DOI 10.1007/s11055-014-9994-2
Neuroscience and Behavioral Physiology, Vol. 44, No. 8, October, 2014

Effects of Neurotensin on the Effects of Pain Stress in Rats with Neurotoxic Lesions to Serotoninergic Structures of the Substantia Nigra of the Brain

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UDC 612.821.6+615

Translated from Zhurnal Vysshei Nervnoi Deyatel'nosti imeni I. P. Pavlova, Vol. 63, No. 3, pp. 384–394, May–June, 2013. Original article submitted March 19, 2012. Accepted October 19, 2012.

The aim of the present work was to study the effects of neurotensin on the behavior of rats with neurotoxic lesions to the serotoninergic structures of the substantia nigra of the brain. Changes in the reproduction of conditioned passive avoidance reactions were analyzed, as were changes in the effects of pain stimulation on the activity of rats in the open field. Administration of 5,7-dihydroxytryptamine into the substantia nigra was found to impair the reproduction of passive avoidance reactions and to weaken the suppressive effects of pain stimulation. Administration of the serotonin 5-HT_{1A} receptor antagonist p-MPPF into the substantia nigra had similar effects on the effects of pain stimulation. Administration of neurotensin into the caudate nucleus before application of pain stimulation prevented the toxin-induced impairment to defensive behavior and its effects on motor activity. Administration of neurotensin into the substantia nigra 24 h after pain stimulation had no marked effect on the passive avoidance reaction but increased motor activity during its reproduction. The effects of giving neurotoxin into the substantia nigra were linked with weakening of the action of pain stress on motor activity. Prevention of the development of this effect in rats after microinjection of neurotensin into the caudate nucleus may be due to recovery of neurotoxin-impaired reproduction of passive avoidance and can be explained by normalization of the balance of interactions between the serotoninergic (5-HT) and dopaminergic systems of the brain.

Keywords: neurotensin, dopamine, serotonin, caudate nucleus, substantia nigra, passive avoidance reaction.

The function of neurotensin in the CNS is linked with regulation of the motivational, emotional, and motor aspects of adaptive behavior. Neurotensin-producing neurons and their projections are widely represented in the CNS, which explains the wide range of effects of this peptide [10]. The highest neurotensin concentrations are seen in areas associated with dopaminergic projections, such as the caudate nucleus, putamen, and nucleus accumbens [20]. In the average brain, the largest numbers of neurotensin-positive cells are found in the ventral tegmental area and substantia nigra [20].

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The role of the dopaminergic systems in the mechanisms of memory are well known [17]. Decreases in catecholamines in the brain after acquisition of conditioned reflexes have been shown to lead to deficiencies in their execution, and this is regarded as a manifestation of retrograde amnesia [8]. It is also known that passive defensive behavior in animals is mediated by fear mechanisms and reflects the state of anxiety [2, 4].

In addition to the widely studied interaction of neurotensin with the dopaminergic system, there are as yet few data evidencing its interaction with 5-HT neurons in the raphe nuclei [21, 24, 27]. The functional role of neurotensin in the raphe nuclei may be linked with modulation of several of the known functions of the 5-HT system, such as stress reactions. We have previously shown that administra-

tion of neurotensin into the nucleus accumbens weakens the differently directed effects of lesions to 5-HT structures in the dorsal raphe nuclei and periacqueductal gray matter of the brain and, depending on the lesions induced, that neurotensin can have a normalizing influence on reproduction of passive pain stimulus avoidance reactions [3]. Administration of neurotensin into the central nucleus of the amygdala also increases the ability of rats to learn these reactions [22].

The aim of the present work was to explain the characteristics of the influences of neurotensin on the behavior of rats with neurotoxic lesions to serotoninergic structures of the substantia nigra of the brain. Changes in the reproduction of conditioned passive avoidance reactions and the effects of pain stimulation on motor activity in rats in an open field were studied.

METHODS

Experiments were performed on 47 white male Wistar rats weighing 250–300 g, kept in animal-house conditions with free access to food and water and with a natural sequence of daily illumination. Animal keeping and experimental manipulations were performed in compliance with the international guidelines "Guide for the Care and use of Laboratory Animals."

Using stereotaxic coordinates [26], metal guide cannulae were implanted bilaterally into the caudate nucleus (AP –1.0, L 2.5, V 4.5) and substantia nigra (AP 4.2, L 1.9, V 7.0). Cannulae were fixed to the skull with two screws and dental cement. Surgery was performed under anesthesia with i.p. ketamine (50 mg/kg) and benzodiazepine (5 mg/kg). Lesions to serotoninergic structures were produced by local administration of the selective neurotoxin 5,7-dihydroxytryptamine into the substantia nigra at a dose of 7 ∝g in 0.7 ∝l of 0.05% ascorbic acid solution.

