# Role of Neurochemical Mechanisms of the Caudate Nucleus in Different Models of Anxiety in Rats

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Microinjections of dopamine, apomorphine, and GABA to the dorsal part of the caudate nucleus decreased the level of anxiety in an illuminated platform avoidance task in rats, while sulpiride and picrotoxin increased it. Intrastriatal injection of serotonin, glutamic acid, yohimbine, and phenylephrine reduced anxiety in threatening situation, but not in the illuminated platform avoidance task. It is suggested that emotional states associated with different kinds of stress are realized through diverse neurochemical systems of the caudate nucleus neuronal network.

**Key Words:** *caudate nucleus; anxiety; neurochemical mechanisms*

Tranquillizers potentiating GABA-ergic transmission are known to suppress fear and deactivate the fear-induced synthesis of catecholaminergic enzymes in the caudate nucleus [4,14]. Aversive stimuli of diverse modalities converging on the caudate nucleus differently change the content of dopamine, GABA, and glutamic acid [8,10,13], or induce asymmetrical changes in their metabolism and synaptic release [11]. Although noradrenaline and serotonin also belong to neostriatal neurotransmitters [2,7,12], their role in the genesis of anxiety is unclear.

In this study, the functional role of different neurotransmitters in anxiety caused by different aversive stimuli was investigated by means of local microinjections of GABA, glutamic acid, monoamines, and their agonists and antagonists into the caudate nucleus.

## MATERIALS AND METHODS

Experiments were carried out on 42 outbred adult male rats weighing 250±70 g. The rats were tested in a box consisting of light (200-W light bulb) and dark compartments and a special section for victim rats separated from the dark compartment by a transparent wall. The light and dark compartments were connected through a hole located 6 cm above the floor. The level of anxiety was measured in rats previously trained to avoid illuminated place (test 1, illuminated platform avoidance) and to avoid light compartment in a threatening situation (test 2, threatening situation avoidance). Painful electric stimulation (45 V) applied to 18 victim rats in the special section of the dark compartment served as a model of threatening situation for spectator rats and was automatically switched off, when spectator rat reached a special platform in the dark compartment. After consolidation of the avoidance reactions, the rats ($n$=24) were anesthetized with ether and chemotrodes for intrastriatal microinjections were implanted in the dorsal part of the caudate nucleus according to stereotaxic coordinates AP=-1.0, L=2.0, and H=3.2. The following drugs (0.5-5%) were used: dopamine GABA, glutamic acid, serotonin creatinine sulphate, agonists of $\alpha_2$ adrenoreceptor, $D_1$ and $D_2$ dopamine receptors (clonidine, apomorphine), and antagonists of $\alpha_1$, $\alpha_2$, $D_1$, and $D_2$ receptors (phentolamine, yohimbine, sulpiride) in doses of 5-50 μg. All drugs were administered in a volume of 1 μl. Retention of the acquired reactions was tested for 2 days before experiments (5-6 days after surgery). The rats with avoidance latency of 2-3 sec in the illuminated platform test and 1-2 sec in the threatening situation test were used in further experiments. The experiments

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consisted of two sessions with a 60 min interval between them. In the first session the baseline indices of anxiety were measured in both behavioral tests, in the second one the same indices were measured 5 min after the intrastriatal injection of one of the drugs. The time spent in the illuminated compartment, the number of crossed squares (motor activity), and the intensity of motivation to reach the dark compartment were measured using a Dekatron-based recording system. The latter index was calculated as the total force developed at the platform of the dark compartment ($I$) related to the time spent on it ($t$) and body weight ($m$) [3]. If these indices revealed the anxiolytic effects, the rats were additionally tested for drug-induced central myorelaxation using the rotarod test.

Control groups for tests 1 and 2 included 5 animals each trained to perform the corresponding avoidance reactions. During the experiment the control rats received 1 μl saline through previously implanted chemotrodes. After the experiments the rats were sacrificed under ether narcosis. The position of chemotrode tips in the dorsal part of the caudate nucleus head was verified morphologically. The data were treated with Stadia software using Wilcoxon—Mann—Whitney and Kolmogorov—Smirnov tests at a significance level of $p<0.05$.

## RESULTS

In a dose of 5 μg, the presynaptic $\alpha_2$-adrenoreceptor agonist clonidine (Clophelinum) blocking the release

**TABLE 1.** Effects of Neurotransmitters, Their Agonists and Antagonists on the Level of Anxiety in Illuminated Platform (Numerator) and Threatening Situation (Denominator) Avoidance Tasks

