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Behavioral effects of novel enterosorbent Noolit on mice with mixed depression/anxiety-like state

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Abstract

The aim of this work was to examine the behavioral effects of a novel lithium-based enterosorbent, Noolit (665 mg/kg), on male mice with mixed depression/anxiety-like state evoked by exposure to repeated social defeats in daily agonistic confrontations. The lithium component allows Noolit to be used as a psychotropic drug. Two experiments are described, in which the therapeutic and preventative effects of chronic Noolit treatment were examined. Response to Noolit was assessed in the plus maze, open field, partition test, and Porsolt's test. In both experiments, Noolit produced obvious anxiolytic and antidepressant effects. Treatment with Noolit fully restored some behavioral parameters in the plus maze and open field in depressed mice and prevented depression that would otherwise have developed. It has been suggested that enterosorbent Noolit can be a potent drug for the treatment of mixed anxiety/depression pathologies and for prevention of mood disorders.

Keywords: Noolit; Lithium; Social stress; Defeat; Depression; Anxiety; Sensory contact model

1. Introduction

The study of gastrointestinal sorption as one of the aspects of prevention and treatment of endo- and exotoxicosis-related diseases (reviews, Borodin et al., 1999; Borodin and Rachkovskaya, 2000a) is a research trend in the Institute of Clinical and Experimental Lymphology of the Siberian Division of the Russian Academy of Medical Sciences and its Clinic (Novosibirsk). It has been shown that enterosorbents can be more efficient and more specific when combined with any drug with known therapeutic properties (reviews, Kartel, 1995; Rachkovskaya, 1996; Borodin and Rachkovskaya, 2000a). One of such composite drugs is Noolit, an enterosorbent with lithium ions immobilized on its surface (Borodin and Rachkovskaya, 2000b). It is expected that, moving down the intestinal tract, Noolit will adsorb metabolites, harmful chemicals and microbial cells, prolong the effects of lithium and prevent overdose, which is an often possibility with some antidepressants (Sarko, 2000). On the other hand, Noolit would have the psychotropic properties known for lithium salts from experiments (Kozlovskii and Prakh'e, 1996; Redrobe and Bourin, 1999a) and clinical practice (reviews, Shastry, 1997; Shelton, 1999; Nolen and Bloemkolk, 2000). It has been shown that lithium maintenance therapy (review, Blacker, 1996) is useful for suicide prevention in patients with manic-depressive disorders (Baldessarini et al., 1999). Furthermore, lithium is suggested to be most appropriate initial treatment of the depressive phase of bipolar disorder (review, Compton and Nemeroff, 2000) and is effective in preventing mood disorders (Denicoff et al., 1997; Bowden, 2000; Serretti et al., 2000). Our earlier data indicate that systematic administration of Noolit reduces immobility in intact mice exposed to Porsolt's test (Borodin et al., 2000a). The aim of this work was to investigate the effects that Noolit might have on mice under simulated clinical conditions. In order to accomplish this, a depression-like state was evoked by chronic social conflicts in C57BL/6J strain male mice under the sensory contact model (Kudryavtseva, 1991). As has been earlier shown (Kudryavtseva et al., 1991a; reviews, Kudryavtseva et al., 1995; Kudryavtseva and Avgustino-

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vich, 1998), social defeat repeatedly experienced in agonistic confrontations leads to dramatic changes in social and individual behaviors, as well as welfare. The etiology, response to treatment, and symptomatology of these changes are analogous to those of human depression. A severe behavioral deficit developed in male mice: after experiencing social defeat for 20 days, they could only demonstrate a passive defense and immobile postures, whilst in the beginning they preferred active defense and withdrawal. These submissive mice, or losers, displayed reduced ambulation in the open field, increased immobility time in Porsolt's test and demonstrated no aggression in any, no matter how provoking the situation. Clear-cut anxiety has been revealed in the plus-maze test and disturbed social behavior in the partition test (Kudryavtseva, 1994; Avgustinovich et al., 1997). Weight loss, decreased plasma testosterone level (Kudryavtseva et al., 1995), immune responsiveness (Devoino et al., 1993), and stress reactivity (Kudryavtseva et al., 1991a) have been demonstrated. Repeated treatment with the antidepressants imipramine (Kudryavtseva et al., 1991a) or tianeptine (Kudryavtseva et al., 1995) decreased immobility in Porsolt's test. Chronic ethanol consumption (Kudryavtseva et al., 1991b) and chronic treatment with ipsapirone (Avgustinovich et al., 1999) reduced anxiety, but did not prevent depression. Using a modified sensory contact technique, treatment with citalopram for 3 weeks reduced anxiety in the light–dark test (Keeney and Hogg, 1999). Chronic unavoidable social stress is believed to be a pathogenic factor that leads to a mixed anxiety/depression state in mice (Kudryavtseva and Avgustinovich, 1998).

We propose an approach to elucidate the effects of Noolit, or any other psychotropic drug, on animals with mixed anxiety/depression states evoked by the chronic social conflict under the sensory contact model. The approach is also good at detecting effects of any drug in preventing experimentally inducible depression or anxiety.

2. Method

2.1. Animals

Adult male mice of the C57BL/6J strain maintained at the Institute were used. The animals were housed under standard vivarium conditions and 12:12-h light regime, food (pellets) and water were available ad libitum. One-month males were weaned and housed in one-litter groups of 8–10 in plastic $36 \times 23 \times 12$-cm cages. Experimental mice were 10–12 weeks of age.