The experimental apparatus for studying passive defensive behavior was a rectangular chamber with a metal floor, divided into two sectors (a dark sector and a brightly illuminated sector) by a vertical partition with an opening near the floor. Rats were initially familiarized with the apparatus. The animals were placed in the illuminated sector and the latent periods of their transfers to the dark sector were measured. These transfers were not accompanied by pain stimulation. After repeated placing of the rat in the illuminated sector, transfers to the dark sector were accompanied by presentation of an unavoidable pain stimulus, which consisted of application of a direct electric current shock (2 mA, 3 sec). During electrical stimulation, the access to the light sector was closed and immediately after termination of the stimulus, the rats were removed from the apparatus and returned to the home cage. Reproduction of the passive defensive reaction was assessed in terms of the latent period of transfer of the rat from the brightly illuminated sector of the chamber to the dark sector in which the animal had received the electric shock. Immediately after entry into the dark sector or at 3 min of failure to enter it, the rat was returned to its cage. Testing of these reactions was performed for three days after presentation of the electric stimulus: at 24, 48, and 72 h. Assessment of the influence of intracerebral microinjections of neurotensin on acquisition of the passive avoidance reaction used doses of peptide given 10 min before or 24 h after presentation of the electric stimulus.

The experimental procedure was as follows: bilateral microinjections of 2.5 ∝g of neurotensin in 0.7 ∝l of physiological saline were given into the caudate nucleus or substantia nigra. Some animals were given the serotonin 5-HT_{1A} receptor antagonist p-MPPF (4-(2'-methoxy)-phenyl-1-[2'-(N-2'-pyridyl)-p-fluorobenzamido]ethylpiperazine) into the substantia nigra at the same dose [30]. Control animals received the same volume of physiological saline only. Microinjections were given using a metal needle positioned 1 mm from the tip of the guide cannula and connected to a microsyringe via tubing. Injections were given by hand at a rate of 1 ∝l/min. The needle was left in the guide cannula for 2 min and then removed and replaced with a metal mandrel. The latent period of entry of animals into the dark sector of the experimental apparatus was measured before application of pain stimuli. The passive avoidance reaction was tested before and 24, 48, and 72 h after pain stimulation.

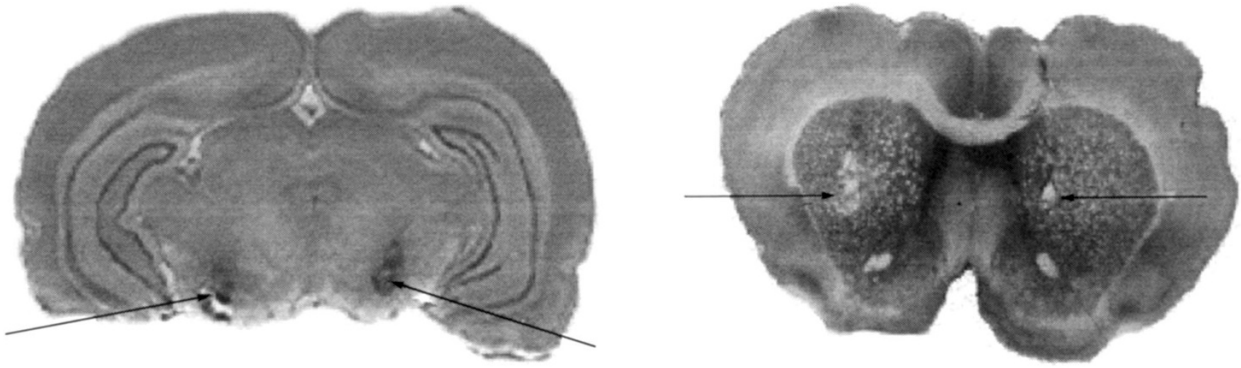
The rats' motor activity was tested in an open field. The open field was a square box with sides of 105 cm, 35 cm high. The floor area was divided into 36 squares. The test lasted 3 min and horizontal motor activity was determined; the total number of squares crossed was counted, along with the number of internal squares of the open field. Testing was performed immediately after pain stimulation and at 24, 48, and 72 h.

Experiments were performed on seven groups of animals: three groups were controls and consisted of six rats each (group 1 received physiological saline into the substantia nigra and caudate nucleus; groups 2 and 3 received physiological saline into the substantia nigra); group 4 received toxin into the substantia nigra (10 rats), group 5 received neurotensin into the caudate nucleus (seven rats), and group 6 received p-MPPF into the substantia nigra (six rats); group 7 received neurotensin into the substantia nigra 24 h after pain stimulation (six rats).

Once behavioral experiments were complete, cannula tip positions in the rats' brains were confirmed morphologically (Fig. 1). Statistical analysis was performed using the Wilcoxon–Mann–Whitney U test for unlinked sets. Differences were regarded as significant at $p \leq 0.05$.

