# NORMALIZATION OF EXTRAPOLATION AVOIDANCE BEHAVIOR OF RATS BY NOOTROPIC DRUGS

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One of the difficulties of studying nootropic drugs is the absence of an adequate set of methods of detecting them. The study of these compounds is based on discovery of the ability of nootropic drugs to weaken the action of amnesic factors on established conditioned reflexes. Avoidance responses to stress situations not requiring preliminary formation are interesting in relation to the study of nootropic activity. The realization of this response can be defined as a level of emotional reactivity, and also as an ability to realize an integral act, leading to avoidance. The normalizing effect of tranquilizers has been discovered by the use of an avoidance model [7] in Bondarenko's modification [1], and its intensity has been shown to depend on the initial emotional state.

The aim of this investigation was to study influences exerted on the avoidance reaction by pyracetam (α-pyrrolidone acetamide), a standard nootropic drug, and by sodium and lithium hydroxybutyrates, as compounds which also exert a nootropic action within a certain dose range [2], and to compare them with the benzodiazepine tranquilizer, phenazepam.

## EXPERIMENTAL METHOD

Experiments were carried out on noninbred male rats weighing 150-180 g. The apparatus for studying extrapolation avoidance behavior, in Bondarenko's modification [1], consists of two coaxial cylinders: an outer one filled with water (t = 22°C) and an inner one, fixed to the wall of the outer one so that its bottom end is immersed in water to a depth of only 2.5 cm. A rat, placed inside the inner cylinder, cannot jump out of it. The only solution enabling avoidance is to dive under the bottom edge of the cylinder, to find a wire mesh ladder fixed to the wall of the outer cylinder and visible by the rat through the transparent wall of the inner cylinder, and to climb up it. The number of ineffective attempts to escape was counted and, if the rat succeeded in escaping from the inner cylinder, the latent period, and the total percentage of animals successfully diving beneath the inner cylinder in the course of 120 sec were also counted. There were two series of experiments: on intact animals (series I) and on animals receiving an injection of cycloheximide (CHX), an inhibitor of protein synthesis (series II). Animals of the following groups took part in series I: control, receiving an injection of 0.9% sodium chloride, and experimental, consisting of animals receiving an injection of one of the test substances in doses given in Table 1. The rats in the experiments of series II were given CHX in a dose of 3 mg/kg combined with 0.9% sodium chloride or with one of the drugs. CHX was given 60 min, and the drug 30 min before testing. All compounds were injected intraperitoneally.

## EXPERIMENTAL RESULTS

In experiments on intact rats the percentage of animals successfully giving an extrapolation avoidance response during a period of observation of 120 sec was 78% in the control group and the average latent period of this response was 38.7 sec (Table 1). The two parameters agree with values described previously [4]. The nootropic drugs studied increased the percentage of animals capable of solving the avoidance problem within the time of observation a little, but the effect reached the level of significance only in the case of administration of lithium hydroxybutyrate and pyracetam. Sodium hydroxybutyrate and pyracetam significantly shortened the latent period of avoidance. A characteristic feature of all the nootropic drugs tested was the marked (up to tenfold) shortening of the interval between the rat entering the outer cylinder and coming out on the mesh ladder, which averaged 3 sec in the

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TABLE 1. Effect of Nootropic Drugs and Phenazepam on Parameters of Avoidance Behavior in Normal Rats and Rats Treated with Cycloheximide (CHX)

