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The Content of c-Fos-Positive Neurons in the Cerebral Cortex and Striatum and Behavioral Characteristics in Rats on Cutaneous Application of Antiseptic Dorogov's Stimulator Paste

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Experiments were performed on three groups of male Sprague–Dawley rats, each of six animals. Group 1 consisted of intact animals; group 2 of animals given zinc paste, and group 3 of animals given fraction 3 of antiseptic Dorogov's stimulator (ASD-3) by application to the skin on the back. Detection of c-Fos-positive neurons in different parts of the brain and studies of behavioral responses demonstrated activation of neurons in the cingulate, motor, and piriform areas of the cerebral cortex and the striatum, with increases in motor and ultrasound activity; there was a correlation between behavioral reactions and activation of neurons in these brain areas. These results provide evidence of the concomitant regulation of behavior by multiple brain structures and that ASD-3 has neurotropic properties.

Keywords: cingulate, motor, and piriform cortex, striatum, antiseptic Dorogov's stimulator, c-Fos, behavioral reactions.

Antiseptic Dorogov's stimulator (ASD) was reported in the first half of the last century, when it was prepared by A. V. Dorogov by deep thermal degradation of animal tissues. The results of animal and human studies described extensive positive pharmacological effects in skin diseases (eczema, neurodermatitis, psoriasis, allergic dermatoses, etc.). A general stimulatory action on the nervous system was also noted [3]. The pharmacological characteristics of the fractions of this formulation confirm the possibility that ASD influences the nervous system, application of the formulation having muscarinic, nicotinic, and cholinomimetic actions [1]. The morphological manifestations of this effect have not been studied. Behavioral acts are known to be regulated by the interaction of many brain structures: the cingulate cortex, which is responsible for complex behavioral reactions and ultrasound vocalizations; the motor cortex, which regulates the clear, selective movements underlying behavior; the somatosensory cortex, responsible for senses from most of the body's receptors; the insular cortex, responsible for the decision-taking process and emotional control of behavior and a center regulating narcotic addiction; the piriform cortex, responsible for emotional control of behavior; and the striatum, which has roles in regulating complex behavioral acts and the operation of the internal organs [16, 21]. Histological evaluation of the event of activation of brain neurons currently relies on determining the

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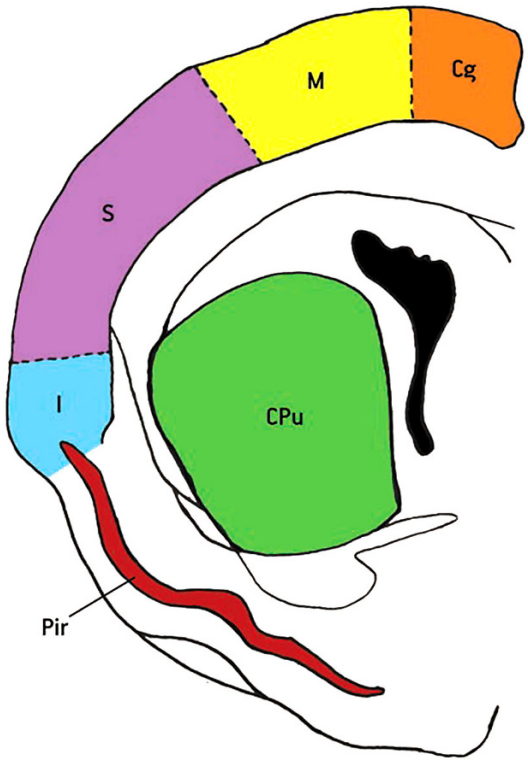

Fig. 1. Diagram of cortical structure and striatum in the right hemisphere of the rat brain. Frontal section at levels 13–17. Structures in which c-Fos-positive neurons were detected: cingulate cortex (Cg); motor cortex (M); somatosensory cortex (S); insular cortex (I); piriform cortex (Pir); striatum (CPu). Diagram by E. V. Sen'.

