the well-known biophysical principles to this discussion. 18,23,25 Thus, it may be assumed that the above mentioned quasi-equilibrium states (designated by K, and K, differ, as is deduced from the dynamics of the basic parameters of definite systems, in the group of the rats under stress and in the control group by some value Δ K; i.e., in this case initial K, transits through the chain of quasi-equilibrium states into final K<sub>f</sub> The transition

is characterized by two parameters that are the most important among all the parameters under investigation: the change in the average frequency of BA and the decreased level of uncertainty in neuronal activity, i.e., the increased number of cells with multimodal distribution and the relevant decrease in the number of neurons with standard distribution of periods of activity. Thereat, different structures of the limbic system are characterized by different degree of change of some separate values. The physical sense of each of the above-mentioned parameters can be represented as follows: 1. A separate impulse of neuron is an electric equivalent of a quantum of energy in metabolic processes which similarity follows the rule "all or nothing". 20,22,24 Obviously, the frequency of BA is a function of this type of energy and, consequently, it characterizes the energy state (behavior) of a certain definite system; 2. Regularity in the activity of a certain definite neuron (similar to the case with the pool of neurons) characterizes the information state (behavior) of that or another system under some certain conditions.

The table shows that the frequency of the discharges decreases under the conditions of stress in both frontal and rear areas of hypothalamus, but it also increases in all remaining formations, i.e., the energy behavior of some separate systems proves to be multidirectional. This multidirectional mature can be explained as follows. Remembering the fact that the frontal hypothalamus is the area of neural hormone synthesis, it will be reasonable to assume that the functionality of the hormone-producing or/and hormone-depositing cells of hypothalamus is controlled by other areas of the brain. Obviously, areas of hypothalamus represent two subsystems with interconnected mechanisms. The unity of these mechanisms predetermines the same negative entropy of tropho- and ergotropic sub-systems of hypothalamus. Separate structures of the limbic system form some type of associate, i.e., a series of interrelated systems where negentropy of hypothalamus can be recompensed by the entropy of other structures. In other words: Here it is possible to observe a kind of balancing "scales" where there are hypothalamic areas on one "scale" and there is hippocampus, amygdale and septum on the other "scale". These "scales" are subject to the first law of  $thermodynamics. This {\it multidirectional} entropy represents$ the unity of all known formations, interconnections, or, according to Sherrington, affiliations<sup>26</sup> of the responses in different structures of interactive activity of the nervous

system. This assumption is even more accentuated when it has been remembered that only hypothalamus possesses secreting function while this function is extrinsic to other systems; besides, synthesis and release of neural hormones and releasing factors, i.e., protein formations, represent activity in chemical sense, represent processes of energy transformation with all the consequences that follow. Then there is a quantitative aspect of the dynamics of the average BA frequency in different areas of hypothalamus under stress.

Based on the idea of neural hormone synthesis as factor of accumulating energy, it could be expected that the decrease in BA frequency in frontal hypothalamus will be more expressed as compared to that in rear hypothalamus. However, the results of the experiments that testify to the opposite bring about the idea that the indicator under consideration can characterize not only and not so much the synthesis of neural secretion as its excretion and release. In this case not only the qualitative but also quantitative dynamics of the indicators of hypothalamus activity under stress become clear which is predetermined by the most close morpho-physiological affinity of its different areas.

Changes in BA have also been identified in other structures (systems) under investigation. Table 1 shows that their dynamics is opposite to that of hypothalamus and it distinguishes different formations in quantitative manner. Thus, the changes in BA in different structures of the limbic complex of rats under stress can be considered as evidence to the fact that amygdale which features the minimal Gibbs energy shift reflecting the change in the energy in the course of a chemical reaction possesses the highest thermodynamic stability under these conditions, as compared to septum and hippocampus formations, i.e., it reveals the minimal changes in the frequency of discharges. However, at the same time it shows maximal changes in the information state that is characterized by the largest amount of neurons with multimodal distribution of values of the activity periods. By contrast to amygdale, in hippocampus the entropy changed considerably proving that this area of the brain is the least stable in terms of thermodynamics; thereat, the deviations from its informational state are minimal. 3,6,25The degree of changing the parameters of BA in septum was less intensive that gives it an intermediate position in between hippocampus and amygdale. Thus, the dynamics of the indicators of activities in the systems of amygdale and hippocampus under the conditions of the experiment are associated with different changes in their different states which is consistent with the results obtained by other researchers. 3,10 This trend in changing indicators of activity of amygdale and of hippocampus proves that the close functional interrelations exist between these systems. In other words, amygdale and hippocampus subordinate each other in the course of developing emotional stress. To determine those relations it seems practicable to make use of the principle of super-structural stabilization. According to this principle, the less chemically stable structures form the superstructure. Consequently, the system is enriched, i.e., it becomes less stable in terms of thermodynamics. Correspondingly, hippocampus as the least stable system forms superstructure in this case, the septum becomes the damping system, and amygdale has to perform the function of a sub-system. This phenomenon intends to preserve maximum entropy (in developing stress conditions in the rats) and it follows from the Gauss' and D'Alembert's principle of least constraint. Pathophysiological sense of the abovementioned judgments is an effort to discover in the limbic structures the most labile area on which the correcting effects should be focused in the course of developing the experimental neurosis under some certain conditions. Insofar as the amygdale complex preserves the maximum thermodynamic stability after the interaction between the systems, it seems logical to assume that amygdale represents exactly the most sensitive formation "on the second scale". Consequently, it is reasonable to assume that under the conditions of the emotional stress situations the sensitive areas of the limbic system (hypothalamus and amygdale) should be affected in order to achieve maximum correcting effect which is consistent with the observations of the clinical physicians<sup>3,10,27,29</sup>.

