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Abstract

Background: There are experimental evidences that chronic social defeat stress is accompanied by development of anxiety- and depression-like state as well as downregulation of serotonergic genes in the midbrain raphe nuclei of male mice. The study was aimed at investigating the effect of chronic lithium chloride (LiCl) administrations on anxiety behavior and the expression of serotonergic genes in the midbrain raphe nuclei of affected mice.

Methods: The pronounced anxiety in male mice was caused by chronic social defeat stress in daily agonistic interactions. After 6 days of social stress, defeated mice were treated with saline or LiCl (100 mg/kg, i.p. two weeks) against the background of continuing agonistic interactions. The level of anxiety was assessed using behavioral tests. The effect of chronic treatment with LiCl on the expression of serotonergic genes in the midbrain rapher nuclei was also studied.

Results: Mild anxiolytic effects of LiCl were found on communication in the partition test. Anxiogenic-like effects assessed using the elevated plus-maze and social interaction tests were found. Chronic LiCl treatment induced overexpression of the serotonergic genes in the midbrain raphe nuclei.

Conclusion: The effects of chronic LiCl treatment depends on the method of treatment, psychoemotional state, and experimental context (tests). It is assumed that chronic administration of LiCl against the background of severe stress and anxiety in defeated animals causes an anxiogenic effect, possibly due to activation of the expression of serotonergic genes, and, as consequences, activation of the serotonergic system as side effect of LiCl.

Keywords: chronic social defeat stress, anxiety, lithium chloride, mice

1. Introduction

Lithium salts are widely used in psychiatric practice in monotherapy regimens as mood stabilizers [1-4], in the supportive therapy of psychoemotional disorders [5], in prevention suicidal behavior in patients with manic-depressive psychosis [6-8]. Lithium is also used at onset of depressive phase in patients with bipolar disorder, for prevention of mood disorders [3,9-12], or relapses in schizophrenia patients with aggressive or suicidal behavior, convulsions *etc* [13-16]. In our study the behavioral effects of lithium-based enterosorbent, called 'Noolit', produced obvious anxiolytic and antidepressant effects in male mice [17].

However, it was similarly shown that patients with bipolar disorder demonstrate lithium responsiveness of two types: they are good responders or poor responders [18]. Lithium is obviously a multitarget drug, which complicates understanding of its mechanism of action [19]. Lithium as a monotherapy or in combination with other drugs is effective in 60% of chronically treated patients, but response remains heterogeneous, and a large number of patients require a change in therapy after several weeks or months [20]. Therefore, there is a great need for the tools that would guide the clinicians in selecting a correct treatment strategy for understanding the variability of response to lithium in the medicine practice across individuals shown in many works [19,21,22]. We agree that important questions regarding the mechanism of lithium action on anxiety and depression remain open [23] despite much practice in application.

In this study, we present experimental data on the chronic treatment with LiCl of anxiety caused by chronic social stress, which leads to the development of mixed anxiety/depression-like state in male mice, similar to that of patients with depression [24-26]. The similarity of symptoms (general behavioral deficit, helplessness, anxiety-like state, decreased communication, etc.), etiology, sensitivity to antidepressants and anxiolytics (imipramine, fluoxetine, diazepam), as well as serotonergic changes in the brain were shown. Results will be compared with effect of the chronic lithium treatment in similar experiments in male mice with repeated aggression experiences in daily agonistic interactions which is accompanied by the development of a whole range of changes in

behavior and psychoemotional state with symptoms of psychosis-like behavior: the aggressive male mice demonstrated an increased number of stereotypical behaviors (self-grooming, rotation, jerks *etc*), enhanced anxiety, hyperactivity and strong aggressive motivation, which, along with other signs, indicated the development of a pathological behavior [27,28].

To investigate the effect of lithium on anxiety behavior, we used the method for screening of psychotropic drugs under simulated clinical conditions, which was proposed earlier [29,30]. This method makes it possible to study the protective properties of drugs under preventive treatment, as well as the therapeutic treatment of animals with behavioral pathology.

2. Methods

2.1. Animals

Adult C57BL/6 male mice were obtained from Animal Breeding Facility, Branch of Institute of Bioorganic Chemistry, RAS (Pushchino, Moscow region, Russia). The animals were housed under standard conditions (12:12 h light/dark regime, switch-on at 8.00 a.m.; food (pellets) and water available *ad libitum*). Mice were weaned at one month of age and housed in groups of 8-10 in plastic cages. All procedures were carried out in compliance with international regulations for animal experiments (Directive 2010/63/EU of the European Parliament and of the Council on the Protection of Animals Used for Scientific Purposes). The protocol for the studies was approved by Scientific Council No9 of the Institute of Cytology and Genetics, SD RAS of March, 24, 2010, N613 (Novosibirsk).

2.2. Behavioral Study

Prolonged experience of chronic social defeat stress, accompanied by strong anxiety in male mice was induced using the sensory contact model [24,28]. Pairs of weight-matched animals were each placed in a cage (28 ×14 ×10 cm) bisected by a perforated transparent partition allowing the animals to see, hear and smell each other, but preventing physical contact. The animals were left undisturbed for three days to adapt to new housing conditions and sensory contact before they were exposed to encounter.

Then, every afternoon (14:00-17:00 p.m. local time), the cage lid was replaced by a transparent one, and 5 min later (the period necessary for mouse activation), the partition was removed for 10 minutes to encourage agonistic interactions. The superiority of one of the mice was firmly established within two or three encounters with the same opponent. The superior mouse would be attacking, biting and chasing another (winners), who would be displaying only defensive behavior (upright postures, sideways postures, freezing or withdrawal, lying on the back). As a rule, agonistic interactions between males are discontinued by lowering the partition if the aggression has lasted 3 min, in some cases, less. Each defeated mouse (defeater, loser) was exposed to the same winners for three days, while afterwards each loser was placed, once a day after the fight, in an unfamiliar cage with an unfamiliar aggressive mouse behind the partition. Each winning mouse remained in its original cage. This procedure was performed once a day and yielded an equal number of winners and losers.