RESULTS

Pain stimulation in the dark sector of the chamber in control animals induced stable passive avoidance reactions whereby the animal either did not enter this sector from the illuminated sector or entered it with longer latent periods on all



A *B*

Fig. 1. Morphological verification of cannula tip positioning (arrows) in the substantia nigra (*A*) and caudate nucleus (*B*) of the brain.

experimental days (Fig. 2, *A*). Pain stimulation was accompanied by suppression of motor activity in the open field in control rats, with subsequent recovery after testing the avoidance reaction (Fig. 2, *B*).

Lesioning of serotoninergic structures of the substantia nigra by local administration of the selective neurotoxin 5,7-dihydroxytryptamine impaired reproduction of the passive avoidance reaction. This impairment was apparent as a reduction in the latent period of transfer of rats from the illuminated sector into the dark sector, where the rats had received painful electrical stimulation the previous day (Fig. 2, *A*). Impairments persisted during the three days of observations and were accompanied by retention of a high level of motor activity in the open field after presentation of pain stimulation (Fig. 2, *B*). Control animals mainly moved around the perimeter of the open field and rarely entered the internal squares (Fig. 3), while operated rats mainly moved within the internal squares of the apparatus.

Neurotensin microinjections into the caudate nucleus of operated rats prior to presentation of pain stimulation led to partial recovery of the reproduction of avoidance reactions, which was apparent as a significant increase in the latency of entry into the dark sector of the chamber (Fig. 2, *A*). Administration of neurotensin also led to recovery of the suppressive effect of pain stimulation on motor activity and, thus, brought values in operated animals closer to those in controls (Fig. 2, *B*).

Figure 4 shows results obtained from studies of the effects of administration of the serotonin 5-HT_{1A} receptor antagonist p-MPPF into the substantia nigra on the effects of pain stimulation and reproduction of the passive avoidance reaction on motor activity in the open field. These results show that control rats displayed a decrease in motor activity immediately after application of the pain stimulus, followed by increases at 24 and 48 h, as compared with baseline. Administration of p-MPPF was followed an increase rather than suppression of motor activity.

Figure 5, *A* and *B* show changes in the reproduction of the avoidance reaction and motor activity after microinjections of neurotensin into the substantia nigra given 24 h after pain stimulation before the first test of the avoidance reaction. Figure 5, *A* shows that this neurotensin treatment had no marked effect on the latent period of excursions to the dark sector of the chamber. Rats of both groups showed persistence of the suppressive effect of pain stimulation on motor activity (Fig. 5, *B*), while neurotensin administration was followed by increases.

DISCUSSION

These studies showed that lesioning of 5-HT structures of the substantia nigra by local administration of the selective neurotoxin 5,7-dihydroxytryptamine led to impaired reproduction of a passive avoidance reaction. This impairment was accompanied by behavioral activation in operated animals, apparent as retention of a high level of motor activity in the open field immediately after presentation of the pain stimulation; operated animals differed from controls in that they moved less around the perimeter of the open field than in the internal squares of the apparatus. This effect of toxin administration may be linked with activation of dopaminergic neurons as a result of weakening of the reciprocal influences of 5-HT structures. This appears also to be supported by evidence that after administration of the serotonin 5-HT_{1A} receptor antagonist p-MPPF into the substantia nigra, the suppressive effect of pain stimulation on motor activity was replaced by an increase.

Avoidable/controllable and unavoidable/uncontrollable stresses are known to elicit opposite responses from the dopaminergic system. This is indicated, for example, by the fact that presentation of series of controllable or conditioned reflex electrical stimuli to the feet of mice increased dopamine release in the nucleus accumbens, while presentation of uncontrollable stimulation decreased dopamine release [11]. The importance of the dopaminergic system