2.2. A technique for generation of depression and anxiety in male mice

To induce a depression-like state in mice a model of sensory contact (Kudryavtseva, 1991) was used. Animals were placed by pairs in steel cages ($28 \times 14 \times 10$ cm) divided into halves by a perforated transparent partition that allowed them to see, hear and sense the smell of the neighbor, but not to contact physically. Test sessions commenced 2 days after adaptation of animals to these new housing conditions (sensory contact). Every afternoon (14:00–17:00 h local time), the steel cover of the cage was replaced by a transparent one, and 5 min later (the period necessary for individuals' activation and habituation to new lighting conditions) the partition was removed for 10 min to allow agonistic interaction. Undoubted superiority of one of the partners was evident within two to three tests in daily social encounters with the same opponent. One partner was seen to attack, bite and chase the other, which only displayed defensive behavior (sideways, upright postures, withdrawal, lying on the back or "freezing"). As a rule, aggressive confrontations between males are discontinued by lowering the partition if the intensive attacks lasted more than 3 min. Then, every day after the test, each defeated member of one pair was paired with the winning member of another pair behind the partition in an unfamiliar cage. The winners were left in their own compartments. The procedure yielded equal numbers of males with an opposite social experience of aggression, accompanied by victories (winners, aggressors) and defeats (defeated mice, losers) in agonistic confrontations. Males after 5 days of individual housing were used as the control. They were thought to be the best as intact controls in the sensory contact model because, in this case, the submissiveness of grouped males would be removed, and effects of social isolation would not yet be acquired (Kudryavtseva, 1991).

2.3. Drug

Enterosorbent Noolit has a mineral (aluminium oxide) matrix with a pre-set structure (pore size 100–1000 Å, specific area up to 300 m^2/g), with a lithium-containing agent fixed to its surface by physical adsorption (Borodin and Rachkovskaya, 2000b). It exists as tasteless, odorless round-shaped white granules less than 1 mm in size. Noolit (665 mg/kg) as a suspension in 0.3 ml 2% starch gel or 0.3 ml 2% starch gel alone (vehicle, "placebo") was carefully injected by a trained investigator per os into the gullet using a syringe and a large-diameter needle with rounded edges. Each session started at 9 a.m., took 5–8 s, and was run daily during the treatment period.

2.4. Behavioral tests

2.4.1. Partition test

The partition test (Kudryavtseva, 1994) is an analogue of the social interaction test widely used for measurement of anxiety in animals (File and Hyde, 1978). The partition test was employed as a tool for estimation of behavioral reactivity of mice to the conspecific behind the transparent perforated partition dividing the experimental cage

($28 \times 14 \times 10$ cm) into halves. The number of approaches to the partition and the total time (seconds) spent near it (moving near the partition, smelling and touching it with one or two fore paws, clutching and hanging on it, putting noses into the holes or even gnawing it) were scored as indices of a reaction to the neighbor. The periods of time over which the males demonstrated the sideways position or "turning away" near the partition were not put on the record. The ratio of the total time of behavioral activity near the partition to the number of approaches yields average time spent near the partition at one approach (seconds).

After a 5-min period of activation (the steel cover of experimental cage was replaced by a transparent one), the behavioral reaction of losers to familiar partners (winners, after 1 day of joint living with him in a common cage through partition) in the neighboring compartment was videotape recorded during 5 min. Then, the familiar partner was replaced by an unfamiliar one (grouped-housed male) and behavioral reaction to the unfamiliar partner was recorded during the next 5 min.

2.4.2. Elevated plus-maze test

The elevated plus-maze is a traditional test for estimating a drug's anxiolytic effects (Lister, 1987). The maze consists of two open arms (25×5 cm) and two enclosed arms ($25 \times 5 \times 30$ cm), arranged so that the two arms of each type were opposite each other and extended from a central platform (5×5 cm). The floor and walls of the maze were constructed from gray opaque Plexiglas material. The maze was elevated to a height of 50 cm above the floor. All measurements were taken in a dimly lit experimental room.

The experimental cage with a mouse was placed in the same room 5 min (period of activation) before exposure to the maze. Over testing period the following conventional measures of plus-maze behavior (Rodgers and Cole, 1994) were recorded during 5 min: (1) open arm time, enclosed arm time and central platform time as a percentage of total testing time; (2) open arm entries, enclosed arm entries and central platform entries as a percentage of total entries; (3) total entries; (4) the number of head dips and (5) the number of passages from one enclosed arm to another were measured. The plus maze was thoroughly cleaned between sessions.

2.4.3. Open-field test

Open-field observations were carried out in a 100×100-cm Plexiglas open field divided into 9×9-cm squares. It was illuminated by a 150-W electric bulb 100 cm above the open-field floor. The experimental cage with mice was placed in the same room 5 min before exposure to the open field (period of activation). After that, each mouse was placed individually in the center of the open field and the following behavioral measures were recorded over a 5-min test period: (1) the number of crossed squares; (2) latency (seconds) to first escape from the center; (3) the number of rearing; (4) defecation — total number of fecal boli. Between sessions the open field was thoroughly washed and dried with napkins.

2.4.4. Porsolt's test (Porsolt et al., 1977)

After 5 min of activation, each mouse was placed in a glass (20 cm height, 9 cm inner diameter) containing water (9 cm of glass height) at $t = 25 \pm 1$ °C for a 5-min period. The total duration of full immobility (seconds) was recorded.