The LiCl was administered during the period of repeated agonistic interactions, expecting to see its protective effects shown earlier for some medicines in our experiments [29]. For that, after 6 days of agonistic interactions accompaning by chronic social stress, defeated mice were treated with saline or lithium chloride (LiCl; Merck, Germany) at a dose of 100 mg/kg, i.p., once a day in the morning at 9-10 AM. Three groups of animals were used in the behavioral experiment: the controls — mice without consecutive experiences of agonictic interactions; defeated males after chronic treatment with saline (Sal-treated losers); defeated males after chronic treatment with LiCl (LiCl-treated losers). After two weeks of LiCl or saline injections the behavior of animals was evaluated in the behavioral tests (one test/day, Fig. 1) which were used for measuring the level of anxiety in different experimental situations, on the background of injections and continuing agonistic interactions.

In order to understand the possible effect of LiCl on the behavior of experimental animals, we conducted an additional experiment that, we hoped, would allow us to explain the effect of this drug. The effect of LiCl (150 mg/kg) on the expression of serotonergic genes in the midbrain raphe nuclei, which contains the bodies of serotonergic neurons, was investigated.

2.3.Behavioral Tests

2.3.1. The Partition Test

Partition test can be utilized as a tool for estimation of mouse behavioral reaction to a conspecific behind the transparent perforated partition dividing the experimental cage into the equal parts [31]. The number of approaches to the partition, and the total time spent near it (moving near the partition, smelling and touching it with nose, one or two paws, putting noses into the holes) are scored during 5 min as indices of reacting to the partner. The time the males show sideways position or "turning away" near the partition is not included in the total time of test. The experimental procedure is as follows: the pair of mice lives together in a cage with a partition. On the testing day, the lid of the cage is replaced by a transparent one. 5 min later (period of activation) behavioral responses of the losers and the controls toward the unfamiliar partner is recorded for 5 min. This test is used for the study of communicativeness (sociability) as well as level of anxiety: it was shown that decrease of partition items correlates positively with indices of anxiety estimated in elevated plus-maze test [31].

2.3.2. Elevated Plus-Maze Test [32].

The elevated plus-maze consisted of two open arms (25×5 cm) and two closed arms (25×5×15 cm) and was placed in a dimly lit room. The two arms of each type were opposite to each other and extended from a central platform (5×5 cm). The maze was elevated to a 50 cm above floor.

The cover of the experimental cage with a mouse was replaced by transparent lid in the same room 5 min before exposure to the plus-maze. The mouse was placed with the nose to the closed arm at the central platform. The following measures were recorded during 5-min: 1) total entries; 2) open arm entries (four paws in open arm), closed arm entries (four paws in closed arm) and central platform entries; 3) time spent in open arms, closed arms, and central platform (center); 4) number of passages from one closed arm to another; 5) number of head-dips (looking down on the floor below the plus-maze); 6) number of peeping when mouse being in closed arms. Indices 2 and 3 are considered as measures of the level of anxiety, indices 1 and 4 are related to locomotor activity, indices 5 and 6 are

considered as risk assessment behavior. Time spent in the closed, open arms and in central platform (center) was calculated in percentages from total testing time. Elevated plus-maze was thoroughly cleaned between sessions. This test we consider as test with low level of aversivity.

2.3.3. Exploratory Activity and Social Interaction Tests

The open-field (36×23 cm) with perforated turned metal wire holder (inverted pencil holder, bottom diameter - 10.5cm) in one of the cage corners was used [33]. Each mouse was placed individually in the opposite from holder corner of the open field for 5 min. This test allows to estimate the exploratory behavior of mice in novel conditions with unfamiliar object - wire holder. This test can be considered as accompanied by expressed stress. Then an unfamiliar group housed male was placed under the wire holder for 5 min to study the reaction of male mice to conspecific in familiar situation (Social interaction test). The following behavioral variables were registered:

1) Automatic registration with EthoVision XT software (version 11.0; Noldus Information Technology, The Netherlands) of the tracking score (distance) during testing time with differentiation of place near the wire holder (5 cm around) of the cage and total time spent in the opposite to wire holder corner;
2) Manual registration with Observer XT (version 7.0; Noldus Information Technology, The Netherlands) was used for following behavior indicators of communicativeness: number and/or duration of: 1) rearing (exploratory activity); 2) grooming (self-oriented behavior ─ licking of the fur on the flanks or abdomen, washing over the head from ear to snout); 3) approaches to the wire holder and total time (s) spent near it (moving near the wire holder, smelling and touching it with nose, one or more paws). The duration of sideways position or “turning away” near the wire holder was not included in the total time. After each test, the open field and wire holder were thoroughly washed and dried with napkins.

Preliminary analysis of LiCl-treated losers’ behavior in exploratory activity test clearly divided animals into two groups ─ the LiCl+sensitive (LiCl+) and LiCl-less sensitive (LiCl-) losers to the

chronic LiCl treatment. As behavioral parameter for division into groups was taken avoidance of novel object (wire holder): LiCl+treated losers did not approach to wire holder at all, seated in the corner only, and did not explore the cage. In the control and Sal-treated and LiCl-treated losers, we observed a natural exploratory activity in novel conditions.