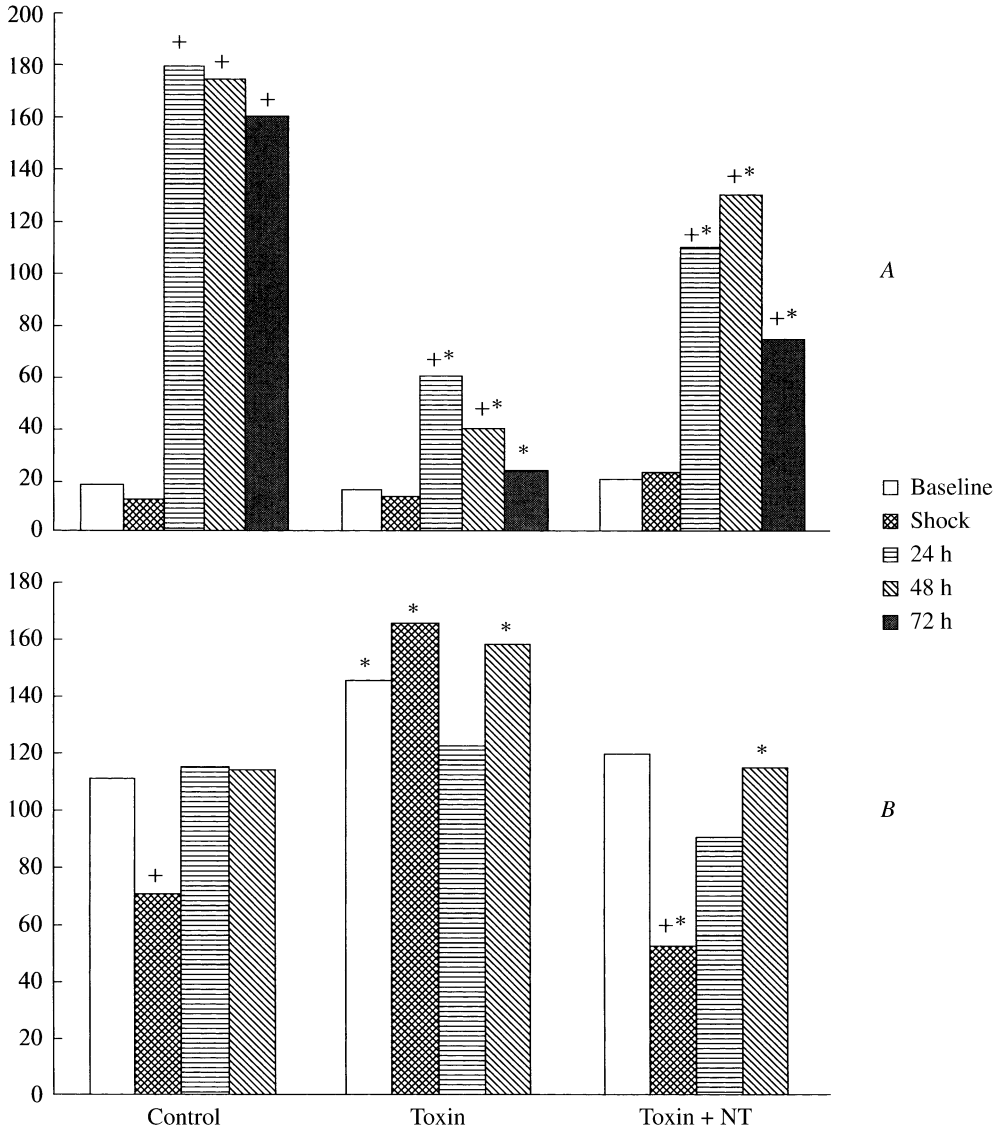


Fig. 2. Reproduction of passive avoidance reactions (*A*) and motor activity in the open field (*B*) in control (Control) rats, rats after administration of 5,7-dihydroxytryptamine into the substantia nigra (Toxin), and after administration of neurotensin into the caudate nucleus in rats with toxic lesions to the substantia nigra (Toxin + NT). *A*) Latency (sec) of transfer of rats from the light sector to the dark sector during training (Baseline), before presentation of pain stimulation (Shock), and 24, 48, and 72 h after pain stimulation (24 h, 48 h, 72 h). *B*) Numbers of squares crossed in 3 min during training (Baseline), immediately after presentation of pain stimulation (Shock), and after presentation of pain stimulation at 24 and 38 h (24 h, 48 h). +Significant differences from baseline; *significant differences between groups, $p \leq 0.05$.

for reproduction of the passive avoidance conditioned reflex is evidenced by results of studies of neuropharmacological actions on dopamine D_2 receptors [1].

We have previously demonstrated [6] that impaired reproduction of passive avoidance reactions occurs in rats after administration of neurotensin into the substantia nigra. This impairment is also accompanied by weakening of the suppressive effect of pain stimulation on motor activity. On the other hand, recovery of passive avoidance reactions in operated rats after administration of neurotensin into the caudate nucleus was accompanied by a decrease in motor activity to the level seen in control animals.

The differences in the effects of neurotensin can be explained on the basis that its effects at different levels of the brain are mediated by different synaptic mechanisms [9, 23]. In the striatum, the action of neurotensin is associated with increases in the efficiency of the negative feedback mechanism mediated by presynaptic inhibition of dopaminergic neurons. Apart from the presynaptic influence at the level of dopaminergic terminals, neurotensin can also increase gluta-

