METHODS

Experimental Model of Combined Pain and Depression Status in Rats

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Combined pain and depression status in rats was created by inducing experimental depressive syndrome (by subchronic injection of MPTP proneurotoxin) in animals with manifest and developing neurogenic pain syndrome induced by preliminary crossing of the sciatic nerve in the hind limb. The neurogenic pain syndrome augmented by some parameters the depressive symptoms and provoked manifestation of signs of depressive behavior in animals treated with saline.

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

Patients with chronic pain often have mental disorders, the most incident of them are depression and pathologically enhanced anxiety [11]. It is assumed that co-existing pain and depression mutually potentiate each other [1]. Mutual effects of pain and anxiety on each other in patients were described not once [12]. However, the cause—effect relationships between affective disorders and pain remain unclear.

We previously showed that the order of treatments in the process of creation of the model of combined depression and pain status in rats largely determined the pattern and severity of the developing shifts. Mutual effects of depression and pain during the development of combined depression and pain status are more pronounced, if the neurogenic pain syndrome (NPS) develops against the background of pronounced depressive syndrome (DS), than in case when DS is induced in the presence of developing NPS [3,4]. We hypothesized that the stage of development of the primary induced neuropathological syndrome, during which the second induced syndrome starts to develop, is essential for the final result.

We studied the development of combined pain and depression status in rats under conditions of DS induction against the background of manifest NPS, induced by preliminary crossing of the sciatic nerve.

MATERIALS AND METHODS

Experiments were carried out on male Wistar rats (350-450 g) in accordance with Regulations of Laboratory Practice in the Russian Federation, approved by the Order No. 267 of Ministry of Health of the Russian Federation of June 19, 2003. The animals were kept under standard vivarium conditions individually at natural light with free access to water and food.

Two series of experiments (n=38 and n=36) were carried out. Similar results were obtained in both, and hence, we present here the united results of the

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two series. The sciatic nerve was crossed in all rats. The dynamics of NPS development was scored by autotomy parameters (involvement of the claws, phalanges, and soles of the operated on limb) using a conventional scale [7] 2 days after surgery and then weekly throughout the experiment. Three weeks after sciatic nerve crossing, the animals were distributed into groups with (n=56) and without autotomy (n=18). Dopamine deficiency-dependent DS was induced by injection of MPTP pro-neurotoxin (synthesized at Institute of Pharmacology) [5]. Some animals with autotomy (group 1; n=38) and without autotomy (group 2; n=13) were intraperitoneally injected with MPTP in a dose of 20 mg/kg (1 ml/kg) daily for 2 weeks. Other animals with autotomy (group 3; n=18) and without autotomy (group 4; n=5) received saline according to the same protocol.

The development of DS was evaluated by hedonic disorders in the sucrose preference test (reduction of preference), by reduction of vital motivation levels (drinking motivation evaluated by reduced daily consumption of fluid and alimentary motivation evaluated by body weight loss as an indirect indicator), and by the development of "behavioral despair" (prolongation of immobilization periods) and biorhythmological disorders (increase of depressiveness rhythmological index) in the forced swimming test [5]. Normally the index of depressiveness does not exceed 1 and duration of immobilization is ≤20 sec [2]. Relative sucrose consumption was additionally evaluated every day in percent of body weight by the formula:

Sucrose drunk, g/Body weight, g×100% [10].

Experimental and control groups before MPTP and saline treatment were similar by initial levels of anxiety, motor activity, fluid consumption, sucrose preference to water, threshold painful reaction, and body weights.

Anxiety was evaluated by a specialized score for evaluation of anxiety and phobia; motor activity was evaluated by standard open field test [9] sciatic nerve crossing, 1 and 2 weeks after the start of MPTP or saline treatment, and 2 weeks after it. Pain sensitivity (painful reaction threshold) was evaluated in the "hot plate" (55°C) test by the latency of the first escape reaction (licking of the fore or hind paw) nerve crossing and then weekly. The development of NPS and DS signs was observed over 12 weeks after axotomy.

The results were statistically processed using nonparametric Kruskal–Wallis unifactorial analysis of dispersions (H test) and subsequent post-hoc analysis by Mann–Whitney U test. Changes within the groups were evaluated by ANOVA analysis of dispersions for repeated measurements (F test; post-hoc analysis by Duncan test) and nonparametric paired Wilcoxon test. The critical level of statistical significance for rejection of the null hypotheses was 0.05.

