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The Effects of Intranasal Administration of Oxytocin on the Behavior of Rats with Different Behavioral Strategies Subjected to Chronic Mild Stress

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Two groups of Wistar rats with opposite behavioral strategies – active and passive – were subjected to chronic mild stress. Half of the group of poststress animals received intranasal oxytocin (at a dose of 0.25 IU); their behavior was tested in the Porsolt test and their consumption of a sweet solution was evaluated. Stressed animals with the active behavioral strategy did not respond to oxytocin. Oxytocin partially restored behavior to prestress levels only in animals starting with the passive behavioral strategy.

Keywords: behavioral strategies, chronic mild stress, oxytocin, Porsolt test, anhedonia.

The psychotropic effects of oxytocin (OT) have received intensive study in recent years [11, 14, 15, 17, 18, 20, 21]. Oxytocin weakens the influences of stress (novelty, jolting, restraint) on behavior in rodents [8, 10] and humans, for example, after presenting images of angry faces [19] and socially evaluative situations (the Trier test) [7]. Changes in behavior after stress are known to depend on the initial features of animals' behavior. Various (and sometimes differently directed) changes in behavior have previously been noted in animals with innate tendencies to active and passive behaviors [4, 31]. In particular, active and passive animals selected from a genetically heterogeneous population of Wistar rats displayed different responses to chronic mild stress. Animals with the passive behavioral strategy demonstrated less depression-like behavior after the stress procedure than animals with the active behavioral strategy [2]. The present report describes our studies on the effects of intranasal administration of OT on the duration of immobility in water (the Porsolt test) and the consumption of sweet solution – these are indicators of depression-like behavior – in Wistar rats with active and passive behaviors.

Methods. All experiments were performed in compliance with international norms for medical-biological studies using animals [13]. The experiments used 60 male Wistar rats aged two months at the beginning of the experiments. Animals were kept in groups of five individuals per cage in standard conditions with free access to feed (dry combined feed for rodents) and water. Experiments were run from 13:00 to 18:00. Animals were accustomed to being handled over a period of two weeks. Animals were then selected on the basis of activity on acquisition of a conditioned passive avoidance reflex. Training to the reflex was performed in a chamber with an electrically conducting floor; the rats were placed on a plastic platform 10 cm in diameter. Premature jumping was prevented using a cardboard cylinder of diameter slightly greater than the diameter of the stand. The test determined the latent period from the moment at which the paper cylinder was removed to the point at which the animal landed on the grid with all four paws, after which the rat received an electric shock of 150–200 μA. The electric shock was not delivered the next day, so only the latent period of landing was recorded. Freezing duration is inversely proportional to the ability to acquire a conditioned active avoidance reflex in a two-way shuttle

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box [1, 11, 28], so the ability to carry out passive avoidance reflects the level of activity of the animal's behavior.

The results of the passive avoidance test were used to divide the animals into groups *A*, with the active, and *B*, with the passive behavioral strategy; group *A* (n = 20) included animals in which the latent period of descending from the platform on test day 2 was shorter than that on day 1. Group *B* (n = 25) included rats in which the latent period was significantly longer on day 2 (on average, group *B* animals sat on the platform for 109 sec longer than on test day 1). Animals with intermediate values were not used in subsequent experiments.

Animals were transferred on the basis of their assignments to groups *A* or *B*. Consumption of sweet (20% sucrose) solution was assessed after a week. The weight of the sucrose solution consumed (g/kg) was measured for each rat in 30-min tests in individual cages. Before the test, a bottle containing sucrose was placed in the cage with the animals for two days to eliminate neophobia reactions.

The expression of the depression-like state was assessed using the Porsolt test. The apparatus consisted of a glass cylinder 20 cm in diameter and 45 cm high, two thirds filled with water. Test duration was 5 min and the duration of immobility was measured.

Rats were then subjected to stress. Animals were subjected to seven different actions over a period of four weeks: tilting the cage, damp litter, food deprivation, water deprivation, social isolation, and inversion of the light regime. The order of aversive actions was changed in random order each week. The chronic mild stress procedure reproduced the previously used protocol [2].

At the end of chronic mild stress, consumption of sweet solution was tested the next day, and the Porsolt test was performed the day after that. Animals received intranasal oxytocin solution (0.25 IU in 20 μl, with 10 μl in each nostril) 20 min before testing. The control group received the same volume of 0.9% NaCl solution.

