Experimental Depressive-Pain Syndrome in Rats with Initial Various Anxiety-Phobic Levels: a Behavioral Study

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Modeling of neurogenic pain syndrome by sciatic nerve transection in rats with pronounced dopamine-deficiency-dependent 1-methyl-4-phenyl-1,2,3,6,-tetrahydropyridine-induced experimental depressive syndrome forms a stable state of combined pain and depression, which can be considered as a model of the pain-depressive syndrome. The neurogenic pain syndrome prolongs the state of behavioral depression in rats irrespective of their initial anxiety level. The depressive symptoms can potentiate the severity of pain syndrome. By a number of indices, more pronounced behavioral changes during the development of pain-depressive syndrome occur in initially nonanxious rats.

Key Words: *pain-depressive syndrome; model; rats*

Numerous clinical studies demonstrated interrelationships between affective states and pain, and specifically, between pain and depression [10,15] and between pain and anxiety, whose clinical manifestations are often similar [8]. Chronic pain can provoke depression and enhance anxiety [13], while depression promotes the onset of various pains [15]. However, in many respects the mechanisms of interdependence between pain and affective disorders are not clear. Elucidation of this problem needs adequate experimental models.

We previously developed a model of experimental depressive syndrome induced in rats by repeated systemic injection of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), a neurotoxin specific for dopaminergic (predominantly, nigrostriatal) neurons [3]. In animals, depressive syndrome manifests in decreased basic motivations (inhibition of orientation and exploratory activity and moderation of food-procuring and drinking motivation), ahedonia, and behavioral despair. In rats with experimental depression the content of dopamine precursor and (to a lesser degree) the transmitter itself in the caudoputamen complex decreased [5], which means that the syndrome depends on dopamine deficiency.

Our aim was to study the character of the development of the depressive syndrome and neurogenic pain syndrome (NPS) in rats injected with MPTP followed by transection of sciatic nerve (SN) as well as to examine the effect of initial anxiety level on the development of these syndromes.

MATERIALS AND METHODS

Experiments were carried out on 85 male albino Wistar rats weighing 350-450 g. The animals were kept individually under standard vivarium conditions with a natural day-night cycle and food and water *ad libitum*. Two experimental series were carried out by the

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same scheme. After primary assessment of the anxiety-phobic score [6], the rats were subdivided into groups of nonanxious rats with initially low anxiety level (<8 points) and anxious rats with initially high anxiety level (>8 points). The test rats in each group were daily injected with MPTP for 16 days (intraperitoneal dose of 20 mg/kg dissolved in 1 ml/kg saline). The drug (Institute of Pharmacology, Russian Academy of Medical Sciences) was dissolved in physiological saline immediately before use. Controls were injected with physiological saline (0.9% NaCl) according to the same scheme. On day 14 of treatment, the left SN was cut in rats of the test and control groups and the severity of depressive symptoms and neurogenic pain was assessed. The dynamics of depressive syndrome in rats with and without NPS was compared in a special series on rats with intact SN.

The development of depressive syndrome was evaluated by reduction of fluid intake (assessment of motivation level), preference of 10% sucrose over water (assessment of hedonic disorders), duration of immobilization (assessment of behavioral despair), increase in depression index (DI) in the forced swimming test (assessment of biorhythmic disturbances; the presence of the depressive component is characterized by DI>1), and weight loss.

The severity of behavioral depression was assessed in points in each animal individually according to the integral score of depression [2] on day 14 after the start of treatment and on day 14 after discontinuation of the treatment, which corresponded to the stages of pronounced behavioral depression and restoration of behavioral activity in MPTP-treated rats with intact SN, correspondingly. Water intake was assessed daily (35 days in the series with intact SN, 35 days in series I and 37 days in series II) and the preference of sucrose over water was calculated in percents. Assessment of swimming behavior was performed on the 9th -10th days of treatment and on 13th-14th days after its discontinuation. The absence of behavioral depression corresponded to low integral score (0-2). The severity of NPS was assessed on weeks 1, 2, and 3 after SN transection according to the standard scale [4] based on the severity of autotomic lesions in claws, phalanges, and the foot of the operated hindlimb.

The data were processed statistically by paired and unpaired parametric Student's *t* tests, unpaired nonparametric Mann—Whitney *U* test, and paired nonparametric Wilcoxon test using Statistica 5.0 software.