2.4.Statistical Analysis

Statistical analysis for behavioral data was performed using either one-way ANOVA for parametric variables or Kruskal-Wallis test with factor "group" (Control, Sal-treated losers, LiCl-, LiCl+treated losers) followed by the Tukey's multiple comparisons post hoc test for parametric variables or Kruskal-Wallis test with Dunn's multiple comparisons post-hoc if the parametric assumptions were not satisfied. To display the variability among the values, the data are presented as a box-whisker plot showing means (*plus sign*), medians (*solid lines*) and 25%/75% quartiles with whiskers indicating 10th and 90th percentiles. All statistical analyses were performed using XLStat software (Addinsoft, www.xlstat.com).

3. Results

3.1. Effects of Chronic LiCl Treatment on Behavior of Defeated Mice in the Partition Test (Fig. 2)

One-way ANOVA revealed the influence of the "group" factor on: number of approaches ($F(2,33) = 6.619$, $P = 0.0038$) and rearing ($F(2,33) = 3.293$, $P = 0.0496$). Kruskal-Wallis test revealed the influence of the "group" factor on: total time spent near the partition ($H = 9.816$, $P = 0.0074$). Based on the Tukey's or Dunn's-multiple comparisons post hoc tests significant differenses for the Sal-treated losers *vs* Controls were found for number of approaches ($P = 0.0027$), total time spent near partition ($P = 0.0053$) and number of rearing ($P = 0.0390$).

Thus, in comparison with the control, Sal-treated losers demonstrated lower communicativeness, as revealed number to and total time spent near the partition as a reaction to the partner in the neighboring compartment, and decreased exploratory activity estimated by number of

rearing behavior. Under the LiCl treatment, these parameters did not yet differ significantly from the control. We can assume that LiCl induced slight anxiolytic effects in the losers.

3.2. Effects of Chronic LiCl Treatment on the Behavior of Defeated Mice in the Elevated Plus-Maze Test (Fig. 3)

One-way ANOVA revealed a significant influence of the factor "group" (Control, Sal-treated losers, LiCl-treated losers) on number of: central platform entries ($F(2,31) = 4.130$, $P = 0.0257$); closed arm entries ($F(2,31) = 4.661$, $P = 0.0170$); passages ($F(2, 31) = 4.717$, $P = 0.0163$), head dips ($F(2,31) = 3.590$, $P = 0.0396$), total entries ($F(2,31) = 4.552$, $P = 0.0185$). Based on the Tukey's multiple comparisons post hoc test revealed in the LiCl-treated vs the controls groups number of: central platform entries ($P = 0.0277$); closed arm entries ($P = 0.0185$); passages ($P = 0.0166$); head dips ($P = 0.0403$); total entries ($P = 0.0192$).

Thus, LiCl causes a decrease in all parameters of locomotor activity, which can be easily explained in the context of a general behavioral deficit that develops in a mixed anxiety/depression-like state [24-26]. This effect of LiCl may be considered also as pro-depressive effects in this experimental context.

Previously, it was repeatedly shown that expressed anxiety is developed in the losers after 20-day social defeat stress, estimated by this test [26,29]. Against the background of the chronic saline treatment, these differences were less pronounced. It can be cautiously concluded that saline itself has a protective antitoxic effect in chronic injections. However, the general decrease in locomotor activity during chronic treatment with LiCl can be regarded as an anxiogenic effect.

3.3. Effects of Chronic LiCl Treatment on the Exploratory Activity of Defeated Mice with Different Sensitivity to LiCl in Novel Situation Toward Unfamiliar Object (empty wire holder, Fig. 4)

According to the level of wire holder avoidance (see description in Materials and Methods) we divided the losers after LiCl injections into two subgroups: LiCl+treated and LiCl-treated losers. One-way ANOVA revealed influence of the factor "group" (Control, Sal-treated, LiCl-treated, LiCl+treated

losers) on the total tracking (F(3,29) = 26.50, $P < 0.0001$) (tracking, cm). Tukey's multiple comparisons test showed significant differences for: Control *vs* Sal-treated ($P = 0.0003$), LiCl-treated ($P < 0.0001$), LiCl+treated losers ($P < 0.0001$); and LiCl+treated *vs* Sal-treated losers ($P = 0.0013$). Kruskal-Wallis test revealed the influence of the "group" factor on the time spent *in the corner* (H = 18.03, $P = 0.0004$) and near *the wire holder* (H = 17.56, $P = 0.0005$). Dunn's multiple comparisons test showed differences for parameters: *in the corner* (sec) - Control *vs* LiCl+treated losers ($P = 0.0027$); Sal-treated losers *vs* LiCl+treated losers ($P = 0.0012$); *near the wire holder* (sec) - Control *vs* LiCl+treated losers ($P = 0.0040$); Sal-treated losers *vs* LiCl+treated losers ($P = 0.0010$).

The LiCl+treated losers were more sensitive to effects of LiCl and displayed less exploratory activity, estimated by total tracking time. Major time they spent in the corner and never were near wire holder. Together with behavior in the plus-maze and partition tests these data may indicate decreased exploratory activity and increased level of anxiety under chronic LiCl treatment.