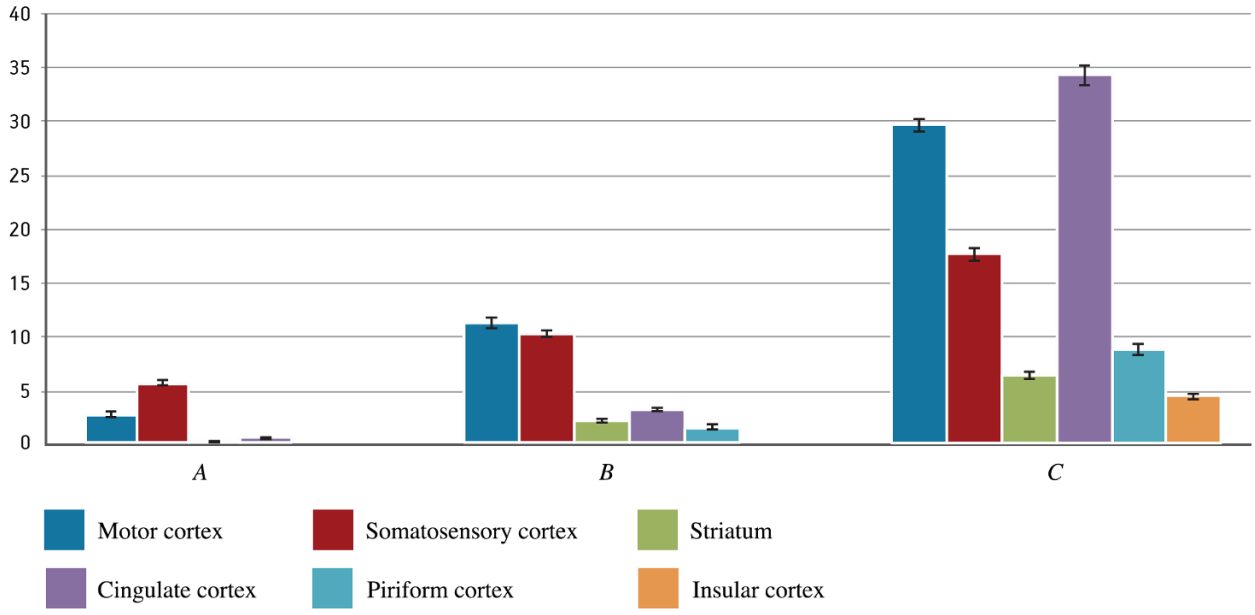

Fig. 2. Numbers of c-Fos-positive neurons in rats in the cerebral cortex and striatum. The horizontal axis shows groups of rats: *A* – intact; *B* – after application of zinc paste, i.e., the formulation base; *C* – after application of zinc paste containing 5% antiseptic Dorogov's stimulator fraction 3; the ordinate shows the number of activated neurons per microscope field. Vertical bars show standard errors.

content of the protein c-Fos (a marker for early neuronal activation) [8, 10, 11, 20].

The aim of the present work was to study morphological changes in neurons in the cerebral cortex and striatum in