| Substance, mg/kg | Per cent of animals succeeding with avoidance (1) | Latent period of avoidance, sec (2) | Number of unsuccessful attempts at avoidance (3) | Number of animals (4) |
|---|---|---|---|---|
| Physiological saline (passive control) | 78 | 38,7±3,06 | 4,7±0,76 | 181 |
| Na-hydroxybutyrate, 50.0 | 90 | 15,3±5,9** | 1,8±1,0* | 20 |
| Li-hydroxybutyrate, 44.0 | 100* | 47,5±15,0* | 15,9±5,0** | 20 |
| Pyracetam, 200.0 | 100* | 20,6±6,2** | 1,6±0,7* | 50 |
| Phenazepam, 0.5 | 100* | 23,2±4,4* | 3,4±1,7 | 10 |
| Phenazepam, 1.0 | 60 | 69,4±13,8 | 7,0±2,4* | 15 |
| CHC, 3.0 | 60 | 54,2±6,8** | 2,7±2,2* | 60 |
| CHX (3.0) + Na-hydroxybutyrate (50.0) | 100++ | 22,8±3,9++ | 7,0±1,8++ | 20 |
| CHX (3.0) + Li-hydroxybutyrate (44.0) | 100++ | 37,9±5,1++ | 6,5±1,5++ | 20 |
| CHX (3.0) + pyracetam (200.0) | 90 | 36,2±1,0++ | 5,7±2,3++ | 17 |
| CHX (3.0) + phenazepam (0.5) | 65 | 53,7±8.8 | 5,0±1,7++ | 20 |
| CHX (3.0) + phenazepam (1.0) | 0+ | 120++ | 0++ | 6 |

Legend. Significant differences between passive and active control. +) Between action of CHX and substances together with CHX; * or +) $p < 0.05$; ** or ++) $p < 0.001$. (Wilcoxon–Mann–Whitney U test) used for parameters 2 and 3. Error of means calculated by Peters' formula using Molderhauer's factor for parameters 2 and 3. Significance of differences for parameter 1 determined by the chi-square method.

control. Phenazepam, in the smallest dose used (0.5 mg/kg), shortened the latent period of avoidance, in agreement with data obtained previously [1], whereas in a dose of 1 mg/kg it prolonged it, evidently because of strengthening of the depressant effect.

In the experiments of series II an attempt was made to disturb the extrapolation avoidance reaction by injecting CHX and studying the effect of the nootropic drugs under these conditions. CHX was found to reduce the percentage of animals capable of solving the avoidance problem: the rats became lethargic, they hung limp in the inner cylinder. Animals which dived into the outer cylinder swam in it longer than rats of the control group (on average by 3 sec) but floated past the mesh ladder without evidently associating its appearance with the possibility of avoiding the stress situation. Pyracetam, sodium hydroxybutyrate, and lithium hydroxybutyrate abolished the damaging effect of CHX on the extrapolation avoidance reaction, and restored the normal values of the parameters studied. Phenazepam, in the smaller of the doses used, did not change the effect of CHX, and in the larger dose it actually potentiated its damaging action on the avoidance reaction.

Thus, in animals with an undisturbed extrapolation reaction pyracetam and sodium hydoxybutyrate accelerate its course. Phenazepam, in the smaller dose, has an effect similar to that of the nootropic drugs, and only in the larger dose does it impair the performance of this reaction. Lithium hydroxybutyrate occupies an intermediate position between the compounds tested. When the avoidance reaction is disturbed by cycloheximide, clear differentiation takes place between the effects of the nootropic drugs and tranquilizers. Pyracetam, the standard nootropic drug, like the two compounds with presumptive nootropic activity, sodium and lithium hydroxybutyrate, abolishes the damaging effect of CHX on the avoidance reaction, whereas phenazepam has no such effect.

The ability of CHX to disturb learning ability was demonstrated previously on conditioned reflexes with positive [8] and negative [6] reinforcement. Data obtained in the present investigation suggest that although the model of avoidance studied is relatively simple from the point of view of motivation and performance, nevertheless its realization does not require the activation of higher integrative processes, against which the damaging effect of CHX, abolished by nootropic agents, is directed. The mechanism of this interaction requires further clarification. The fact that pyracetam [9], like sodium hydroxybutyrate [3], increases the rate of RNA, DNA, and protein synthesis, whereas CHX reduces it, suggests that the ability of these substances to abolish the behavioral effects of CHX may be due to the opposite direction of their action on synthesis of information macromolecules. The possibility cannot be ruled out that activation of the biogenic amine system, and, in particular, an increase in the rate of dopamine turnover in brain tissue, which has been described for pyracetam [8], and accumulation of dopamine under the influence of sodium hydroxybutyrate [9], may also play a role in the realization of the normalizing effect of nootropic drugs.