3.4. *Effect of Chronic LiCl Treatment on the Reaction of Mice Toward Unfamiliar Partner in the Social Interaction Test* (Fig. 5)

One-way ANOVA indicate a significant influence of the factor "group" (Control, Sal-treated, LiCl-treated, LiCl+treated losers) on the number of *approaches* to partner (F(3,31) = 11.35, $P < 0.0001$) and *total time spent* near the wire holder (Approaches, sec) (F(3,31) = 20.98, $P < 0.0001$). Kruskal-Wallis test revealed the influence of the "group" factor on the time of *self-grooming* (H = 9.816, $P = 0.0212$). Tukey's multiple comparisons test was used for the following behavioral parameters (Figure 5): *approaches* (N) — Control *vs* LiCl+treated losers ($P < 0.0001$), Sal-treated loser *vs* LiCl+treated losers ($P = 0.0008$), LiCl-treated *vs* LiCl+treated losers ($P = 0.0020$); *approaches* (sec) — Control *vs* Sal-treated losers ($P < 0.0001$), LiCl-treated losers ($P = 0.0017$), LiCl+treated losers ($P < 0.0001$); Sal-treated losers *vs* LiCl+treated losers ($P = 0.0083$), LiCl-treated losers *vs* LiCl+treated losers ($P = 0.0073$). Dunn's multiple comparisons test was used for the time of *self-grooming* (sec) — Control *vs* Sal-treated losers ($P < 0.0133$).

This test we considered as measuring the level of communicativeness with the unfamiliar partner in conditions which has already become familiar during 5 minutes before introduction of partner. During previous 5 minutes the mice realized that they were not in danger, and they began shortly to examine the cage.

Chronic LiCl treatment induced strong anxiogenic effect in LiCl+treated losers in comparison with the control and Sal-treated losers which was estimated by decreased number of approaches to and total time spent near the holder with partner (approaches, sec). Time spent in the corners was significantly higher in comparison with all groups. Anxiogenic effects in LiCl-treated losers was significantly less differing in comparison with the control mice.

3.5.Effects of Chronic LiCl Treatment on the Expression of Serotonergic Genes in the Midbrain Raphe Nuclei of Defeated Mice (Fig. 6)

The measurement data is provided by the BioRad Amplifier software (USA). In comparison with the controls and Sal-treated losers chronic LiCl treatment induced overexpression of the *Tph2* gene encoded the limiting enzyme of serotonin synthesis (both $P < 0.01$); the *Slc6a4* gene, encoded serotonin transporter ($P < 0.05$ and $P < 0.01$, respectively); the *Htr1a* gene, encoded $5HT_{1a}$ receptors (for both $P < 0.01$); and the *Htr5b* genes, encoded $5HT_{5b}$ receptors ($P < 0.01$ and $P < 0.05$, respectively) in the midbrain raphe nuclei of defeated mice.

Some discrepancy between the data obtained earlier in [34] and the data of these studies can be explained by the lack of complete identity of these groups. Earlier, we did not administer chronically the saline to the losers as in this experiment. There is also the possibility that saline itself may have a protective antitoxic effect with chronic injections decreasing effect of CSDS.

4. Discussion

A very important aspect is the varied sensitivity of animals of the same group to drugs, in particular in this work, to LiCl. When studying the behavior of aggressive [35] and defeated male mice (this

experiment) in the social interaction test we found that animals of one group split into subgroups according to different sensitivity to LiCl: only 40% of aggressive and defeated males were sensitive to chronic lithium treatment. Naturally, the question arises: why inbred animals split into sensitive and less sensitive groups to drug under obviously identical experimental conditions? One of the plausible reasons, as supposed in [36,37], could be the differences in prenatal and early postnatal development, which have been overlooked in the standardized experimental setting. However, in our opinion, a more possible assumption is that the baseline psychoemotional statuses of experimental animals grown in group-housed conditions in Animal House are different. Mice are known to form despotic dominance hierarchy with one male being dominant and others being subordinates [38]. Social status leaves an imprint on the behavior and brain neurochemistry of mice. In this study we got additional evidence that the effect of a drug may depend on the psychoemotional state of an individual. In other words, the neurochemical background can modify the effect of a drug, sometimes to the opposite of the expected effect.

Moreover, some effects of a drug can be detected in one situation (test) and not manifested in another. Apparently, the cause is that the leading motivation developed in the experimental conditions underlies many (but not all) forms of behavior. Sometimes it could be a struggle between two opposite motivations (ambivalence), for example, fear and communicativeness (the test of social interactions), or anxiety and exploratory activity in the elevated plus-maze test. The balance between the leading motivational components is situational, is expressed through a psychoemotional state, and, consequently, the underlying neurochemical background that may mediate the effect of the drug. The division of defeated male mice into the subgroups, susceptible or resilient to the effects of CSDS, was also observed by other researchers in numerous studies [39-41 *etc*].

In our behavioral studies chronic LiCl injections into intact male mice for two weeks was shown to produce anxiolytic effects in our previous experiment: the anxiety level estimated in the elevated plus-maze and social interaction tests decreased [35]. In the present experiment, under preventive treatment of defeated male mice on the background of severe anxiety, the effect of LiCl correlated with

the behavioral scores in different tests. Slight anxiolytic effects were observed in the partition test and decreased exploratory activities in the elevated plus maze test. Apparent anxiogenic effects on LiCl-treated losers in the social interaction test may be a result of a frightening situation. In the group of defeated mice LiCl produced marked variable effects: about 40% of the LiCl+treated losers demonstrated pronounced anxiety in new situation toward novel object and unfamiliar partner under wire holder.

Earlier in similar experiments, the LiCl was administered preventively to aggressive males on the background of agonistic interactions, as well as therapeutically to males with the 20-day period of repeated aggression in the subsequent 2 week period without agonistic interactions [35]. Preventive chronic LiCl injections to aggressive male mice were shown to induce pronounced anxiogenic effects similar to those in the losers: LiCl further enhanced anxiety, which was shown earlier for male mice with repeated experience of aggression in the partition test and elevated plus-maze test [35,42]. In the social interactions test about 40% of aggressive mice demonstrated pronounced anxiety following LiCl treatment with a decrease of communicativeness and exploratory activity as compared with the controls. Therefore, the anxiogenic effect of LiCl is likely to be consequence of the stress that accompanies agonistic interactions common for aggressive and defeated mice.