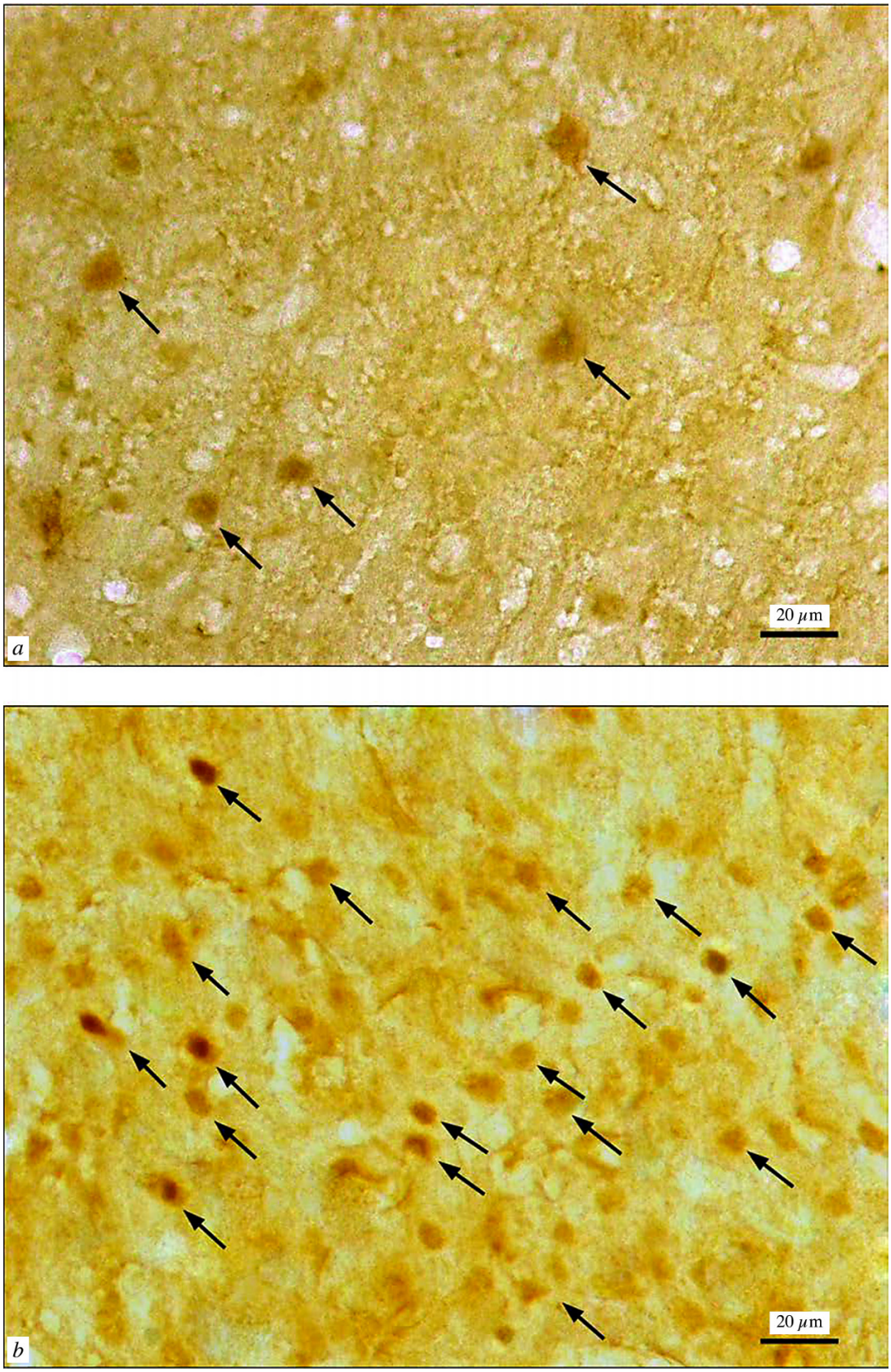

Fig. 3. Motor (*a*, *b*) and piriform (*c*, *d*) cortex in rats receiving zinc paste containing antiseptic Dorogov's stimulator fraction 3 (*b*, *d*) and base only (*a*, *c*). Arrows show c-Fos-positive neurons with different staining intensities. Histochemical reaction for c-Fos. Objective ×40, ocular ×20.