RESULTS

During MPTP injections, the severity of behavioral depression in nonanxious and anxious rats was similar in all series (Table 1). However, during recovery of behavioral activity after discontinuation of MPTP,

TABLE 1. Severity of Depressive Syndrome (Points) in Initially Nonanxious and Anxious Rats Treated with MPTP and Physiological Saline in Combination with Sciatic Nerve Transection during Treatment and after Withdrawal (*M*±*m*)

Experimental series		Anxiety level, points	Severity of depression	
			during treatment	after withdrawal
Intact SN				
nonanxious	MPTP (*n*=8)	5.8±0.5	6.0±1.2^{+}	0.8±0.4^{o}
	0.9% NaCl (*n*=9)	6.2±0.3	0.8±0.5	0.3±0.1
anxious	MPTP (*n*=8)	9.1±0.3*	5.6±0.7^{+}	1.2±0.3^{o}
	0.9% NaCl (*n*=8)	9.7±0.4*	0.4±0.2	0.7±0.3
SN transection, series I				
nonanxious	MPTP (*n*=8)	4.9±0.8	4.0±1.1^{++}	2.8±0.2
	0.9% NaCl (*n*=8)	3.6±0.8	0.6±0.3	1.1±0.5
anxious	MPTP (*n*=5)	8.0±0.7*	4.4±1.6^{++}	2.4±1.4
	0.9% NaCl (*n*=3)	9.8±0.9*	0.0	0.7±0.7
SN transection, series II				
nonanxious	MPTP (*n*=8)	5.4±0.6	5.3±1.4^{++}	3.8±0.8
	0.9% NaCl (*n*=9)	5.8±0.5	1.7±0.8	1.9±0.8
anxious	MPTP (*n*=5)	9.8±1.0*	7.0±0.9^{+}	2.8±1.0
	0.9% NaCl (*n*=6)	9.8±0.3*	1.0±0.5	1.3±1.0

Note. *p<0.01 compared to the corresponding nonanxious subgroup (Mann—Whitney *U* test); ^{+}p<0.01, ^{++}p<0.05 compared to the corresponding control (Mann—Whitney *U* test); ^{o}p<0.05 compared to the same subgroup at the previous stage of examination (paired nonparametric Wilcoxon test).

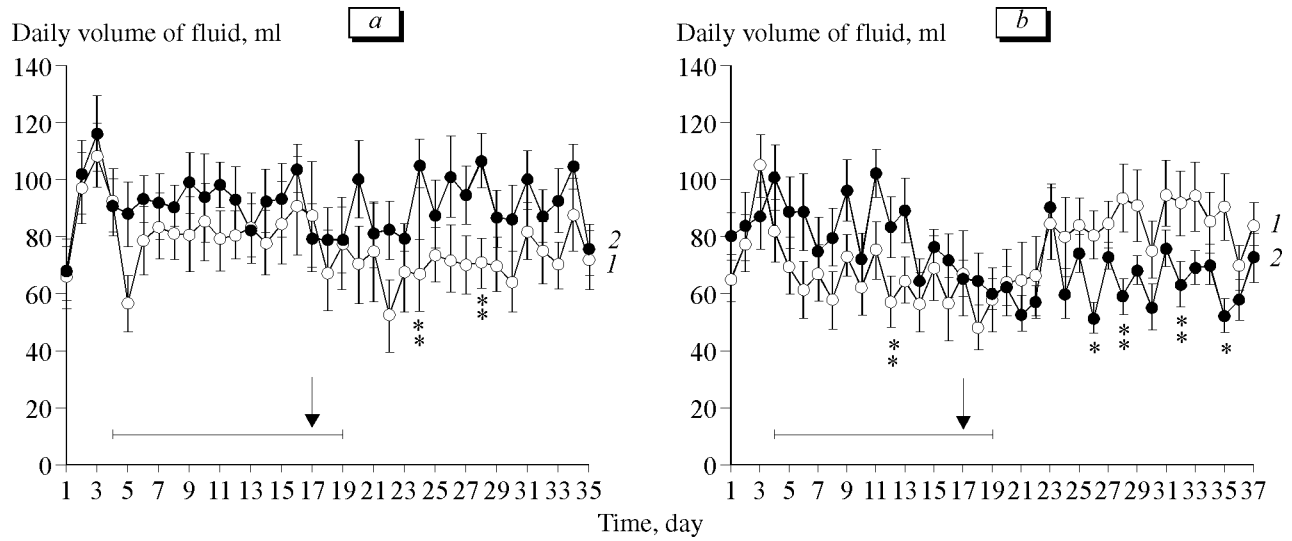

Fig. 1. Daily fluid intake in series I (*a*) and II (*b*) in the groups of initially nonanxious rats with depressive syndrome (*1*) and in controls (*2*). Line shows period of injections and arrow marks the day of sciatic nerve transection. **p*<0.01, ***p*<0.05 compared to the control (unpaired parametric Student's *t* test).

when the behavior of test rats with intact SN returned to normal, severe depression was still observed in nonanxious and anxious test groups with NPS in both experimental series. Thus, irrespective of the initial anxiety level, prolongation of MPTP-induced depressive state was observed in depressive rats with NPS. No depressive syndrome developed in control animals in all series throughout the experiment.