Under therapeutic treatment of aggressive males during the no fight period, the anxiolytic effect of LiCl became evident in the social interaction test which was characterized by an increased interest to the partner under the wire holder [35]. However, in the elevated plus-maze and partition tests no effects of LiCl were detected. In the study of the diazepam effect, similar data were obtained in slightly different experimental context: acute administration of the drug to mice with a short-term experience of aggression induced anxiogenic effect, whereas in the males with a long-term experience of aggression diazepam had an anxiolytic effect [43]. Similar data were also obtained in other work: the effects of the anxiolytic chlordiazepoxide on aggressive behavior of animals with different social status differed [44].

Thus, the effects of chronic LiCl treatment can depend on the method of treatment (preventive, therapeutic), psychoemotional state developing under positive or negative social experience of animals (intact, aggressive, defeated), experimental context (tests), and can have both anxiogenic and anxiolytic effect or no effect whatsoever as shown in our study (Table 1). Moreover, the response to new conditions, as well as to the anxiolytics, differs in mice of different strains, which may be associated with the features of hereditary defined anxiety (state or trait) [45-47] which develops in behavioral tests. In the experiments, male mice of C57BL/6J strain with hereditary defined enhanced level of "trait" anxiety [47,48] were used. Our data may be useful for understanding the variability of response to lithium in the medicine practice across individuals shown in many works [19-22].

The central role of the brain serotonergic system in the mechanisms of stress, anxiety, depression and bipolar disorder [49-50], as well as in the neural plasticity [51-54] was shown in numerous studies. It is widely believed that serotonergic dysbalance is a key pathophysiological mechanism in major depression. We put forward an idea, that different effects of LiCl treatment depends on brain serotonergic activity changing under daily agonistic interactions in mice. We have shown an interaction between developing anxiety and depression and dynamic changes in serotonergic brain activity that progress in affected mice [26]. It was shown that social defeat stress induces strong anxiety from the first days of agonistic interactions which is accompanied by the activations of the serotonergic system. After 20 days of chronic social stress, downregulation of serotonergic genes expression in depressive mice was shown in the midbrain raphe nuclei [34,55], which contains the majority of serotonergic neuron bodies. These data indicated, together with the development of hypofunction of the serotonergic system at the stage of pronounced depression [26], a link between stress, serotonergic activity, anxiety- and depression-like states shown also in other studies in animals and humans [45,46,51-54,56]. These data also showed that chronic preventive administration of LiCl against the background of severe stress and anxiety in animals with experience of aggression or social defeats produced an anxiogenic effect.

In this article we represent our first data which have demonstrated the activation of serotonergic *Tph2, Slc6a4, Htr1a,* and *Htr5b* gene expression in the midbrain raphe nuclei under preventive lithium

treatment, that, supposedly, may lead to activation of the serotonergic system activity, and as consequences, to development of anxiety as side effect of LiCl. Our data is in agreement with other pharmacogenomic studies which have identified candidate genes that may be sensitive to antidepressants and mood stabilizers, in particular, to the lithium: as example, serotonergic *Htr2a, Htr1a, Slc6a4, Maoa,* and *Tph* genes [review, 57]. These results may be useful for understanding the mechanisms of psychotropic LiCl action through the increased serotonergic gene expression and thereby serotonergic activity. However, it's necessary to take into consideration that numerous other genes associated both with lithium exposure and bipolar disorder were also identified [21,22,57-60], and differential expression of these genes in brain tissue samples from patients and healthy controls was studied [61]: lithium exposure significantly affected 1108 genes, 702 of which were upregulated and 406 genes were downregulated. Our neurogenomic data, obtained in last years by whole transcriptomic analysis, also revealed changes in the expression of mitochondrial [62], ribosomal [63-64], monoaminergic [65-68], autistic [69] genes as well as changes in the expression of neurotrophic, transcription factors [70,71] and collagen [72] genes specific for brain regions in mice with mixed anxiety/depression-like state. It confirms that there are various mechanisms that may account for the effects of lithium [73,74] on the neurochemical, cellular, and genomic levels. It is becoming apparent that the study of molecular mechanisms of neuroplasticity provides the most promising basis for understanding the pathogenesis of chronic anxiety, depression and efficacy of anxiolytics and antidepressants. Our behavioral approach can be useful for understanding the effects of lithium in order to study in details the neurogenomic mechanisms of drug action in psychoemotional disorders.

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Author Contributions

DAS received behavioral and brain data, analyzed and processed data statistically, wrote manuscript text; ILK received brain materials; AGG contributed to behavioral data acquisition, IVB organized an experiment, revised statistics and text of manuscript critically; NVT and KOB analyzed neurogenomic data; NNK performed study design, analyzed and interpreted data, wrote the main manuscript text. All authors gave final approval.

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Compliance with Ethical Standards

The authors declare that all ethical standards are met.

Conflict of Interest

The authors declare that they have no conflict of interest.

Ethical Approval

All procedures were carried out in compliance with international regulations for animal experiments (Directive 2010/63/EU of the European Parliament and of the Council on the Protection of Animals Used for Scientific Purposes). The protocol for the studies was approved by Scientific Council No 9 of the Institute of Cytology and Genetics SD RAS of March, 24, 2010, N 613 (Novosibirsk).

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Legend to the Table 1

Table 1. Effects of chronic LiCl treatment on the anxious behavior of mice with alternative social experience

Legends to the Figures

Fig. 1. Scheme of experiment: chronic LiCl and/or saline treatment of defeated mice. Behavioral tests: Day 21 – the partition test; Day 22 – the elevated plus-maze test, Day 23 – the social interaction test.