RESULTS

Sciatic nerve crossing led to the development of NPS in 76.5% operated rats. The dynamics of NPS development over 3 weeks after surgery was similar in animals later included in groups 1 and 3. By the time of MPTP and saline treatment, the mean autotomy score was 5.6±0.7 and 5.3±0.8, respectively.

High mortality was observed in groups 1 and 2 during treatment (47.4 and 38.5% of initial number of animals). In group 1, 19 rats died during MPTP treatment and one rat on day 9 after MPTP was discontinued (Fig. 1, *a*). In group 2, 5 animals died on days 1-3 of MPTP treatment. Hence, 4 subgroups were formed: 2 subgroups consisted of animals dead at various stages of the experiment (1A, n=20, and 2A, n=5) and 2 subgroups of survivors (1B, n=18, and 2B, n=8). Differences in the dynamics of NPS development in subgroups 1A and 1B were detected. The severity of autotomy in subgroup 1A was lower on days 4-9 after sciatic nerve crossing than in subgroup 1B. The progress of autotomy on days 9-21 was similar in these subgroups. Autotomy progressed more intensely after the start of treatment in subgroup 1A: on day 10 (day 31 after nerve crossing) autotomy in subgroup 1A animals was higher than in subgroup 1B (p<0.00, U test; Fig. 1, *b*). Animals of subgroups 1A and 1B did not differ from group 3 rats by autotomy severity at all terms of the study. It is noteworthy that animals of subgroups 1A and 2A did not differ significantly from animals of subgroups 1B and 2B by the initial behavioral characteristics.

Sucrose preference decreased in animals treated with MPTP in comparison with the initial level: from day 4 of treatment ($F_{(14.238)}$=16.374, p=0.000 and $F_{(11.55)}$=3.029, p=0.003) in subgroups 1A and 1B and from day 6 of treatment in subgroup 2B ($F_{(14.98)}$=5.874, p=0.000). Sucrose preference in subgroup 1B remained low after treatment compared to initial level over 9 days of observation ($F_{(15.255)}$=8.658, p=0.000). In subgroup 2B, sucrose preference remained low for only 2 days after MPTP discontinuation ($F_{(15.105)}$=4.603, p=0.000). No reduction of sucrose preference to water was detected in groups 3 and 4 (Fig. 2).

Sucrose preference in subgroup 1B was lower than in group 3 during treatment (days 7-14) and after it (days 1-2): the maximum value H(3,N=49)=15.996, p=0.001 on day 1 after treatment discontinuation; the minimum value H(4,N=58)=9.902, p=0.042 on day 9 of treatment. In subgroup 2B, reduction of sucrose preference in comparison with group 4 reached the