LITERATURE CITED

1. N. A. Bondarenko, V. A. Kamysheva, M. F. Mineeva, and A. V. Val'dman, Byull. Éksp. Biol. Med., No. 1, 20 (1981).
2. R. U. Ostrovskaya and S. S. Trofimov, Byull. Éksp. Biol. Med., No. 2, 170 (1984).
3. R. U. Ostrovskaya, S. S. Trofimov, N. M. Smol'nikova, and Yu. V. Burov, All-Union Symposium on Mediators in the Genetic Regulation of Behavior [in Russian], Novosibirsk (1986).
4. S. Tuneva, Ts. Tencheva, N. Tyutnelkova, and M. Nikolova, Mediko-Biol. Inform., No. 6, 8 (1980).
5. S. L. Shelekhov and A. V. Val'dman, Byull. Éksp. Biol. Med., No. 8, 59 (1983).
6. S. H. Barondes and H. D. Cohen, Science, 160, 556 (1968).
7. H. D. Hendersen, J. Psychol., 75, No. 19 (1970).
8. L. K. Rägo, L. H. Allikmets, and A. M. Zarkovsky, Naunyn-Schmiederberg's Arch. Pharmakol., 318, 36 (1981).
9. R. H. Roth, G. B. Doherty, and J. B. Walters, Brain Res., 189, 556 (1980).
10. G. Schmaltz and P. Marcant, Physiol. Behav., 30, No. 6, 845 (1983).

# SPINAL GANGLIONIC NEURONS OF RATS: A MODEL FOR THE STUDY OF CENTRAL SEROTONIN RECEPTORS

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Radioligand investigations have revealed two populations of serotonin receptors (SR) in brain membranes, of which one ($S_2R$) is labeled by $^3$H-spiroperidol, the other ($S_1R$) by $^3$H-serotonin; $^3$H-lysergic acid diethylamide (LSD) labels both SR populations [9]. More recent studies have shown that $S_1R$ can be subdivided into several subtypes [4, 7].

The results of electrophysiological investigations confirm this view that there are two populations of SR. The action of serotonin and stimulation of the nuclei raphe intensified spontaneous discharges or depolarization of neurons in the frontal cortex and dorsal hippocampus and of spinal motoneurons [1, 5, 10]; these effects, moreover, were abolished by LSD, cyproheptadine, methysergide, and other substances displacing $^3$H-spiroperidol from its binding, methylsergide, and other substances displacing $^3$H-spiroperidol from its binding with $S_2R$. However, serotonin antagonists did not affect serotonin-induced inhibition of neuronal activity in the frontal cortex, hippocampus, amygdala, and hypothalamus [6, 10]. These facts are evidence that $S_1R$ mediate inhibition and $S_2R$ mediate excitation of functions of central neurons. The concrete mechanisms of serotonin-inducted excitation or inhibition of nerve cell functions have not been adequately studied.

The investigation described below showed that $S_1R$ and $S_2R$ are represented in membranes of rat spinal ganglionic neurons, and the mechanisms of the excitatory and inhibitory action of serotonin on these nerve cells also were studied.

## EXPERIMENTAL METHOD

Experiments were carried out on isolated lumbar spinal ganglia of adult rats. After isolation and removal of the meninges a spinal ganglion was fixed to the floor of a perfusion chamber, where it was superfused with salt solution of the following composition (in mM): NaCl 124, KCl 2, $K_2HPO_4$ 1.25, $NaHCO_3$ 25, $CaCl_2$ 2, $MgSa_4$ 1, glucose 10. The solution was aerated intensively with a mixture of 95% $O_2$ + 5% $CO_2$; the pH of the solution was 7.4. The experiments were carried out at a temperature of 27°C.

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