Fig. 2. Effects of chronic LiCl treatment on the behavior of defeated mice in the partition test. Los Sal ― Sal-treated losers, Los LiCl ― LiCl-treated losers. Values are shown as means (*plus sign*), amedians (*solid lines*), and 25%/75% quartiles in a box-whisker plot with whiskere indicating 10th and 90th percentiles. * ― $P < 0.05$, **― $P < 0.01$ *vs* controls; N=12 for each group.

Fig. 3. Effects of chronic LiCl treatment on the behavior of defeated mice in the elevated plus-maze test. Los Sal – Sal-treated losers, Los LiCl – LiCl-treated losers. * ― $P < 0.05$ *vs* controls, Tukey's multiple comparisons post hoc test (N = 12 for each group). Values are shown as means (*plus sign*), medians (*solid lines*) and 25%/75% quartiles in box-whisker plot with whiskers indicating 10th and 90th percentile.

Fig. 4. Effects of LiCl on the exploratory behavior of defeated mice in new situation and toward novel object (holder). Los Sal – Sal-treated losers, Los LiCl – LiCl-treated losers. Values are shown as means (*plus sign*), medians (*solid lines*) and 25%/75% quartiles in box-whisker plot with whiskers indicating 10th and 90th percentile. ** ― $P < 0.01$, *** ― $P < 0.01$ *vs* controls; ## ― $P < 0.01$, ### - $P < 0.001$ *vs* Sal-treated losers (*Tukey's multiple comparisons post hoc test*); Control (N=9); Sal-treated losers (N=11), LiCl-treated losers (N=7), LiCl+treated losers (N=5).

Fig. 5. Effects of LiCl on the behavior of defeated mice in the social interaction test as a reaction to the partner under wire holder. Los Sal – Sal-treated losers, Los LiCl – LiCl-treated losers. Values are shown as means (*plus sign*) and medians (*solid lines*) and 25%/75% quartiles in box-whisker plot with whiskers indicating 10th and 90th percentile; For the control (N=9), Sal-treated losers (N=11), LiCl-treated losers (N=7), LiCl+treated losers (N=5). ** — $P < 0.01$, *** — $P < 0.01$ *vs* controls; ## — $P < 0.01$, ### — $P < 0.001$ *vs* Sal-treated losers; $$ — $P < 0.01$ *vs* LiCl-treated losers.

Table 1. Effects of chronic LiCl treatment on the anxious behavior of mice

Tests	Preventive treatment of the losers	Preventive treatment of the winners (48)	Therapeutic treatment of the winners (48)	Intact males (48)
Partition	Anxiolytic effects	Anxiogenic effects	No effects	-
Plus-maze	Anxiogenic effects (?)	Anxiogenic effects	No effects	Anxiolytic effects
Social interactions	Anxiogenic effects (40% of mice)	Anxiogenic effects (40% of mice)	Anxiolytic effects	Anxiolytic effects

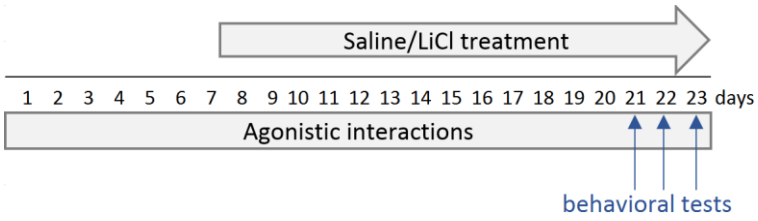

Figure 1. Scheme of experiment: chronic LiCl and/or saline treatment of defeated mice. Behavioral tests: Day 21 – the partition test and agonistic interaction; Day 22 – the elevated plus-maze test, Day 23 – the social interaction test.

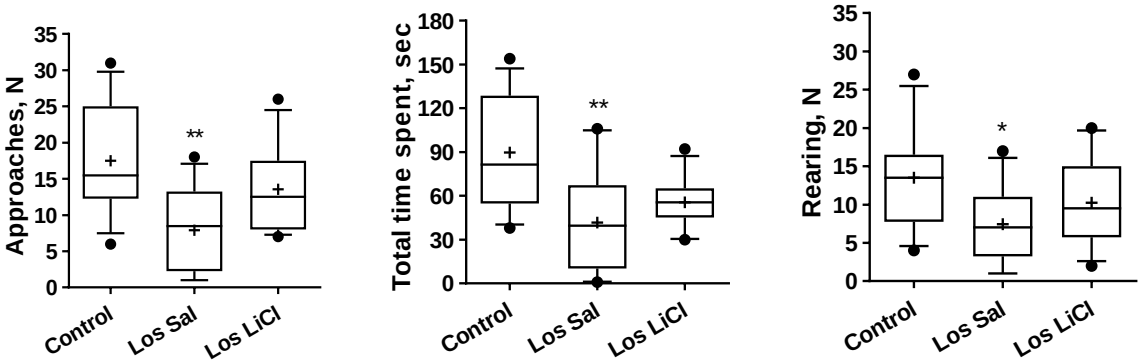

Figure 2. Effects of chronic LiCl treatment on the behavior of defeated mice in the partition test. Los Sal ─ Sal-treated losers, Los LiCl ─ LiCl-treated losers. Values are shown as means (*plus sign*) and medians (*solid lines*) and 25%/75% quartiles in a box-whisker plot with whiskers indicating 10th and 90th percentiles. * $P < 0.05$, ** $P < 0.01$ *vs* the controls; N=12 for each group.