The contribution of various symptoms of the depressive syndrome into prolongation of the depressive state was different. Reduction in fluid intake of various degrees was observed in test rats with intact SN during injections of MPTP followed by a rapid (2-5 days) normalization of fluid intake after discontinuation of the drug [2], which is also observed in this study. No significant changes in this parameter were observed in the test nonanxious rats during injection of MPTP, but on days 5 and 9 after discontinuation of the drug (the period of restoration of behavioral activity) fluid intake decreased (Fig. 1, *a*). Reduction of fluid intake was significant during injection of MPTP to anxious rats in series II, and it was also observed on days 1, 2, 5, and 11 after discontinuation of treatment. It should be noted that reduced in fluid intake was also observed in control nonanxious rats in series II on days 7, 9, 13, and 16 after discontinuation of physiological saline injections (Fig. 1, *b*), which means this symptom of the depressive syndrome developed in control nonanxious rats with NPS. However, the severity of behavioral depression assessed by the integral score did not significantly increase in this subgroup (Table 1).

In series II in nonanxious test rats DI remained high during recovery of behavioral activity (Table 2), while in the test groups in other series at this stage it did not significantly surpass 1. Thus, the severity of depressive syndrome assessed by DI during recovery was higher in series II in nonanxious rats with developing NPS compared to anxious rats. In control rats in all series ID did not surpass 1 throughout the observation period.

The parameters of preference of sucrose over water, the duration of immobilization in the forced swimming test, and weight loss revealed no any significant differences between the rats of control and test groups, and also between nonanxious and anxious rats of the corresponding groups.

These data indicate that NPS prolongs the state of behavioral depression in the rats with dopamine-deficient-dependent depressive syndrome irrespective of initial anxiety level. At the same time, the nonanxious rats are more inclined to behavioral depression during the development of NPS than the anxious rats.

Three weeks 3 after SN transection, the severity of pain syndrome in series I depressive rats (not subdivided according to the initial anxiety level) was higher than that in control rats (8.1±1.0 and 3.5±1.1 according to *U*-test, correspondingly). This phenomenon was not observed in series II (7.2±1.4 and 6.3±1.2, correspondingly), probably due to more intensive autotomy in control rats. This difference can be explained by the appearance of depressive symptoms in control rats of this series. The data show that the development of depressive symptoms can potentiate pain syndrome.

When the rats were subdivided into the groups according to the initial anxiety level, it was found that during 3 weeks after SN transection, the severity of

pain syndrome in initially nonanxious series I depressive rats was higher than in the corresponding control rats ($p<0.05$ during entire observation period). A pronounced tendency to potentiation of autotomy was observed in initially anxious depressive rats only 3 weeks after SN transection (Fig. 2). In series II, autotomy was similar in nonanxious and anxious rats of test and control groups throughout the observation period. It seems that initial anxiety level can determine the development of pain syndrome in the animals with experimental behavioral depression.

The pathogenic mechanisms underlying the revealed dependence in the development of depressive and pain syndromes in animals on initial anxiety level are unknown. However, there are clinical and experimental data indicating that the initial anxiety level is determined by receptor density and sensitivity in some cerebral neurotransmitter systems [11,14]. Stressed rats with different anxiety level assessed by the reaction to novel environments demonstrated variations in gene expression in some cerebral structures (hippocampus, rhinencephalon, orbital cortex, cingulate gyrus, dorsal striatum, and paraventricular nucleus of hypothalamus), which reflects peculiarities of their CNS [12].

We previously showed that the development of experimental MPTP-induced depressive syndrome is associated with hyperactivation of some structures in the striatum of rat brain [1]. These structures contain neurons responsible for the nociceptive response of various modalities [9]. It is possible that during the development of depressive syndrome, striatum hyperactivation produces different plastic changes in striatal neurons mediating the nociceptive response in initially nonanxious and anxious rats.