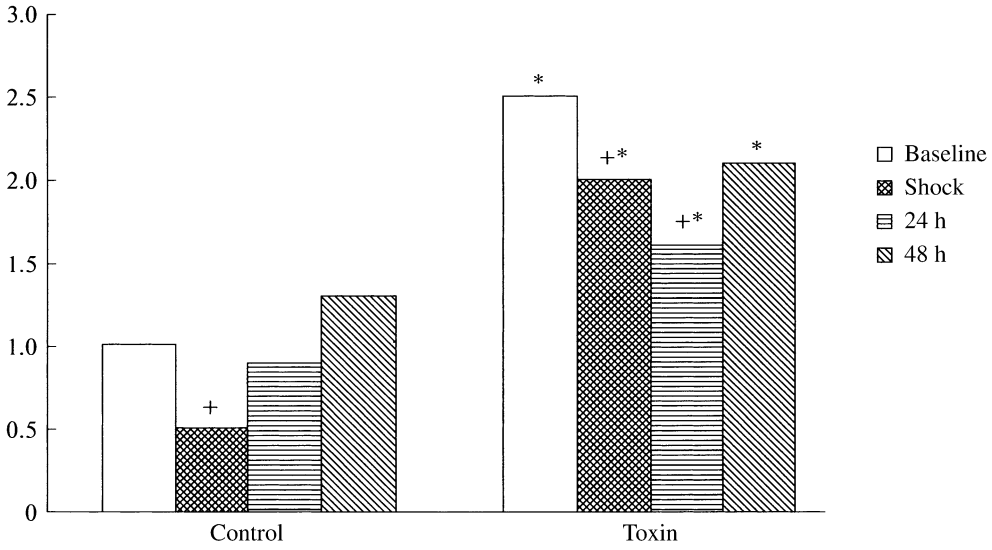


Fig. 3. Effects of pain stimulation and reproduction of the passive avoidance reaction on horizontal motor activity in the internal squares of the open field in control rats (Control) and rats after administration of toxin into the substantia nigra (Toxin). The vertical axis shows the number of internal open field squares crossed (relative units) in relation to the baseline value in control rats. For further details see caption to Fig. 2.

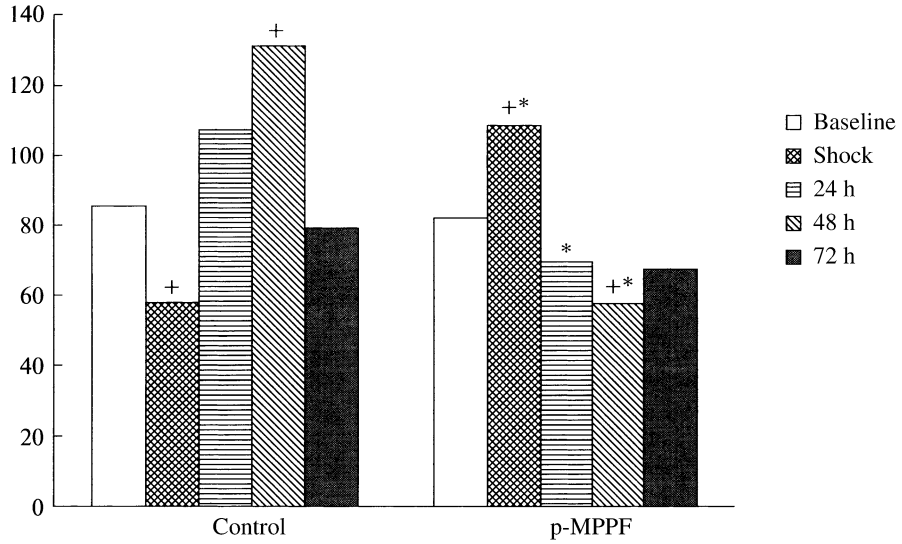


Fig. 4. Effects of pain stimulation and reproduction of the passive avoidance reaction on horizontal motor activity in the open field in control rats (Control) and rats after administration of p-MPPF into the substantia nigra. The vertical axis shows the number of squares crossed in 3 min. For further details see caption to Fig. 2.

mate release in the striatum and gamma-aminobutyric acid release in the substantia nigra [16]. At the level of the substantia nigra, the effect of neurotensin is associated with both the stimulatory action on dopaminergic neuron bodies and blockade of somatodendritic dopamine D_2 autoreceptors, leading to increases in the synthesis and release of dopamine from terminals [15, 23]. On behavioral testing, local administration of neurotensin into this area in our experiments induced increases in motor activity. In addition, administration of neurotensin into brainstem structures increased the reinforcing properties of psychostimulators [28], promoting weakening of the negative emotional state of rats in conditions of defensive behavior.

In addition, the effects of neurotensin may be associated with actions not only on dopamine structures, but also 5-HT structures. Data have been reported [4, 5] providing