2.5. Experimental design

2.5.1. Experiment 1. Therapeutic effects of Noolit on mice with mixed anxiety/depression state

Twenty-day agonistic confrontations under the sensory contact model evoked a mixed anxiety/depression state in the losers. Then the fighting was stopped, and a perforated transparent partition, which prevented any physical contact, was put down permanently between the losers and winners. At that point, Noolit was administered to half of the losers (Noolit-treated losers, or NTL in figures), and half received starch gel (vehicle-treated losers, or VTL in figures) for 18 days. The animals were exposed to the partition test on Day 35, the plus-maze on Day 36, the open field on Day 37 and Porsolt's test on Day 38. Testing commenced at 2–5 p.m. Animals with a history of 5-day individual housing were used as controls.

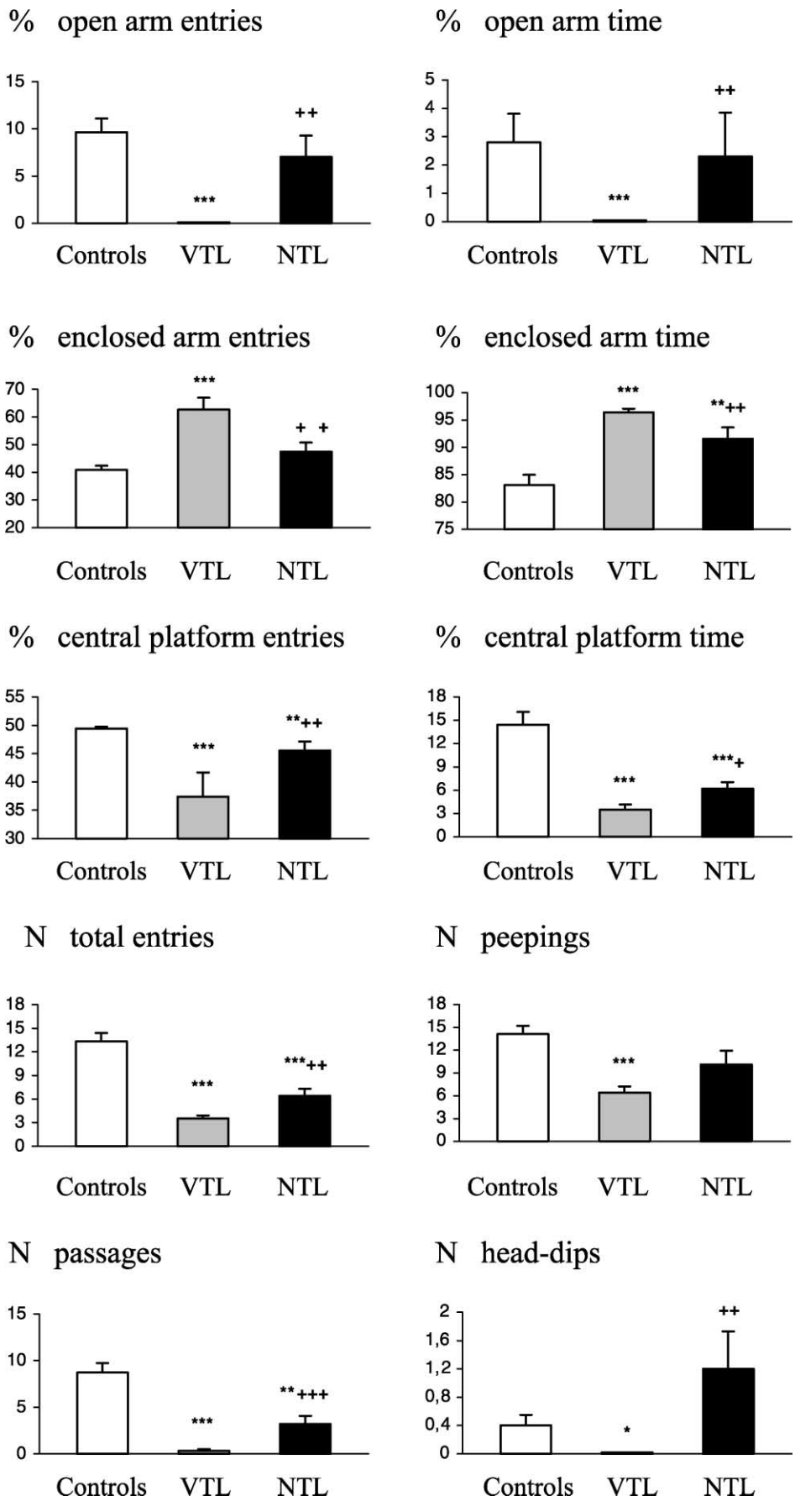
2.5.2. Experiment 2. Noolit in preventing of anxiety and depression in mice

On Day 6 of social confrontations, half of the losers were treated with starch gel (vehicle), and half with Noolit and starch gel. Treatments and agonistic confrontations continued from Day 6 through Day 24. The losers of both groups were exposed to the partition test on Day 21, plus-maze on Day 22, open field on Day 23, and Porsolt's test on Day 24. Testing commenced at 2–5 p.m. Animals with a history of 5-day individual housing were used as controls.

All procedures were in compliance with European Communities Council Directive of 24 November 1986 (86/609/EEC).