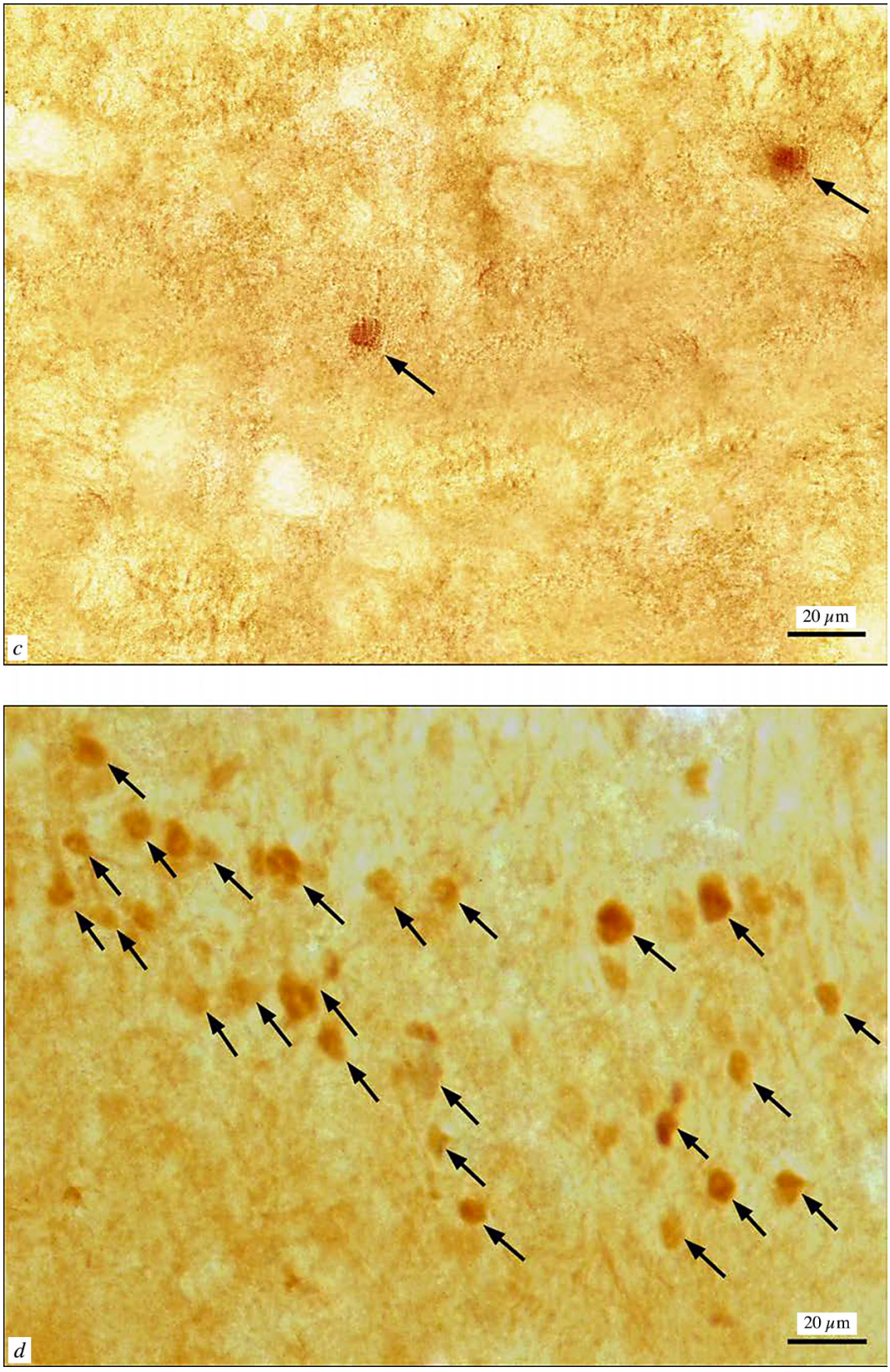

Fig. 3. Continued.

TABLE 1. Behavioral and Ultrasound Activity in Rats ($\bar{x} \pm s_{\bar{x}}$)

Study parameter	Groups of animals		
	control – intact animals	animals given zinc paste, i.e., formulation base	animals given antiseptic Dorogov's stimulator
Duration of motor acts, sec	6.5 ± 2.7	40.2 ± 1.2*	74 ± 10*,#
Rate of movement, mm/sec	0.40 ± 0.20	2.9 ± 0.4*	6.5 ± 0.7*
Distance covered, m	0.30 ± 0.20	2.6 ± 0.3*	5.9 ± 0.6*,#
Total number of behavioral acts	155 ± 34	130 ± 15*	413 ± 13*,#
Number of cries	9 ± 4	6.7 ± 1.5	49 ± 7*,#
Number of suprathreshold cries	0	1.7 ± 0.7*	19 ± 5*,#
Frequency of cries, Hz	49364 ± 47	49191 ± 150	48512 ± 78*,#

*Significant differences compared with control group; #compared with animals given formulation base, $p \le 0.05$.

TABLE 2. Correlation Coefficients between the Numbers of c-Fos-Positive Neurons in Brain Areas and Behavioral Reactions in Rats on Application of Antiseptic Dorogov's Stimulator

Brain area	Behavioral parameters	Correlation coefficient (r)
Motor cortex	Mean movement speed, mm/sec	0.93
Striatum		0.92
Piriform cortex		0.92
Cingulate cortex	Cries	0.94
	Mean frequency of cries, Hz	0.92
	Suprathreshold cries	0.91

rats after cutaneous application of zinc paste containing 5% ASD-3 (ASD fraction 3).