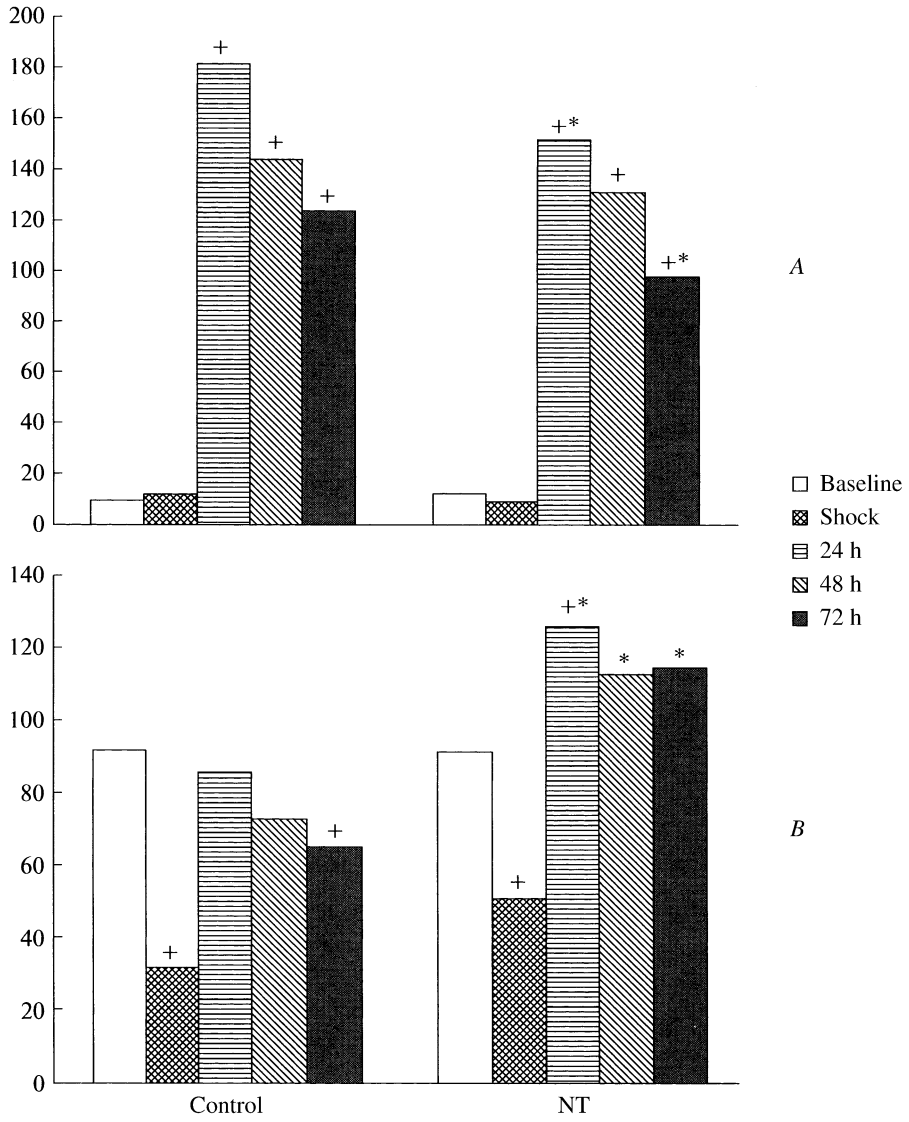


Fig. 5. Reproduction of passive avoidance reactions (*A*) and motor activity in the open field (*B*) in control (Control) rats and rats after administration of neurotensin into the substantia nigra (NT). Vertical axes: *A*) Latency of transfer of rats form the light sector, sec. *B*) Number of squares crossed in 3 min. For further details see caption to Fig. 2.

evidence of the involvement of dopamine and serotonin in regulating two different processes mediating reproduction of conditioned passive avoidance reactions. It is suggested that the involvement of dopamine is more linked with the neuronal mechanisms of the information process determining the behavioral strategy, while serotonin is more involved with emotiogenic memory mechanisms. The role of serotonin in the mechanisms of stress resistance in animals is well known [13, 19]. According to published concepts, reproduction of uncontrollable defensive reactions and their behavioral effects are due to hypersensitivity of serotoninergic neurons in the dorsal raphe nucleus [18, 29]. Studies of neuron activity showed that unavoidable electrical stimulation of the tail in rats led to impairment of 5-HT_{1A} receptor-mediated inhibition of neuron spike activity in the dorsal raphe nucleus. These effects were seen only during the behavioral effects of uncontrollable stress.

Our previous experiments [7] showed that administration of the serotonin 5-HT_{1A} receptor agonist 8-hydroxy-2(di-n-propylamino)tetralin (8-OH-DPAT) into the substantia nigra, like administration of neurotensin, induced sharp weakening of avoidance reactions, while administration into the dorsal raphe nucleus produced the opposite effects. It was also shown that animals given neurotensin into the substantia nigra at the beginning of the training process showed increases in the concentrations of serotonin and its metabo-

lite 5-hydroxyindoleacetic acid in the caudate nucleus of the brain [7]. Published data [4] indicate that these differences in the effects of neurotensin can be explained by the fact that its influence in the substantia nigra can be mediated by actions at the postsynaptic level, while actions in the dorsal raphe nucleus are on somatodendritic 5-HT_{1A} autoreceptors.

According to published data, the passive defensive behavior of animals is mediated by fear mechanisms and reflects the state of anxiety. Studies reported in [2] showed that each level of anxiety corresponds to a particular dynamic of the extinction of passive avoidance reactions. Highly anxious animals were characterized by the absence of extinction of reactions and stable reproduction of memory traces. On this basis it can be suggested that impairments to the reproduction of passive defensive reflexes after lesioning of serotoninergic structures of the substantia nigra appear to reflect weakening of anxiety. This is also indicated by weakening of the suppressive influence of painful electrical stimulation on the motor activity of rats in an open field. Administration of neurotensin into the caudate nucleus before application of pain stimulation countered the effects of the toxin, thus restoring the animal's reaction to pain stimulation.