2.6. Statistics

The statistical significance of the differences of the behavioral parameters was tested using two-tailed Mann–Whitney U test. That for the response to the familiar and unfamiliar partner in the partition test was tested using Wilcoxon matched pairs T test. The vehicle-treated mice were compared with the controls to see if a mixed anxiety/depression state established. Noolit-treated mice were compared with the vehicle-treated ones to see if the drug has had any effect. Noolit-treated mice were compared with the control animals to see how effective the Noolit treatment was.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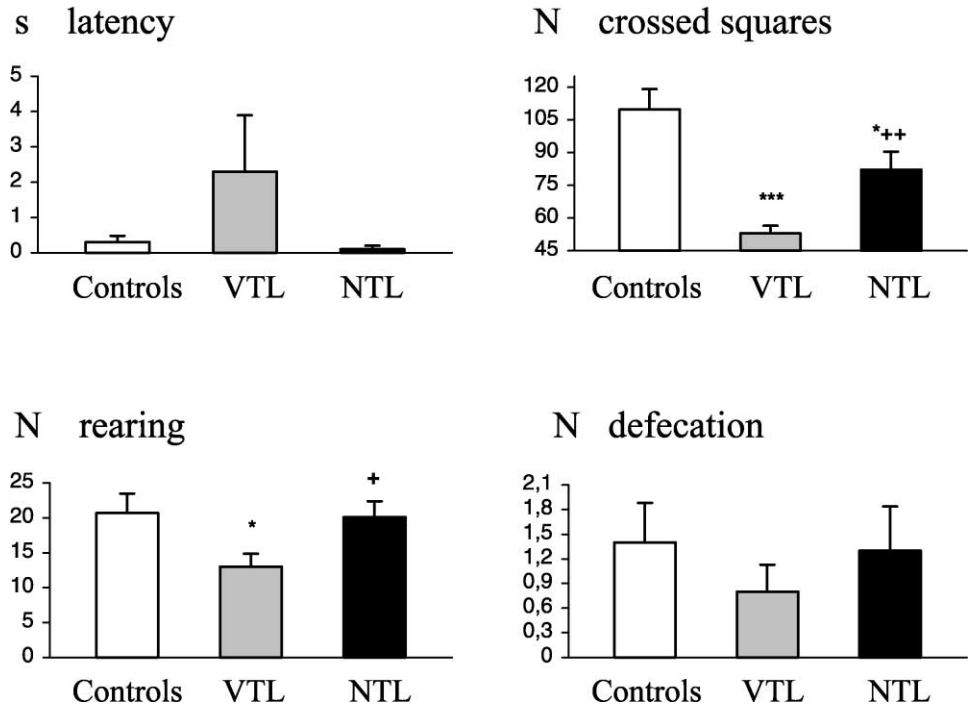

Fig. 2. Therapeutic effects of Noolit on the behavior of male mice in the open-field test. Data are presented as mean ± S.E. VTL, vehicle-treated losers; NTL, Noolit-treated losers. * $P<.05$; *** $P<.001$, differs from the control males. + $P<.05$; ++ $P<-.01$, differs from vehicle-treated losers, *U*-criterion.

Each experimental group comprised 9–11 animals.

3. Results

3.1. Experiment 1. Therapeutic effects of Noolit on mice with mixed anxiety/depression state

3.1.1. Plus-maze test (Fig. 1)

Percentage open arm time ($U=5$; $P<.0001$) and entries ($U=5$; $P<.0004$), percentage central platform time ($U=0$; $P<.0001$) and entries ($U=6$; $P<.0006$), total entries ($U=0$; $P<.0001$), passages ($U=0$; $P<.0006$), levels of peeping ($U=3$; $P<.0002$) and head dip ($U=35$; $P<.04$) were decreased, and the percentage enclosed arm time ($U=0$; $P<.0001$) and entries ($U=0$; $P<.0001$) increased in the vehicle-treated mice compared to the control animals (Fig. 1). Obviously, Noolit reduced anxiety: the percentage open arm time ($U=20$; $P<.005$) and entries ($U=20$; $P<.005$); percentage central platform time ($U=20.5$; $P<.02$) and entries ($U=13.5$; $P<.005$), total entries ($U=17.5$; $P<.01$), passages ($U=11$; $P<.001$), and the level of head dip ($U=25$; $P<.01$) were increased, and the percentage enclosed arm time ($U=17.5$; $P<.01$) and entries ($U=13.5$; $P<.005$) were decreased in Noolit-treated losers compared to the vehicle-treated losers. The level of peeping did not change significantly ($P>.05$).

There were significant differences between the Noolit-treated and control mice in percentage central platform time ($U=8$; $P<.001$) and entries ($U=19.5$; $P<.009$), total entries ($U=11$; $P<.001$), passages ($U=11.5$; $P<.002$) and percentage enclosed arm time ($U=15.5$; $P<.005$). There were no significant differences in percentage open arm time and entries, percentage enclosed arm entries, or levels of head dip and peeping ($P>.05$).

3.1.2. Open-field test (Fig. 2)

Crossed squares ($U=1.5$; $P<.0001$) and rearing ($U=30$; $P<.05$) were decreased in the vehicle-treated losers compared to the control mice (Fig. 2). There were no significant differences in defecation or latency to first escape from the center ($P>.05$). Crossed squares ($U=18$; $P<.015$) and rearing ($U=23$; $P<.04$) were increased in the Noolit-treated losers compared to the vehicle-treated ones. There were significant differences between the Noolit-treated mice and controls in the number of crossed squares ($U=30$; $P<.05$).