Group sizes were: group *A* before stress n = 20, after stress + OT n = 10, after stress + NaCl n = 10; group *B* before stress n = 25, after stress + OT n = 12, after stress + NaCl n = 13.

Video recordings were made of the animals' behavior using a Sony DCR-HC17E PAL (Japan) video camera and a Logitech webcam (Switzerland). Statistical analysis was run using the nonparametric Mann–Whitney test for independent sets and the Wilcoxon test for dependent sets in SPSS (PASW Statistics 18.0). The level for statistical significance was $\alpha < 0.05$. Data are presented as mean ± standard error.

Results. Immobility durations in the Porsolt test are shown in Fig. 1. For rats of group *A*, the duration of immobility before stress was 4.7 ± 1.0 sec, compared with 48.8 ± 6.1 sec in the poststress NaCl group and 50.8 ± 5.1 sec in the poststress OT group. In animals of group *B*, immobility before stress lasted 12.9 ± 2.8 sec, compared with 56.3 ± 3.4 sec in the poststress NaCl group and 38.6 ± 3.7 sec in the poststress OT group. The increase in the duration of immobility after stress is evidence of development of a depression-like state in all the animals subjected to the stress procedure. Animals in group *B*, with the passive behavioral strategy, demonstrated a significantly shorter period of immobility in the Porsolt test after pretest administration of OT than the NaCl-treated group. This indicates the OT reversed the effect of the stress procedure in animals of group *B*, with the passive behavioral strategy.

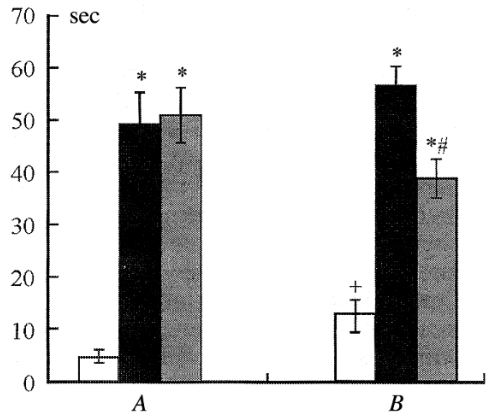

Fig. 1. Duration of immobility in the Porsolt test. The vertical axis shows duration of immobility in the Porsolt test; the horizontal axis shows groups of animals: *A*) with the active and *B*) with the passive behavioral strategy. Light columns show animals before stress; black columns show poststress rats given physiological saline (NaCl); gray columns show poststress rats given oxytocin (OT). Here and henceforth: mean values with standard errors. *Significant differences between pre- and post-stress animals, $p < 0.05$; #significant differences between the NaCl and OT groups, $p < 0.05$; +significant differences between groups *A* and *B*, $p < 0.05$.

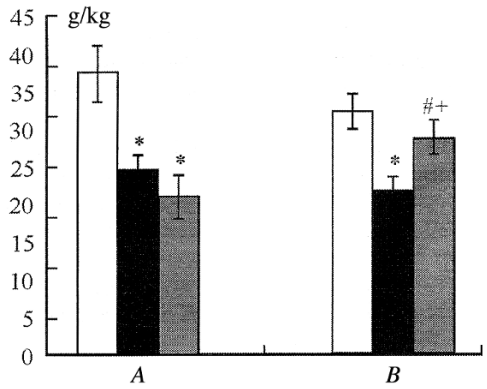

Fig. 2. Test for consumption of 20% sucrose solution. The vertical axis shows the quantity of solution consumed, g/kg body weight; the horizontal axis shows groups of animals. For further details see caption to Fig. 1.

Consumption of sucrose solution is shown in Fig. 2. In the case of rats of group *A*, consumption of sucrose solution amounted to 37.5 ± 3.7 g/kg, compared with 24.4 ± 2.2 g/kg in the poststress NaCl group and 21.0 ± 2.9 g/kg in the post-

stress OT group. Consumption was 32.3 ± 2.2 g/kg in animals of group *B* before stress, compared with 21.6 ± 2.2 g/kg in the poststress NaCl group and 28.8 ± 2.3 g/kg in the OT group. Intranasal administration of oxytocin significantly increased the consumption of sucrose by rats of group *B* with the passive behavioral strategy but produced no changes in consumption in rats of group *A*, with the active behavioral strategy.