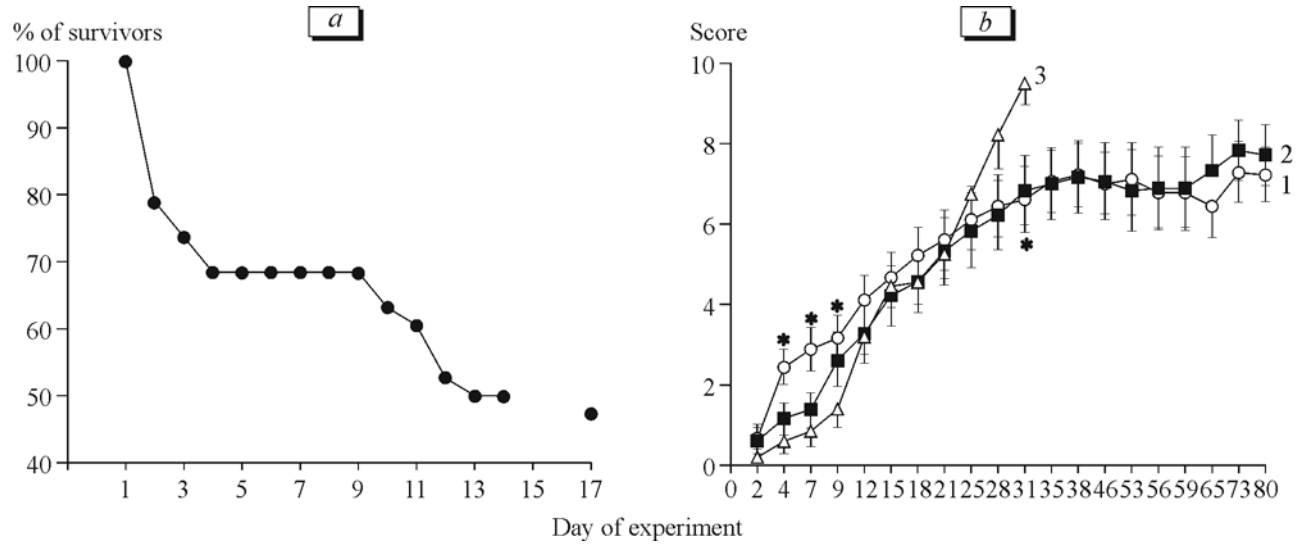

Fig. 1. Mortality (*a*) and intensity of autotomy (*b*) after induction of experimental DS in the presence of NPS formed after sciatic nerve crossing. *a*: group 1; *b*: subgroup 1B (*1*), group 3 (*2*), subgroup 1A (*3*). **p*<0.05 compared to subgroup 1A.

level of statistical significance on days 11-13 of treatment, but not after it (Fig. 2, *a*).

The decrease in daily fluid consumption in comparison with the initial level was detected only in subgroup 1B starting from day 2 of treatment until the end of observation ($F_{(29.493)}$=7.368, p=0.000). A trend to reduction of fluid consumption ($F_{(29.203)}$=1.411, p=0.089) was observed in subgroup 2B. An increase of daily consumption of fluid during ($F_{(14.56)}$=4.089, p=0.000) and after saline injections ($F_{(15.60)}$=4.184, p=0.000) was detected in group 4, while in group 3 fluid consumption did not differ from the initial level. Comparison of the groups revealed no significant differences in this parameter throughout the study.

Animal body weights in subgroups 1A, 1B, and 2B decreased significantly during MPTP treatment in comparison with the initial values. This parameter returned to normal after drug discontinuation in subgroups 1B and 2B (Fig. 3). Body weight did not change during saline injections in group 3 and increased in group 4. After discontinuation of saline treatment, body weights in these groups increased in comparison with the initial level. Changes in body weights in subgroups 1A, 1B, and 2B differed from those in groups 3 and 4 for all four periods of the study ($F_{(4.53)}$=14.490, p=0.000; $F_{(4.47)}$=4.294, p=0.005; $F_{(4.44)}$=10.337, p=0.000; $F_{(3.45)}$=4.959, p=0.005, respectively). Body weight gain in group 4 after 1 week of treatment was statistically more pronounced than in group 3.

Sucrose consumption in subgroup 1B decreased in comparison with the initial value at all terms of observation ($F_{(4.68)}$=17.281, p=0.000; Table 1), while in subgroup 2B it decreased only during week 1 of MPTP treatment ($F_{(4.28)}$=2.987, p=0.034). Sucrose consumption in these subgroups increased after MPTP discontinuation. In subgroup 1A, sucrose consumption decreased after 1 week of MPTP treatment ($F_{(1.8)}$=8.863, p=0.018). In groups 3 and 4, no appreciable changes in this parameter were noted. Sucrose consumption in subgroup 1B was lower than in group 3 during treatment: $H(3N=49)$=9.246, p=0.026 after 1 week of treatment; a pronounced trend to reduction was detected after 2 weeks: p=0.059 (U test)).