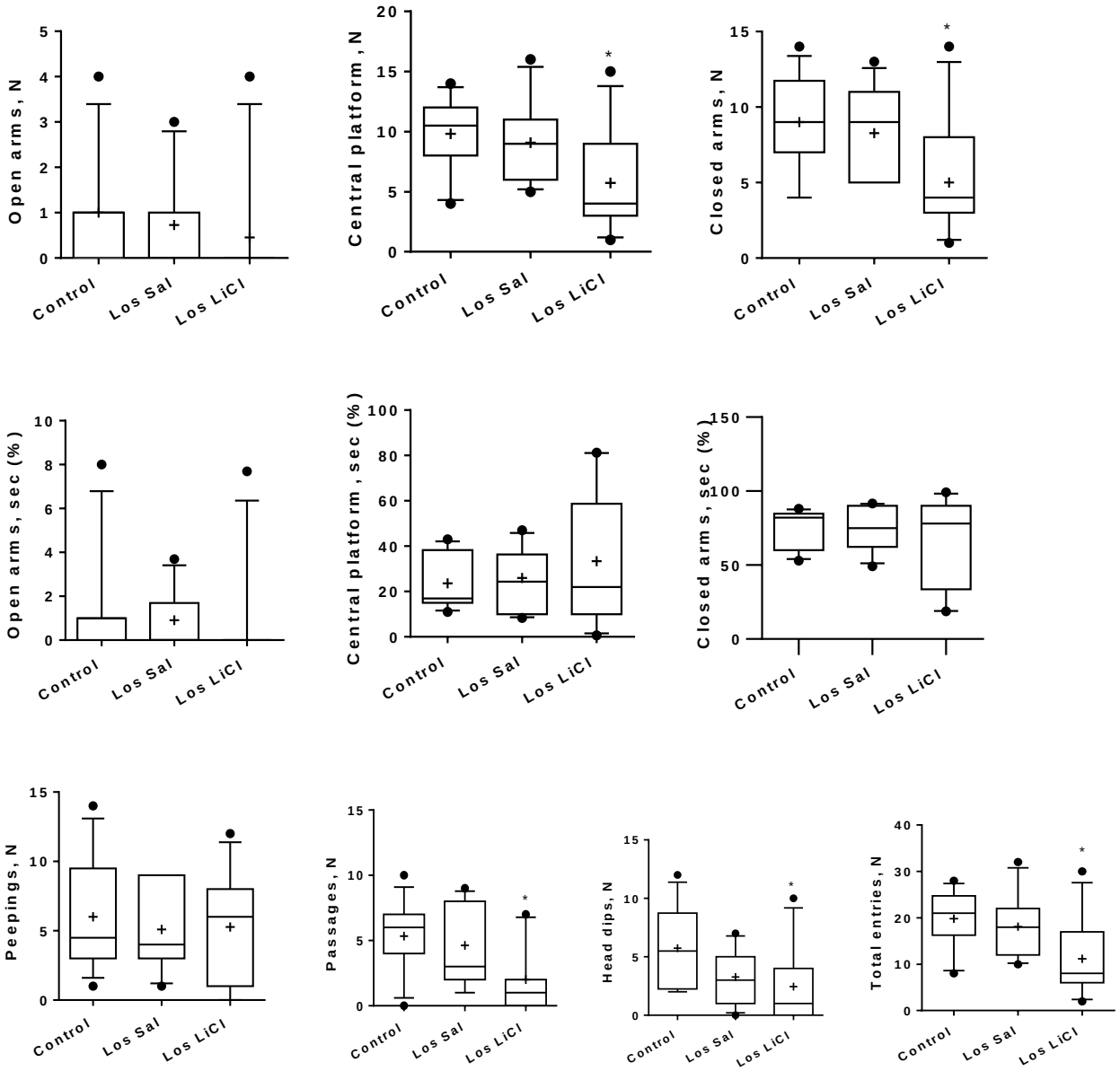

Figure 3. Effects of chronic LiCl treatment on the behavior of defeated mice in the plus-maze test. Los Sal – Sal-treated losers, Los LiCl – LiCl-treated losers. * — $P < 0.05$ *vs* controls, Tukey's multiple comparisons post hoc test; N = 12 for each group. Values are shown as means (*plus sign*) and medians (*solid lines*) and 25%/75% quartiles in box-whisker plot with whiskers indicating 10th and 90th percentile. * $P < 0.05$ *vs* the control group (*Tukey's multiple comparisons post hoc test*).