On the other hand, an important effect of central administration of neurotensin is its antinociceptive action [14], which can occur without involvement of the opioid system of the brain. Thus, it can be suggested that impairment to reproduction of avoidance reactions may result from this action of neurotensin. However, as shown by our previous data [6, 7], impairment to avoidance reactions occurred after administration of neurotensin both before and after pain stimulation. In other words, the antinociceptive action of neurotensin is not a leading factor in the induced impairment to defensive behavior.

In addition, the present study showed that administration of neurotensin into the substantia nigra 24 h after pain stimulation and before testing the avoidance reaction had no significant effect on reproduction of the reaction. These data appear to provide evidence that the effect of neurotensin on the reproduction and suppression of avoidance reactions are determined by the characteristics of its action on emotional status dominant in animals during application of pain stimulation.

According to the hypothesis that serotonin has a dual role, this monoamine increases anxiety by acting on forebrain structures, but suppresses it via actions in the periacqueductal matter [18, 25]. This leads to the suggestion that the normalizing effect of neurotensin administration on passive avoidance behavior may be associated with selective actions on these 5-HT projections.

CONCLUSIONS

1. Administration of the neurotoxin 5,7-dihydroxytryptamine, selective for 5-HT structures, was found to impair the reproduction of passive avoidance reactions and weakened the suppressive effects of pain stimulation. Administration of the serotonin 5-HT_{1A} receptor antagonist p-MPPF into the substantia nigra had a similar influence on the effects of pain stimulation.

2. Administration of neurotensin into the caudate nucleus of the brain before application of pain stimulation prevented the toxin-induced impairment to passive defensive behavior and its effects on motor activity.

3. Administration of neurotensin into the substantia nigra 24 h after pain stimulation had no marked effect on the passive avoidance reaction but increased motor activity during reproduction.

4. The effect of giving neurotoxin into the substantia nigra of the brain was associated with weakening of the effects of pain stress on motor activity in the animals. The prevention of this effect in rats after neurotensin microinjections in the caudate nucleus may be due to recovery of the neurotoxin-impaired reproduction of the passive avoidance reaction and is explained by normalization of the balance of the interaction between the serotoninergic and dopaminergic systems of the brain.

REFERENCES

1. N. I. Dubrovina, E. V. Popova, and R. Yu. Il'yuchenok, "Compensatory-restorative effects of quinpirole on extinction of a conditioned skill and amnesia in mice with alternative behavioral stereotypes," *Eksperim. Klin. Farmakol.*, **64**, No. 3, 13–16 (2001).
2. N. I. Dubrovina and R. A. Tomilenko, "Characteristics of the extinction of a conditioned passive avoidance reflex in mice with different levels of anxiety," *Ros. Fiziol. Zh.*, **91**, No. 9, 1013–1020 (2005).
3. E. V. Miroshnichenko, A. V. Stavrovskaya, N. P. Shugalin, et al., "Changes in the emotional state of rats on reproduction of passive avoidance reactions after administration of neurotensin into the nucleus accumbens of the brain," *Zh. Vyssh. Nerv. Deyat.*, **60**, No. 6, 738–745 (2010).
4. G. F. Molodtsova, "Metabolism and receptor binding of serotonin in brain structures on reproduction of a conditioned passive avoidance reaction," *Zh. Vyssh. Nerv. Deyat.*, **54**, No. 4, 433–441 (2004).
5. G. F. Molodtsova, "Different roles of dopamine and serotonin in the process of reproduction of a conditioned passive avoidance reaction in rats," *Zh. Vyssh. Nerv. Deyat.*, **56**, No. 2, 242–246 (2006).
6. N. P. Shugalev, A. S. Ol'shanskii, G. Hartmann, and L. Lenard, "Effects of neurotensin on execution of active and passive avoidance motor reactions in rats with lesioned serotoninergic neurons," *Zh. Vyssh. Nerv. Deyat.*, **55**, No. 2, 247–252 (2005).
7. N. P. Shugalev, A. V. Stavrovskaya, A. S. Ol'shanskii, et al., "Serotoninergic mechanisms of the effects of neurotensin on passive avoidance behavior in rats," *Zh. Vyssh. Nerv. Deyat.*, **57**, No. 3, 341–346 (2007).
8. C. S. Alves, R. Andreatini, C. Cunha, et al., "Phosphatidylserine reverses reserpine-induced amnesia," *Eur. J. Pharmacol.*, **404**, 161–167 (2000).
9. T. Antonelli, M. C. Tomasini, K. Fuxe, et al., "Receptor-receptor interactions as studied with microdialysis. Focus on NTR/D2 interactions in the basal ganglia," *J. Neural Transm.*, **114**, No. 1, 105–113 (2007).
10. N. Azmi, C. Norman, C. H. Spicer, and G. W. Bennett, "Effects of a neurotensin analogue (PD149163) and antagonist (SR142948A) on the scopolamine-induced deficits in a novel object discrimination task," *Behav. Pharmacol.*, **17**, No. 4, 357–362 (2006).