3.1.3. Porsolt's test (Fig. 3)

A significantly longer immobility time was observed in the vehicle-treated than control mice ($U=0.5$; $P<.0001$)

Fig. 1. Therapeutic effects of Noolit on the behavior of male mice in the plus-maze test. Data are presented as mean ± S.E. VTL, vehicle-treated losers; NTL, Noolit-treated losers. * $P<.05$; ** $P<.01$; *** $P<.001$, differs from the control males. + $P<.05$; ++ $P<.01$; +++ $P<.001$, differs from vehicle-treated losers, *U*-criterion.

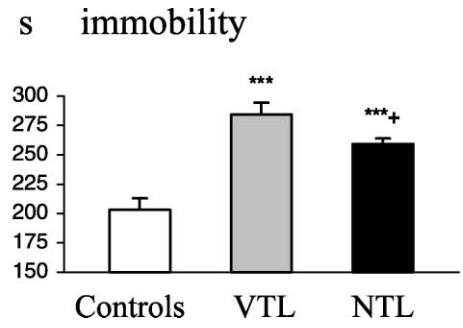

Fig. 3. Therapeutic effects of Noolit on the behavior of male mice in Porsolt's test. Data are presented as mean ± S.E. VTL, vehicle-treated losers; NTL, Noolit-treated losers. *** $P<.001$, differs from the control males. + $P<.05$; differs from vehicle-treated losers, *U*-criterion.

(Fig. 3). It was shorter in Noolit-treated losers compared to the vehicle-treated ones ($U=20$; $P<.04$). Nevertheless, the differences between the Noolit-treated mice and controls were significant ($U=4$; $P<.0003$).

3.1.4. Partition test (Fig. 4)

Approaches to the partition in response to the familiar ($U=17.5$; $P<.002$) and unfamiliar ($U=32.5$; $P<.04$) partner were significantly fewer, and the total time spent near the partition in response to the familiar ($U=19.5$; $P<.004$) and unfamiliar ($U=29$; $P<.02$) partner was shorter in the vehicle-treated mice compared to the controls (Fig. 4). There was no significant difference in the average time spent near the partition per approach ($P>.05$). Unlike the losers, the control mice responded more actively to the unfamiliar than familiar partners ($T=10$; $P<.02$).

There were no significant differences between Noolit-treated and vehicle-treated losers in the number of approaches, or total or average time spent near the partition ($P>.05$). In a manner similar to that of the control mice, the average time spent near the partition by Noolit-treated mice was longer in response to the unfamiliar than familiar partner ($T=11$; $P<.02$).

3.2. Experiment 2. Noolit in preventing of anxiety and depression in male mice

3.2.1. Plus-maze test (Table 1)

Percentage centre platform time ($U=7.5$; $P<.0003$) and entries ($U=25$; $P<.01$), total entries ($U=17.5$; $P<.003$), passages ($U=10.5$; $P<.001$) and the level of peeping ($U=5$; $P<.0002$) were decreased, and percentage enclosed arm time ($U=7$; $P<.0003$) was significantly increased in the vehicle-treated losers compared to the controls (Table 1). There were no significant differences in percentage open arm time and entries, enclosed arm entries, or head dip ($P>.05$). Noolit improved most behavioral parameters: percentage open arm time ($U=29$; $P<.03$) and entries ($U=25.5$; $P<.02$), total entries ($U=29$; $P<.04$), passages ($U=19.5$; $P<.005$) were increased, whilst the percentage enclosed arm time ($U=27$; $P<.03$) and entries ($U=24$; $P<.02$) were decreased in Noolit-treated losers compared to the vehicle-treated ones. There were no significant differences in percentage centre platform time and entries, or level of peeping ($P>.05$). There were significant differences between the Noolit-treated losers and controls in percentage centre platform time ($U=14$; $P<.001$) and entries ($U=28$; $P<.02$), percentage enclosed arm time ($U=28.5$; $P<.02$), percentage open arm entries ($U=26$; $P<.01$), total entries ($U=30$; $P<.03$), passages ($U=30$; $P<.025$) and level of peeping ($U=15$; $P<.001$).

3.2.2. Open-field test (Table 1)

Crossed squares ($U=11$; $P<.0004$) and the level of rearing ($U=3$; $P<.0001$) were decreased, and defecation