Discussion. Animals with the passive type of behavior – group *B* – reacted to chronic mild stress more weakly than animals of group *A*, with the active type of behavior. The duration of immobility in the Porsolt test in intact animals of group *B* was 1.5 times greater than that in animals of group *A*. After chronic mild stress, the duration of immobility increased threefold in animals of group *B* and 10-fold in those of group *A*. Decreases in the consumption of sweet sucrose solution were by 30% in rats of group *B* and 40% in group *A* rats. The lower reactivity of passive rats compared with active was consistent with our previous data obtained in a model of chronic mild stress [2]. The data obtained in the present study also support our previous conclusion that stress reactivity of animals with the passive behavioral strategy is greater, based on a model of uncontrollable pain stress in rats of genetically bred strains with high and low rates of acquisition of a conditioned active avoidance reflex (KHA and KLA rats) [3, 4].

Animals with opposite behavioral strategies also showed different responses to administration of OT. Treatment with OT significantly reduced the duration of immobility in the Porsolt test in stressed rats of group *B* but had virtually no effect on stressed rats of group *A*. Administration of OT restored sucrose consumption in stressed group *B* rats to the levels seen in the intact group but had no effect on the consumption of sweet solution by stressed animals of group *A*. Thus, the stress-protective effect of OT was seen only for animals of group *B*, with the passive type of behavior.

The different responses to OT demonstrated here acquire special value when we consider that chronic mild stress is a recognized model for depressive disorder in humans. Chronic mild stress was first proposed as a model of anhedonia, consisting of a reduction in the consumption of sweet solutions [30]. Subsequent studies showed that chronic mild stress was followed by changes in other behavioral measures, such as motor and exploratory activity, anxiety levels, aggressive and sexual behavior [29], and the activity of CNS structures traditionally linked with depressive disorders [16, 22, 25]. The overall set of changes seen in rats and mice subjected to chronic mild stress allows these changes to be characterized as symptoms of a depression-like state.

The great advantage of chronic mild stress as a model of depression is the absence of any strong action such as pain, as used in many other models, for example unavoidable pain stimulation. In chronic mild stress, the main factor affecting the animals' behavior is not the impossibility of avoiding the aversive action to which the animal is unable to adapt, but the unpredictability of the changes in the living conditions. This model reproduces the so-called "everyday" stress experienced by humans [23, 24]. "Everyday stress" refers to numerous events which people find unpleasant, each of which alone cannot produce persisting changes in mental status but which when occurring unpredictably in large numbers result in mental and behavioral disorders. "Everyday stress" is regarded as the main cause of depressive disorders of the endogenous class, i.e., those for which the patient has no history of mentally traumatizing events. Thus, chronic mild stress is a model of endogenous depression in humans and is very suitable for studies of the mechanisms of the formation and treatment of this pathology.

Because of its stress-protective action, oxytocin has received intense study as a factor in mental and behavioral disorders (for reviews see [9, 26]). The relationship between the reaction to oxytocin on the one hand and the initial tendency to passive or active behavior on the other is very interesting. This interest comes from differences in the oxytocinergic system in rats with genetically determined behavioral strategies [5, 6, 12]. The activity of the neurohypophyseal oxytocinergic system in intact animals was greater in rats with passive behavior than in rats with active behavior; after stress, there was a decrease in the activity of the neurohypophyseal oxytocinergic system only in animals with the passive behavioral strategy.

Finally, further studies of the mutual dependence of the functions and mechanisms of regulation of the oxytocin system and behavioral strategies in animals are required. However, the link between the type of response to stress and the oxytocinergic system is already clear. Furthermore, oxytocin can be recommended as a stress-protective agent: oxytocin will be effective for this purpose only for patients with an initial passive behavioral strategy.

Thus, the present study shows that the stress-protective effect of oxytocin is seen only in animals with the passive behavioral strategy. Administration of oxytocin after chronic mild stress normalizes measures in the Porsolt test and the anhedonia test only in animals which initially demonstrated a tendency to passive behavioral reactions to stress – freezing. Oxytocin did not alter behavior after chronic mild stress in those animals which initially demonstrated a low tendency to freezing in response to stress.

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