Both clinical and experimental data revealed a close interdependence in the changes of dopaminergic

TABLE 2. Depression Index in Initially Nonanxious and Anxious Rats with MPTP-Induced Depressive Syndrome and Depression-Free Rats Treated with Physiological Saline in Combination with Sciatic Nerve Transection during Treatment and after Withdrawal ($M\pm m$)

Experimental series	During injections	After drug discontinuation
Intact SN		
nonanxious		
MPTP (n=8)	2.1±0.5**	0.6±0.2^{+}
0.9% NaCl (n=9)	0.8±0.3	0.3±0.1
anxious		
MPTP (n=8)	2.2±0.3*	1.1±0.4^{++}
0.9% NaCl (n=8)	0.5±0.1	0.7±0.2
SN transection, series I		
nonanxious		
MPTP (n=8)	2.5±0.7*	1.0±0.2**
0.9% NaCl (n=8)	0.3±0.1	0.3±0.2
anxious		
MPTP (n=5)	1.9±0.6**	0.6±0.3
0.9% NaCl (n=3)	0.4±0.2	1.0±0.6
SN transection, series II		
nonanxious		
MPTP (n=8)	1.7±0.6	2.3±0.4
0.9% NaCl (n=9)	0.7±0.2	0.6±0.2
anxious		
MPTP (n=5)	1.6±0.5	1.2±0.8
0.9% NaCl (n=6)	0.6±0.3	0.8±0.8

Note. *$p<0.01$, **$p<0.05$ compared to the corresponding control (unpaired Student's t test); $^{+}p<0.01$, $^{++}p<0.05$ compared to the same subgroup at the previous stage of examination (paired Student's t test).

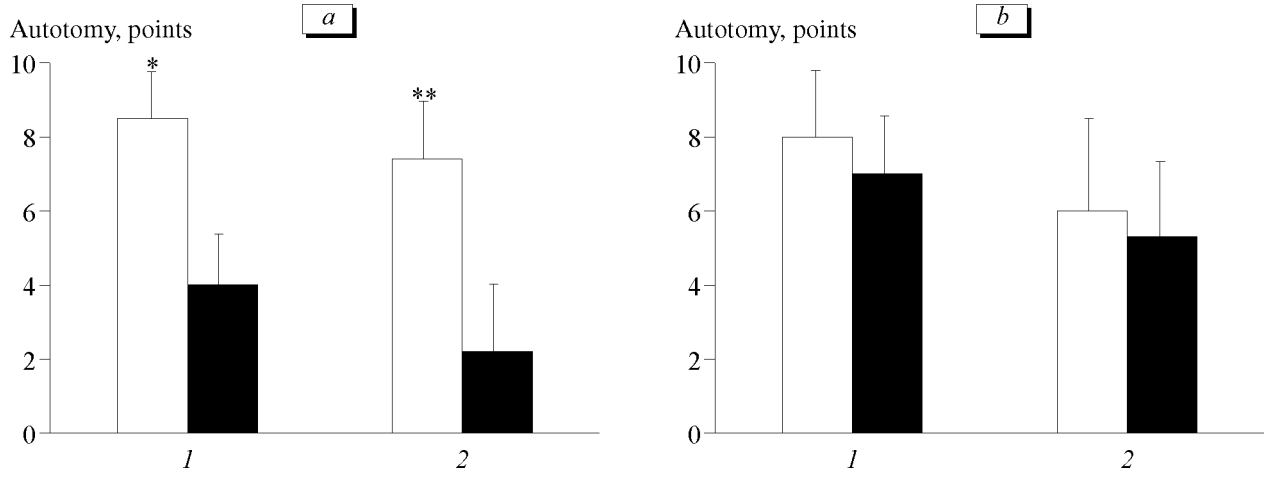

Fig. 2. Autotomy in nonanxious (*1*) and anxious (2) rats with depressive syndrome (light bars) and in control rats (solid bars) 3 weeks after sciatic nerve transection in series I (*a*) and II (*b*). *$p<0.05$, **$p<0.1$ compared to the corresponding control (unpaired nonparametric Mann—Whitney U test).

system activity and nociceptive sensitivity. The central nigrostriatal dopaminergic system plays an important role in integration of nociceptive and sensorimotor functions [9], while mesolimbic system mediates (at least, partially) the persistent tonic pain, and enhancement of dopamine release in this system produces analgesia [7]. Experimental MPTP-induced syndrome is characterized by moderation of functional activity of nigrostriatal dopaminergic system. It can be assumed that one of the mechanisms underlying the interaction between the pain and depressive syndromes in this model is insufficiency of the central dopaminergic systems.

On the whole, these data make it possible to consider the stable combined depression and pain as a model of the depressive-pain syndrome. In addition, the results attest to intermodulating effects between experimental pain and depressive syndromes.

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