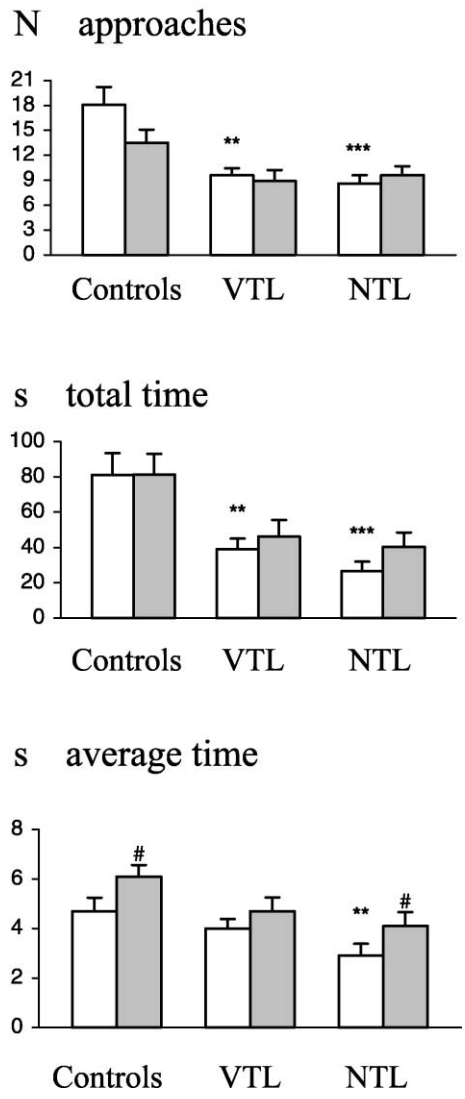

Fig. 4. Therapeutic effects of Noolit on the behavior of male mice in the partition test. Data are presented as mean ± S.E. VTL, vehicle-treated losers; NTL, Noolit-treated losers. White column, reaction to the familiar partner; gray column, reaction to the unfamiliar partner; ** $P<.01$; *** $P<.001$, differs from control males, *U*-criterion. # $P<.05$, differs from reaction to the familiar partner, *T*-criterion. (Significant differences between reactions of animal groups to the unfamiliar partners are not presented.)

Table 1
Preventative effects of Noolit on the behavior of male mice in different tests

Tests, behavioral parameters	Controls	Vehicle-treated losers	Noolit-treated losers
Plus-maze test			
Open arm time, %	1.31 ± 0.55	0.36 ± 0.16	1.61 ± 0.56^{+}
Central platform time, %	8.92 ± 1.00	4.12 ± 0.51***	4.88 ± 0.46***
Enclosed arm time, %	89.53 ± 1.57	96.00 ± 0.49***	93.79 ± 0.73*$^{+}$
Open arm entries, %	4.38 ± 1.24	4.43 ± 1.90	13.95 ± 2.84**$^{+}$
Central platform entries, %	46.85 ± 0.70	42.88 ± 1.27**	44.20 ± 0.85*
Enclosed arm entries, %	48.77 ± 1.78	52.68 ± 2.79	41.85 ± 2.71^{+}
Total entries, *N*	11.08 ± 1.72	4.45 ± 0.45**	6.09 ± 0.53*$^{+}$
Passages, *N*	6.58 ± 1.45	0.82 ± 0.26***	2.36 ± 0.39*$^{++}$
Peepings, *N*	12.33 ± 1.10	5.91 ± 0.67***	7.00 ± 0.89***
Head dips, *N*	0.42 ± 0.19	0.09 ± 0.09	0.64 ± 0.28
Open field test			
Latency, s	0 ± 0.00	0.00 ± 0.00	0.15 ± 0.15
Crossed squares, *N*	136.50 ± 7.49	83.50 ± 7.52***	72.92 ± 6.49***
Rearing, *N*	40.67 ± 3.05	19.33 ± 2.26***	18.08 ± 2.09***
Defecation, *N*	0.92 ± 0.47	1.92 ± 0.43*	2.08 ± 0.38*
Porsolt's test			
Immobility time, s	172.25 ± 13.79	215.00 ± 7.40**	186.0 ± 7.00^{++}
Partition test			
Approaches, *N*			
familiar partner	13.17 ± 1.09	6.45 ± 0.89***	5.45 ± 0.96***
unfamiliar partner	14.75 ± 1.35	7.45 ± 1.63**	7.45 ± 1.80**
Total time, s			
familiar partner	91.17 ± 5.68	35.18 ± 8.12***	18.45 ± 3.33***
unfamiliar partner	141.92 ± 11.75$^{\#\#}$	86.45 ± 18.04*$^{\#}$	46.82 ± 13.42***$^{\#}$
Average time, s			
familiar partner	7.42 ± 0.67	5.60 ± 0.89*	3.18 ± 0.71***$^{+}$
unfamiliar partner	10.00 ± 0.78$^{\#}$	10.18 ± 2.20	4.34 ± 0.99***$^{+}$

Data are presented as mean and S.E.
* $P<.05$: differs from controls, *U*-criterion.
** $P<.01$: differs from controls, *U*-criterion.
*** $P<.001$: differs from controls, *U*-criterion.
$^{+}$ $P<.05$: differs from vehicle-treated losers, *U*-criterion.
$^{++}$ $P<.01$: differs from vehicle-treated losers, *U*-criterion.
$^{\#}$ $P<.05$: differs from familiar partner, *T*-criterion.
$^{\#\#}$ $P<.01$: differs from familiar partner, *T*-criterion.

($U=39.5$; $P<.05$) increased in the vehicle-treated mice compared to the controls. There was no significant difference in latency to first escape from the center ($P>.05$). Noolit did not affect behavior: there were no significant differences between the Noolit-treated losers and vehicle-treated losers in any parameters ($P>.05$). There were significant differences between the Noolit-treated mice and the controls in crossed squares ($U=5$; $P<.0001$), levels of rearing ($U=4.5$; $P<.0001$) and defecation ($U=39.5$; $P<.03$).

3.2.3. Porsolt's test (Table 1)

Immobility was significantly increased in the vehicle-treated mice compared to the controls ($U=24$; $P<.005$), decreased in Noolit-treated mice compared to the vehicle-treated mice ($U=30$; $P<.009$), and identical in Noolit-treated mice and the controls ($P>.05$).