Forced swimming test showed greater number of active swimming periods in subgroups 1B and 2B during treatment in comparison with groups 3 and 4

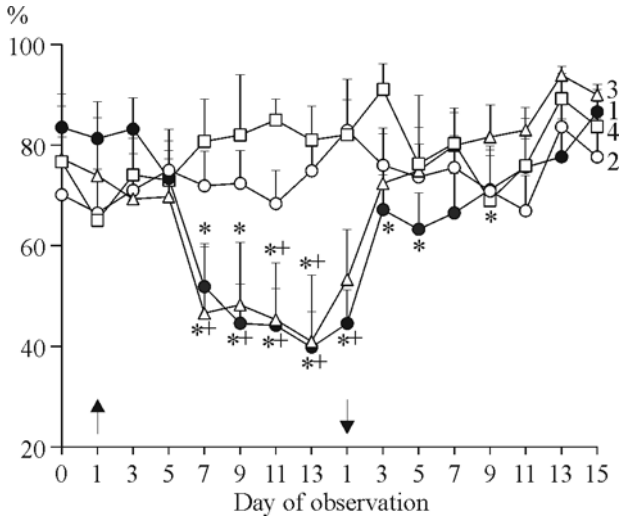

Fig. 2. Dynamics of sucrose preference in experimental and control rats. *1*) subgroup 1B; *2*) group 3; *3*) subgroup 2B; *4*) group 4. Arrows show the start and end of treatment. p<0.05 compared to: *initial values; +corresponding control group.

TABLE 1. Sucrose Consumption (%) in Experimental and Control Groups (*M*±*m*)

Group	Before treatment	Treatment		After treatment	
		week 1	week 2	week 1	week 2
1B	20.2±2.3	7.2±1.3*°	7.5±1.7*	14.2±1.6*ˣ	15.9±1.8*⁺ˣ
3	14.0±2.8	13.1±1.6	12.7±1.9	13.9±2.0	11.1±2.1
2B	16.2±3.8	7.9±2.6*	10.8±4.5	14.7±3.2⁺	15.1±2.7⁺
4	7.6±2.2	10.3±2.6	8.2±1.3	9.3±1.2	8.6±2.1
1A	16.7±2.8	12.8±3.2*	—	—	—

Note. $p<0.05$ compared to: *initial level; ⁺level after 1 week of MPTP treatment; ˣlevel after 2 weeks of MPTP treatment; °group 3.

animals ($H(3,N=49)=10.727$, $p=0.013$; $p<0.05$ by U test in both cases). A trend to longer active swimming was observed in experimental groups compared to controls ($p<0.08$ by U test; Table 2). After treatment discontinuation, a significantly longer duration ($H(3,N=49)=9.012$, $p=0.029$) and greater number of active swimming periods ($H(3,N=49)=9.009$, $p=0.029$) in comparison with the control were observed only in the groups with autotomy ($p<0.05$ by U test in both cases). The duration of immobilization and index of depressiveness in experimental and control animals did not differ during all periods of the study and were within the normal range of values.

The hot plate test showed reduction of painful reactions threshold compared to the initial values only in subgroup 1B and in group 3 during all periods of the study (Table 3). A trend to elevation of painful reaction threshold was noted in subgroups 1A and 2A in comparison with subgroups 1B and 2B 1 week after sciatic nerve crossing.

The level of anxiety virtually did not change in any of the groups throughout the entire experiment.

Changes in motor activity differed in the groups injected with MPTP and saline. Motor activities of animals in subgroups 1B and 2B virtually did not change during MPTP treatment and deareased after its discontinuation ($p<0.005$ by Duncan's test in both cases). Motor activities of rats in groups 3 and 4 decreased during saline treatment and remained low after it ($p<0.005$ by Duncan's test in all cases).

High mortality was observed in groups treated with MPTP starting from week 3 after sciatic nerve crossing compared to just rare deaths in groups without nerve crossing. No mortality was observed in our previous study with MPTP injected on day 3 after nerve crossing [4]. High mortality observed in the present study could be caused by higher sensitivity of rats to MPTP during delayed periods after sciatic nerve crossing, presumably due to neuroplastic and neurochemical changes in the CNS [8] developing as a result of injury to the peripheral compartments of the nervous system [13].