By contrast, to the energy-related behavior, the informational behavior in all systems under investigation proved unidirectional and mostly simultaneous, i.e., the parameter irrelevant to thermodynamics was perceived by the time. Hence, there are definite limitations that have be put on construing the following judgments. It is a well-known fact that emotions have been generated at different "stages" of the brain and at each "stage" of the brain; the emotion acquires that or another component element. <sup>17,20,25,30,26</sup> Assuming this as the basis refer to Table 1 where it is shown that the least relative increase in regularity in neuronal activity under stress is characteristic for

hippocampus, and the maximum value of multimodal neurons under the same conditions is observed in amygdale. This difference can be explained from the perspectives of the Gauss's and D'Alembert's principle. In this regard the increased number of multimodal distributions of BA values testifies of the regularity or of the constrictions in the activity of the pool of neurons under stress and, vice versa, the number of stochastic

standard distributions characterizes the uncertainty in the central nervous system and the minimum of the shift in the uncertainty should correspond to the superstructure. Consequently, from informational perspective, amygdale plays the part of the sub-system and hippocampus forms the superstructure.

Thus, both energy-related and informational indicators of the background activity of neurons of amygdale prove

Table 1. Dynamics of limbic system neuron activity in conflict situation with rats

Structures *	Control				Experiment (stress)				ΔW',	$\Delta K_{_{ m MM}}$
-	K	Wʻ	LN	MM	K	Wʻ	LN	MM	%	%
Hippocampus	47	5.32	18	29	51	6.88	19	32	+ 26.9	+ 3.6
Septum	44	6.19	16	28	49	6.94	12	37	+ 10.8	+11.9
Amygdale	51	5.73	10	21	54	6.28	20	34	+ 8.8	+21.7
Hypothalamus frontal	55	5.62	31	24	59	5.0	24	35	- 8.9	+15.7
Hypothalamus rear	44	7.15	30	14	47	6.08	28	19	- 14.9	+ 8.7

Note: \* - general population of the values of the interpulse periods was approximated by the normal distribution in case of Control and in case of Experiment.

#### Legend:

K - number of neurons under investigation.

W' - frequency of neuron discharges (imp/s).

LN – number of neurons with logarithmic normal distribution of the values of inter-pulse periods.

MM – number of neurons with multimodal distribution of the values of inter-pulse periods.

ΔW' - change in frequency of neuron discharges (%) as compared to the control value assumed as 100%.

 $\Delta K_{MM}$  – change in number of neurons (% of the control value) with multimodal distribution of the values of inter-pulse periods.

that amygdale can be considered as regulator in the process of organizing cortical-hypothalamus and hypothalamuscortical interactions in developing emotional stress under conditions of the prolonged sexual conflict situations.

## 5. Conclusion

- It is established that the formation of the state of "stress" in long-term sexual conflict situation in rats modifies the excitability of limbic system structures. Thus different structures of the limbic system are characterized by different degrees of changes in individual variables.
- The hippocampus, amygdaloid complex of nuclei, the septum pellucidum, the anterior and the posterior hypothalamus in rats are characterized by different ratios of intervals interspike value distribution types, beyond the approximation of normal distribution according to the Central limit theorem of the probability theory.
- In the development of emotional stress in terms of group isolation the ratio of the number of neurons

- with multimodal and lognormal distribution ranges of intervals of activity values is changed in limbic brain structures of rats.
- The frequency of discharge decreases during the stress in both areas of the hypothalamus, but increases in the rest of the formations, i.e., the energy behavior of individual systems is bidirectional. The increase in the number of neurons with multimodal distribution of values of intervals indicates the bit ordering of their activities under stress.
- Changes in various structures of the limbic complex in rats under stress suggest that the amygdala has a minimal change in background activity as compared with septum and hippocampus, and the maximum number of neurons with multimodal distribution of inter-pulse intervals values.
- The obtained data show the degree of the similarity and differences in the functional organization of the limbic system structures and indicate the unequal excitability of its different areas.
- The pathophysiological meaning of the above results is the discovery of the maximally unstable

place in the limbic structures, whereon corrective influence should be directed in the development of experimental neurosis in certain circumstances.

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