3.2.4. Partition test (Table 1)

Approaches to the partition in response to the familiar ($U=10.5$; $P<.0006$) and unfamiliar ($U=19$; $P<.004$) partners were significantly fewer, and the total time spent near the partition in response to the familiar ($U=8$; $P<.0004$) and unfamiliar ($U=32.5$; $P<.04$) partners as well as the average time spent near the partition in response to the familiar partner ($U=27$; $P<.02$) were significantly shorter in the vehicle-treated mice compared to the controls. In total, both vehicle-treated mice ($T=8$; $P<.03$) and controls ($T=5$; $P<.008$) spent more time near the partition in response to unfamiliar than familiar mice. The control mice spent more time near the partition for one approach in response to the unfamiliar than familiar mice ($T=10$; $P<.02$), while vehicle-treated mice did not ($P>.05$).

Noolit had little effect on behavior. There were no differences between the Noolit-treated losers and vehicle-

treated losers ($P > .05$) in total time spent near the partition or number of approaches to it ($P > .05$). However, the average time spent near the partition in response to the familiar ($U = 26.5$; $P < .025$) and unfamiliar ($U = 27$; $P < .03$) mice was shorter in the Noolit-treated than vehicle-treated mice. There were significant differences in total time spent by the Noolit mice near the partition in response to the familiar and unfamiliar mice ($T = 5$; $P < .04$), and likewise for the other animal groups. There were significant differences between the Noolit-treated mice and the controls in the number of approaches to the partition in response to the familiar ($U = 9$; $P < .0004$) and unfamiliar ($U = 24$; $P < .009$) mice, total time spent near the partition on the familiar ($U = 0$; $P < .00$) and unfamiliar ($U = 9$; $P < .0004$) mice, and average time spent on the familiar ($U = 10$; $P < .0006$) and unfamiliar ($U = 11$; $P < .0007$) mice.

4. Discussion

Experiencing social defeat repeatedly and having to share a cage with a winner throughout the experiment, even though separated by a perforated transparent partition, evokes a state in the losers analogous to depression in humans (review, Kudryavtseva and Avgustinovich, 1998). Under the sensory contact model, social defeat, negative emotions, permanent anxiety produced by fear over being attacked, threatened and so on were considered the possible etiologic factors in the development of experimental depression. In general, unavoidable psychoemotional social stress produced mainly by anticipation of bad development of events can be considered a major psychopathogenic factor. The physical component (pain) is not strong here, because in our experiments, close agonistic interactions normally last for seconds and we would not let them go on for more than 3 min. However, because of sensing the aggressor, anticipation, fear and anxiety stay throughout the study (for days and weeks) and lead to obvious consequences for the losers.

Depression- and anxiety-like states developed in Experiments 1 and 2, which were confirmed by comparison of the controls and vehicle-treated (''placebo'') losers in the behavioral tests. Reduced ambulation (e.g., reduced level of rearing and crossed squares in the open field, passages in the plus maze, and approaches in the partition test) suggests that the losers suffered a behavioral deficit in all tests, similar to those in other experiments (Kudryavtseva et al., 1991a, 1995). Increased immobility time in Porsolt's test suggests development of depression. Decreased percentage open arm entries and time and increased enclosed arm entries and time (the classical plus-maze anxiety parameters all) do favour anxiety. It is possible that these conditions evoke generalized anxiety, since sociability (response to the conspecific) assessed in the partition test, too, revealed an anxiety component in the losers' behavior, namely reduced sociability: The number of approaches and the total time spent near the partition as a reaction to the partner in the neighboring compartment of the common cage were decreased in the vehicle-treated losers compared to the control animals. If emotionality in bright light open-field conditions could, following Griebel's (1995) and Ramos and Mormede's (review, 1998) suggestion, be regarded as a measure of anxiety, we might say that open-field behavior, too, confirms pronounced anxiety.

As was demonstrated in Experiment 1, Noolit significantly improved the psychoemotional state of the otherwise depressed mice following postfight 2-week treatment. The enclosed arm parameters (percentage time and entries) were decreased, and the center platform parameters (percentage time and entries) were increased in Noolit-treated losers compared to the vehicle-treated ones, thus revealing obvious anxiolysis. The level of peeping and head dip, percentage open arm time and entries and the percentage enclosed arm entries fully restored, suggesting a high efficiency of Noolit. In the open field, the level of rearing fully restored also, and the number of crossed squares was significantly increased in Noolit-treated losers compared to the vehicle-treated mice. These changes may be interpreted as a significant reduction in behavioral deficit and an enhancement in exploratory activity following treatment with Noolit.

In the partition test, the Noolit-treated mice were responsive to the unfamiliar mouse, as were the controls. Therefore, treatment with Noolit reduced fear and anxiety. Although losers and winners lived side by side in the sensory contact conditions, the psychoemotional state of the former improved significantly. An antidepressant effect of Noolit was also revealed in Porsolt's test. It could therefore be concluded that Noolit exerted pronounced antidepressant and anxiolytic effects on the depressed mice in the less stressful postfight conditions, when the winner was still nearby, but it could not do any physical harm to the loser. Additionally, Noolit enhanced exploratory activity and reduced behavioral deficit.