The data indicate that the course of experimental dopamine deficiency-dependent DS in rats, caused by repeated systemic treatment with MPTP pro-neurotoxin 3 weeks after sciatic nerve crossing, differed in animals with and without NPS. The depressive symptoms were more pronounced during MPTP treatment

TABLE 2. Characteristics of Swimming Behavior in the Forced Swimming Test in Experimental and Control Groups (*M*±*m*)

Group	Duration of active swimming, sec		Number of active swimming periods	
	during treatment	after treatment	during treatment	after treatment
1B	184.2±17.1	206.4±20.6*	10.9±0.7*	11.1±1.0*
3	142.0±22.8	129.2±15.2	8.6±1.0	7.8±0.7
2B	163.8±24.6	178.5±25.6	11.40.7⁺	10.3±1.8
4	94.0±20.2	133.8±27.5	6.6±1.0	6.6±1.4

Note. $p<0.05$ compared to: *group 3; ⁺group 4.

in animals with NPS, judging from reduced drinking motivation and ahedonism (stable reduction of sucrose consumption). The recovery of hedonic behavior was slower in rats with NPS after MPTP treatment (judging from reduced sucrose preference and reduced sucrose consumption) and signs of disadaptation behavior persisted in forced swimming test (higher duration and number of active swimming periods in comparison with the control).

The results attest to more severe course of DS in rats with NPS, which developed after axotomy in comparison with animals without signs of NPS. In other words, the presence of pain symptoms aggravated the severity of DS developing against the background of these symptoms. NPS impaired body weight gain and prevented the increase in daily fluid consumption during saline treatment, *i.e.* suppressed alimentary and drinking motivation, which is characteristic of experimental DS. These results confirm our previous hypothesis according to which experimental NPS can provoke manifestation of DS in rats [3]. However, we failed to detect the opposite effect (effect of depression on pain symptoms) in the present study: the severity of NPS was statistically the same in the DS and control groups. Reduced pain reaction threshold was observed only in rats with NPS in experimental and control groups. We previously showed that NPS induction in the presence of manifest DS was associated with more intense mutual effects of depression and pain on each other, while after DS induction in the presence of unfolding NPS only the effect of depression on pain development was observed, but not vice versa [3,4]. Analysis of the development of combined pain and depression status simulated by various protocols does not confirm the idea assumed in clinical practice, according to which pain and depression always aggravate each other [1]. Presumably, this mutual aggravation is a result of sustained pain and depression in patients. The prognosis for early stages of combined experimental pain and depression status is determined by the sequence of the syndrome development and degree of initial neuropathological syndrome, during which the second induced syndrome appears.

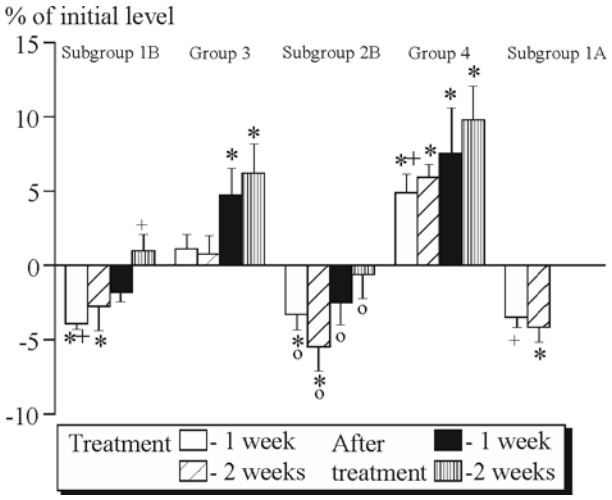

Fig. 3. Body weight changes during treatment and during recovery of behavioral activity after treatment. $p<0.05$ compared to: *initial value; +group 3; °group 4.

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TABLE 3. Painful Sensitivity Threshold (sec) in Experimental and Control Animals ($M \pm m$)

Group	Before sciatic nerve crossing	1 week after sciatic nerve crossing	1 week of treatment	1 week after treatment	3 weeks after treatment
1B	12.3±0.5	7.9±0.7*	8.4±0.8*	9.3±0.6*	9.8±0.9*
3	13.4±0.7	9.6±0.9*	10.1±1.2*	9.3±0.8*	8.3±0.8*
2B	11.7±1.8	9.4±1.1	8.0±1.4	10.1±1.5	11.6±2.1
4	10.6±1.4	10.0±1.7	8.2±1.3	8.8±1.0	7.6±0.8
1A	13.7±0.9	10.3±1.0+	10.3±1.5	—	—
2A	14.0±1.0	13.8±1.5°	—	—	—

Note. *$p<0.05$ compared to initial level; +$p<0.09$ compared to subgroup 1B; °$p<0.09$ compared to subgroup 1B.

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