Materials and Methods. Experiments were performed on male Sprague–Dawley rats weighing 100–120 g from the Pushchino branch of the Institute of Bioorganic Chemistry, Russian Academy of Sciences. Animals were kept in animal-house conditions in the Retinoidy Preclinical Study Center. The main regulations for the keeping and care of rats were compliant with the regulations for the facilities, equipment, and maintenance of animal houses [4]. Rats were kept in controlled environmental conditions and received complete granulated feed and purified water without restriction. Before the study, animals were transferred to a different location for a 14-day adaptation period, after which the following groups were formed (each of six animals): group 1 consisted of intact animals; group 2 consisted of animals receiving base substance (zinc paste); group 3 received ASD-3. Rats of groups 2 and 3 received base and ASD (2 g/kg) twice daily for seven days to an area of 4 cm^2 of the spine between the shoulder blades. At 2 h after the last application, a time-synchronized Laboras-Sonotrack (Metric, Holland) complex [6] was used to record motor activity, ultrasound vocalizations, and suprathreshold cries for 15 min. Animals were then anesthetized with Zoletil 100 (Virbac, France). After intracardiac perfusion with Immunofix (Bio-Optica, Italy), brains were fixed for 1 h in 15% sucrose solution in Immunofix. Sections of thickness 20–40 μm were cut on a Thermo Scientific HM 430 frozen microtome (Microm GmbH, Germany) with a KS 34 rapid freezing apparatus; c-Fos-positive (activated) cells were detected with the avidin-biotin method with detection using 3,3'-diaminobenzidine (Santa Cruz, USA).

Studies addressed the cingulate, motor, somatosensory, insular, and piriform areas of the cortex and the striatum (Fig. 1), corresponding to sections at brain atlas levels 13–17 [15]. c-Fos-positive neurons were counted in three microscope fields for each animal (objective ×40, ocular ×20) using the program AxioVision (Carl Zeiss, Germany) using an Axioskop 2 microscope (Carl Zeiss, Germany). Statistical analysis of significant differences in mean values (Student's t test) and analysis of correlational relationships between behavioral reactions and the numbers of neurons (correlation coefficient r) was run in Statistica 6.1 [7].

Results. Expression of the c-Fos gene in rats of group 1 was detected at baseline levels. Animals of group 2 showed greater numbers of c-Fos-positive neurons, particularly in the motor and somatosensory areas of the cortex. Rats receiving ASD showed a sharp increase in the number

of activated neurons in all the areas studied (Fig. 2), especially in the cingulate, motor, and piriform areas of the cortex and the striatum (Fig. 3). Results from analysis of behavioral activity in rats after use of ASD-3 demonstrated increases in the duration and rate of movement, the distance covered, and the total number of behavioral acts recorded (Table 1). Analysis of measures of ultrasound vocalizations showed that rats receiving ASD-3 produced more ultrasound and at lower frequency (see Table 1). Correlation analysis in the group of animals given ASD-3 identified a significant relationship between the number of c-Fos-positive neurons and behavioral activity in the rats (Table 2).

Discussion. These experiments revealed a link between activation of neurons in the motor and piriform areas of the cortex and the striatum on the one hand and mean rates of movement on the other, supporting the existence of corticostriate and corticocortical interactions in the brain. These data are consistent with results from studies on laboratory rodents [21]. The existence of these links has also been described by other authors [13, 19]. Correlation analysis revealed a relationship between the number of activated neurons in the motor and piriform areas of the cortex and the striatum on the one hand and motor activity on the other in lab rats. This supports extensive published data on links between the cortex and subcortical nuclei of the brain and the execution of motor acts and their control [2, 5, 12, 14]. These studies showed that cutaneous application of ASD-3 was accompanied by activation of neurons in the striatum and the cingulate, motor, and piriform areas of the cortex, along with changes in the animals' behavior. Sayin et al. [18] noted from their studies that administration of citalopram to female rats correlates with anxiety behavior and increased content of c-Fos-positive neurons in the cingulate cortex, amygdaloid body, and paraventricular thalamic nucleus. This is evidenced by results from a study by Babaev et al. [9], where actions on neuroligin proteins led to activation of c-Fos-positive neurons in zones associated with anxiety.

Our study revealed a correlational relationship between the number of c-Fos-positive neurons in the cingulate cortex and measures of ultrasound vocalizations in the group of animals given ASD-3. Pertsov et al. [17] also noted that different emotional states in rats are accompanied by changes in the parameters of ultrasound vocalizations. Increases in c-Fos protein in the somatosensory and insular cortex of the brain is evidence of neuron activation in response to the process of the cutaneous application of ASD-3 and its associated sensations.

Thus, application of zinc paste containing 5% ASD-3 fraction to the skin of the interscapular areas of Sprague–Dawley rats led to a significant increase in the number of c-Fos-positive neurons in the cingulate, motor, insular, and piriform cortex, as well as in the striatum, along with an increase in motor and ultrasound activity in rats. Correlation analysis results provide evidence of a high probability that there is a link between the number of activated neurons and animals' behavioral activity.

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