| Substance | Dose, μg | Time spent in light compartment, sec | Motor activity, number of crossed squares in light compartment | Achievement motivation, arb. units | Myorelaxant effect, % of rats slipped off rotating bar |
|---|---|---|---|---|---|
| Saline 0.9% (control) | 1μl | 3.6±0.40 / 2.4±0.51 | 5.6±0.25 / 5.2±0.37 | 0.29±0.04 / 0.26±0.03 | 0 / 0 |
| Clonidine | 5 | 4.0±0.45 / 2.8±0.58 | 5.6±0.40 / 5.2±0.49 | 0.27±0.03 / 0.26±0.06 | 0 / 0 |
| | 10 | 6.0±0.63* / 5.0±0.45* | 5.6±0.40 / 5.4±0.4 | 0.29±0.04 / 0.27±0.03 | 40 / 40 |
| Phentolamine | 5 | 4.4±0.51 / 3.4±0.40 | 5.8±0.37 / 5.4±0.40 | 0.28±0.02 / 0.26±0.04 | 0 / 0 |
| | 10 | 6.2±0.58* / 5.2±0.37* | 6.0±0.55 / 5.4±0.25 | 0.29±0.03 / 0.27±0.05 | 40 / 40 |
| Phenylephrine | 10 | 4.0±0.55 / 5.4±0.51* | 5.6±0.25 / 5.6±0.40 | 0.31±0.02 / 0.29±0.04 | 0 / 0 |
| Serotonin | 20 | 4.0±0.71 / 5.0±0.37* | 5.8±0.49 / 5.4±0.40 | 0.30±0.05 / 0.26±0.02 | 0 / 0 |
| Glutamic acid | 10 | 3.6±0.40 / 5.6±0.51* | 5.6±0.40 / 5.8±0.37 | 0.31±0.02 / 0.27±0.02 | 0 / 0 |
| Yohimbine | 10 | 4.4±0.60 / 4.8±0.49* | 5.6±0.40 / 5.2±0.49 | 0.30±0.02 / 0.26±0.03 | 0 / 0 |
| Dopamine | 10 | 6.6±0.51* / 2.6±0.40 | 6.0±0.32 / 5.2±0.37 | 0.31±0.04 / 0.27±0.06 | 0 / 0 |
| Apomorphine | 10 | 7.0±0.55* / 2.8±0.37 | 6.4±0.40 / 5.2±0.37 | 0.28±0.02 / 0.30±0.04 | 0 / 0 |
| GABA | 10 | 5.8±0.37* / 2.6±0.40 | 5.6±0.51 / 5.2±0.37 | 0.29±0.05 / 0.26±0.04 | 0 / 0 |
| Picrotoxin | 5 | 1.8±0.37* / 3.0±0.32 | 5.4±0.25 / 5.4±0.25 | 0.48±0.03* / 0.27±0.02 | 0 / 0 |
| Sulpiride | 50 | 1.8±0.37* / 2.6±0.25 | 5.0±0.45 / 5.2±0.37 | 0.49±0.03* / 0.25±0.05 | 0 / 0 |

**Note.** *$p\leq0.05$ in comparison with the control.

of noradrenaline from chatecholaminergic terminals and the postsynaptic $\alpha_1$- and $\alpha_2$-adrenoreceptor antagonist phentolamine did not change the level of anxiety in the two tests. In higher doses (10 μg) the two adrenergic drugs reduced anxiety in both behavioral tests as evidenced by significantly longer time spent by rats in the illuminated compartment. However, this anxiolytic effect was associated with motor deficit, since these doses significantly reduced the muscular tone (Table 1).

The fear-induced anxiety was resistant to chemical stimulation of the caudate nucleus with phenylephrine (Mesatone, postsynaptic $\alpha_1$-adrenoreceptor agonist ), serotonin, glutamic acid, and presynaptic $\alpha_2$-adrenoreceptor antagonist yohimbine potentiating the release of noradrenaline from catecholaminergic terminals. None of these drugs modulated avoidance reactions in the illuminated platform test (Table 1). In the same test dopamine, apomorphine, and GABA showed a distinct anxiolytic effect increasing the time spent by rats in the illuminated compartment. This effect was specific for fear-induced anxiety [3], since none of the drugs affected motor activity and muscular tone (Table 1). Picrotoxin and sulpiride (GABA and dopamine antagonists, respectively) had no effect on motor activity, but potentiated anxiety and stimulated mechanisms controlling innate preference of darkness: these drugs significantly shortened the time spent by rats in the light compartment (Table 1). It can be concluded that in fear-motivated behavior the neuronal matrix of anxiety in the dorsal part of the caudate nucleus processes aversive stimuli through activation of dopaminergic and GABA-ergic rather than adrenergic, serotoninergic, and glutamatergic mechanisms. This conclusion is in line with previous reports showing that activation of the GABA-ergic transmission by neostriatal injection of the GABA agonist muscimol impairs aversion-motivated avoidance reactions [9], while imipramine, which stimulates the striatal release of dopamine during performance, improves biological adaptation to stress [8].

In the task with a threatening situation and free choice between the light and dark compartments, stimulation of the caudate nucleus with serotonin, glutamic acid, yohimbine, and phenylephrine effectively reduced anxiety without affecting locomotor activity (Table 1), while other test drugs exerted no significant effects. These data indicate that adrenergic, glutamatergic, and serotoninergic, but not GABA-ergic and dopaminergic mechanisms are involved in the formation of a neuronal matrix of anxiety in the dorsal part of the caudate nucleus under conditions of aversive zoosocial stimulation [3]. This conclusion is supported by previous data on diverse effects of aversive stimuli of different modalities on binding of serotonin 1A receptor agonist $^3$H-8-hydroxy-2-(di-n-propylamino)tetraline ($^3$H-8-OH-DPAT)[6]. In complex behavioral tests for zoosocial interactions, serotonin and its agonists suppressed fear and increased the number of punished reactions in a conflict situation [5].

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