11. S. Cabibl and S. Pugi-Allegra, "Opposite responses of mesolimbic dopamine system to controllable and uncontrollable aversive experiences," *J. Neurosci.*, **4**, No. 5, 3333–3340 (1994).
12. K. C. Corley, T. H. Phan, W. P. Daugherty, et al., "Stress-induced activation of median raphe serotoninergic neurons in rats is potentiated by the neurotensin antagonist, SR 48692," *Neurosci. Lett.*, **319**, 1–4 (2002).
13. B. R. Dilts, M. R. Novitzki, T. H. Phan, et al., "Neurotensin inhibits the activation of midbrain – serotoninergic neurons produced by random inescapable sound," *Brain Res.*, **742**, No. 1–2, 294–298 (1996).
14. P. R. Dobner, "Neurotensin and pain modulation," *Peptides*, **27**, No. 10, 2405–2414 (2006).
15. C. S. Fawaz, P. Martel, D. Leo, and L. E. Trudeau, "Presynaptic action of neurotensin on dopamine release through inhibition of D_2 receptor function," *BMC Neurosci.*, **10**, 96 (2009).
16. L. Ferraro, M. C. Tomasini, K. Fuxe, et al., "Mesolimbic dopamine and cortico-accumbens glutamate afferents as major targets for the regulation of the ventral striato-pallidal GABA pathways by neurotensin peptides," *Brain Res. Rev.*, **55**, No. 1, 144–154 (2007).
17. P. S. Goldman-Rakic, "The cortical dopamine system: role in memory and cognition," *Adv. Pharmacol.*, **42**, 707–711 (1998).
18. F. G. Graeff, "On serotonin and experimental anxiety," *Psychopharmacology*, **163**, 467–476 (2002).
19. R. Grahn, M. Will, S. Hammack, et al., "Activation of serotonin-immunoreactive cells in the dorsal raphe nucleus in rats exposed to an uncontrollable stressor," *Brain Res.*, **826**, 35–43 (1999).
20. L. Jennes, W. E. Stumpf, and P. W. Kalivas, "Neurotensin: topographical distribution in rat brain by immunohistochemistry," *J. Comp. Neurol.*, **210**, 211–224 (1982).
21. J. Tolas and G. K. Aghajanian, "Neurotensin and the serotoninergic system," *Prog. Neurobiol.*, **52**, 455–468 (1997).
22. K. László, K. Tóth, E. Kertes, et al., "The role of neurotensin in passive avoidance learning in the rat central nucleus of amygdala," *Behav. Brain Res.*, **226**, No. 2, 597–600 (2012).
23. M. Legault, P. Congar, and F. J. Michel, "Presynaptic action of neurotensin on cultured ventral tegmental area dopaminergic neurones," *Neuroscience*, **111**, 177–187 (2002).
24. A. H. Li, T. H. Yeh, and P. P. Tan, "Neurotensin excitation of serotonergic neurons in the rat nucleus raphe magnus: ionic and molecular mechanisms," *Neuropharmacology*, **40**, 1073–1083 (2001).
25. S. Maier, B. Kalman, and R. Grahn, "Chlordiazepoxide microinjected into the region of the dorsal raphe nucleus eliminates the interference with escape responding by inescapable shock whether administered before inescapable shock or escape testing," *Behav. Neurosci.*, **108**, 121–130 (1994).
26. G. Paxinos and C. Watson, *The Rat Brain in Stereotaxic Coordinates*, Academic Press, San Diego, 2nd edition (1986).
27. P. Petkova-Kirova, A. Rakovskaya, G. Zaekova, et al., "Stimulation by neurotensin of dopamine and 5-hydroxytryptamine (5-HT) release from rat prefrontal cortex: Possible role of NTR1 receptors in neuropsychiatric disorders," *Neurochem. Int.*, **53**, No. 68, 355–361 (2008).
28. P. P. Rompre, "Psychostimulant-like effect of central microinjection of neurotensin on brain stimulation reward," *Peptides*, **16**, 1417–1420 (1995).
29. R. R. Rozeske, A. K. Evans, M. G. Frank, et al., "Uncontrollable, but not controllable, stress desensitizes 5-HT_{1A} receptors in the dorsal raphe nucleus," *J. Neurosci.*, **31**, No. 40, 107–115 (2011).
30. R. J. Thielen and A. Frazer, "Effects of novel 5-HT_{1A} receptor antagonists on measures of postsynaptic 5-HT_{1A} receptor activation in vivo," *Life Sci.*, **56**, PL163–PL168 (1995).