Examination of Noolit effects on the mice being defeated during confrontations (Experiment 2) revealed its preventative properties. Noolit prevented the depression-like state that might otherwise have developed in the losers under the influence of social pathogenic factors: There was no difference between Noolit-treated losers and controls in immobility time (Porsolt's test). The effects of Noolit in preventing anxiety were revealed in the plus maze: the percentage enclosed arm time and entries were significantly decreased, and total entries, passages and percentage open arm time increased in the Noolit-treated losers compared to the vehicle-treated mice. However, in the open field, the behavioral parameters did not change following treatment with Noolit: The number of crossed squares and the level of rearing were significantly reduced in the Noolit-treated losers compared to the control mice, and were the same as in vehicle-treated losers. It is possible that Noolit did not affect anxiety significantly. Judging by the number of approaches and the total time spent near the partition in the partition test, the respective responses to the familiar and

unfamiliar mice in Noolit-treated and vehicle-treated losers were identical. Moreover, the average time spent near the partition per approach was significantly reduced following Noolit treatment. On the one hand, that could be considered an anxiogenic effect, because there was an additional reduction in sociability. Importantly, similar effects were observed in experiments on losers challenged with imipramine (reviews, Kudryavtseva et al., 1991a): Imipramine had an antidepressant effect in Porsolt's test, none in the open field, and a slight anxiogenic effect in the partition test. Anxiogenic effects have been reported for some antidepressants (including imipramine) (Allikmets et al., 1995). Unwanted as they might be, they are signaling that the drug's action is underway and are likely to disappear after a period of treatment longer than 2 weeks. It is possible that the anxiolytic effects observed in the plus maze (but not in the open field and partition test) result from less aversion in this testing situation. Overall, these data suggest that Noolit prevents the development of generalized anxiety. Presumably, it is because Noolit has reduced anxiety that the depression-like state does not develop.

It is possible that the favorable psychotropic effects of Noolit in male mice with mixed anxiety/depression-like states are primarily due to the well-known therapeutic properties of lithium ions. It has been shown that lithium is a noncompetitive inhibitor of the enzyme inositol monophosphatase (review, Lenox et al., 1998; Fauroux and Freeman, 1999). Lithium reduces the cortisol level in drug responders suggesting a regulatory effect on hypothalamic–pituitary–adrenal axis activity (Rybakowski et al., 1999). The effect of lithium depends on tryptophan hydroxylase variants in humans (Serretti et al., 1999) and lithium may act through serotonin 5-HT_{1B} receptors as shown in an animal model (Redrobe and Bourin, 1999b). Chronic administration of lithium increases the density of the serotonin uptake site in cortical regions suggesting an increase in the number of serotonin transporters in brain regions containing nerve terminals of serotonergic neurons (Carli and Reader, 1997). It may be supposed that the therapeutic action of lithium is due to effects it takes on serotonergic neurotransmission, since depression in humans is accompanied by changes in brain serotonergic activity (reviews, Kostowski et al., 1989; Curzon, 1988; Delgado, 2000; Ressler and Nemeroff, 2000). Our previous investigations provide evidence that changes in serotonin metabolism and receptors (review, Kudryavtseva et al., 1995; Avgustinovich et al., 1998) depend on the depth of depressive pathology (i.e., the duration of agonistic confrontations) in mice. It is likely that the enterosorbent prolongs the effect of lithium, performs endodetoxification, which adds to welfare of the depressed individuals and prevents overdose. It has also been shown that Noolit exerts favorable effects (Borodin et al., 2000b) on psychogenic immune deficiency developing in the losers due to repeated social defeat (Devoino et al., 1993; Popova et al., 1996; Gryazeva et al., 1999) effected by the sensory contact model.

The sensory contact model (or, alternatively, the model of chronic social conflicts) is unique in that it allows the investigator to examine neurochemical and physiological changes, spanning between norm and depression-related pathology, and to study the mechanisms of depression at separate stages of development in animals. Any drug can be tested for efficacy on the neurochemical background modified by experimentally induced depression under simulated clinical conditions. This approach can be useful in the search for novel antidepressants and anxiolytics, and the experimental study of the mechanisms of their action. Previous to the experiments being reported herein, we had examined the effects of Noolit on the behavior of intact (nondefeated) mice (Borodin et al., 2000a), and the only finding was an antidepressant effect in Porsolt's test (the others were the open field, exploratory activity, and plus maze). This implies that the effects of Noolit (or any other antidepressant or anxiolytic) might be dependent on the neurochemical background, which, in turn, can be modified by pathogenic factors. Our approach allows the therapeutic and preventative properties of the drug, as well as its efficiency as anxiolytic and antidepressant, to be studied experimentally, and thus employed, it can be useful in having a better understanding of the drugs' action.

Anxiety, phobias and depression are the most widespread psychoemotional pathologies produced by stress in humans. There is a wealth of evidence suggesting the comorbidity of anxiety and phobia, and major depression associated with greater symptom severity (Kaufman and Charney, 2000). Anxiety can either lead to or coexist with depression (Stahl, 1993; Rouillon, 1999). The quest for an appropriate pharmacological therapeutic strategy that could warrant simultaneous correction of anxious and depressive states in individuals continues (Montgomery and Judge, 2000; Nutt, 2000). Finally, our study suggests that enterosorbent Noolit may represent a novel therapeutic agent for the successful treatment of mixed depression/anxiety states, and prevention of environmentally induced mood disorders.

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