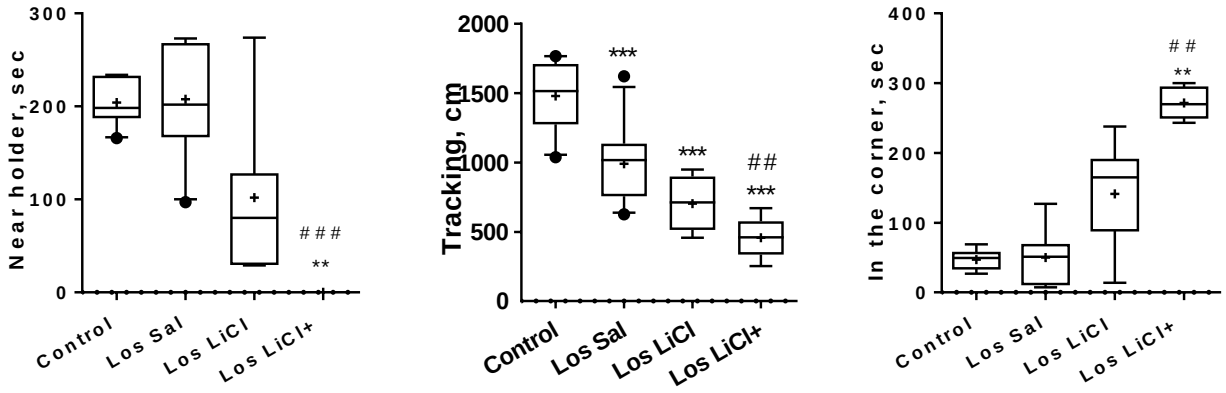

Figure 4. Effects of LiCl on the exploratory behavior of defeated mice in new situation and toward novel object (holder). Los Sal – Sal-treated losers, Los LiCl – LiCl-treated losers. Values are shown as means (*plus sign*) and medians (*solid lines*) and 25%/75% quartiles in box-whisker plot with whiskers indicating 10th and 90th percentile. ** — $P < 0.01$, *** — $P < 0.01$ *vs* controls; ## — $P < 0.01$, ### - $P < 0.001$ *vs* Sal-treated losers (*Tukey's multiple comparisons post hoc test*); Control (N=9); Sal-treated losers (N=11), LiCl-treated losers (N=7), LiCl+treated losers (N=5).

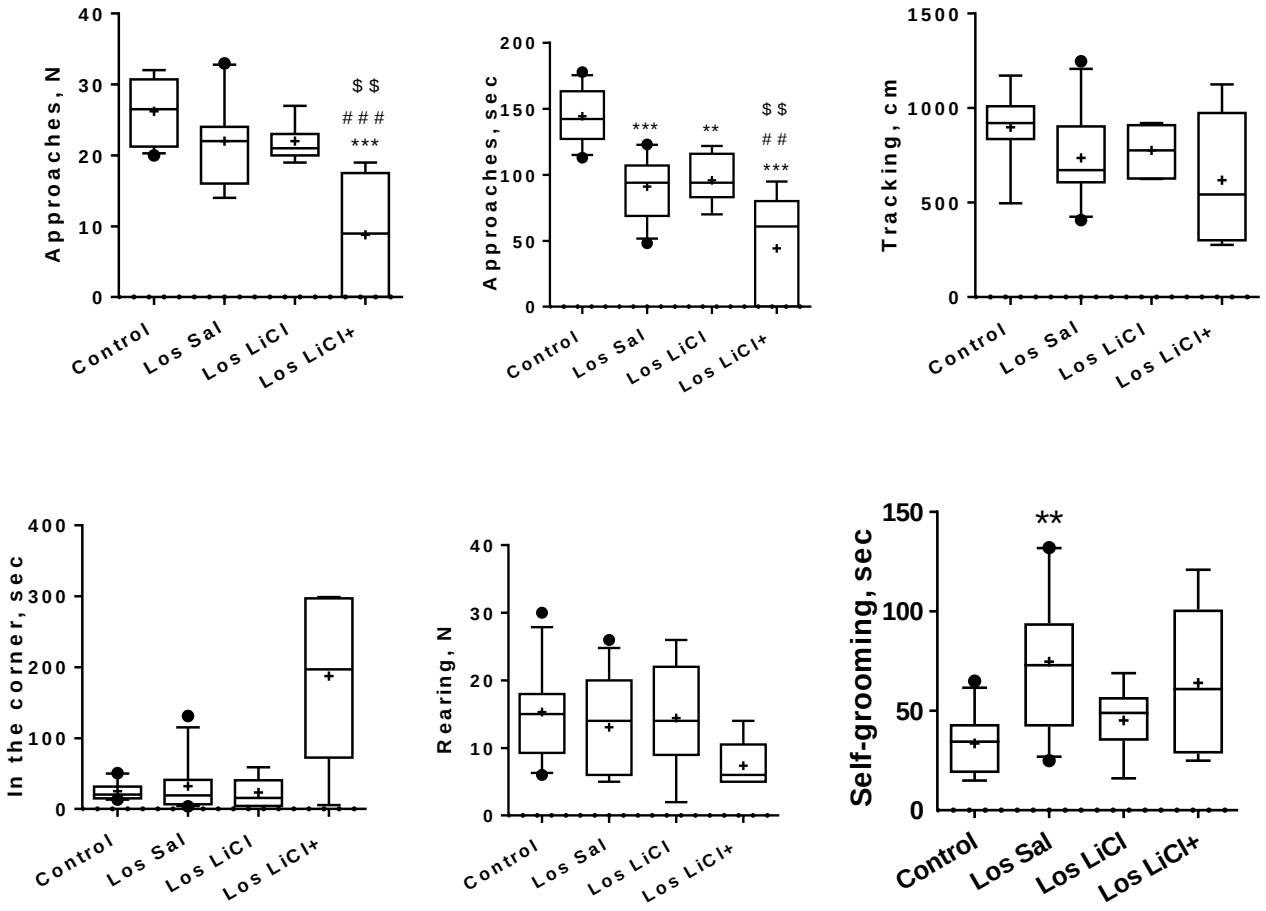

Figure 5. Effects of LiCl on the behavior of defeated mice in the social interaction test as a reaction to the partner under wire holder. Los Sal – Sal-treated losers, Los LiCl – LiCl-treated losers. Values are shown as means (*plus sign*) and medians (*solid lines*) and 25%/75% quartiles in box-whisker plot with whiskers indicating 10th and 90th percentile; For the control – N=9, Sal-treated losers – N=11, LiCl-treated losers – N=7), LiCl+treated losers – N=5. ** — $P < 0.01$, *** — $P < 0.01$ *vs* controls; ## — $P < 0.01$, ### — $P < 0.001$ *vs* Sal-treated losers; $$ — $P < 0.01$ *vs* LiCl-treated losers.

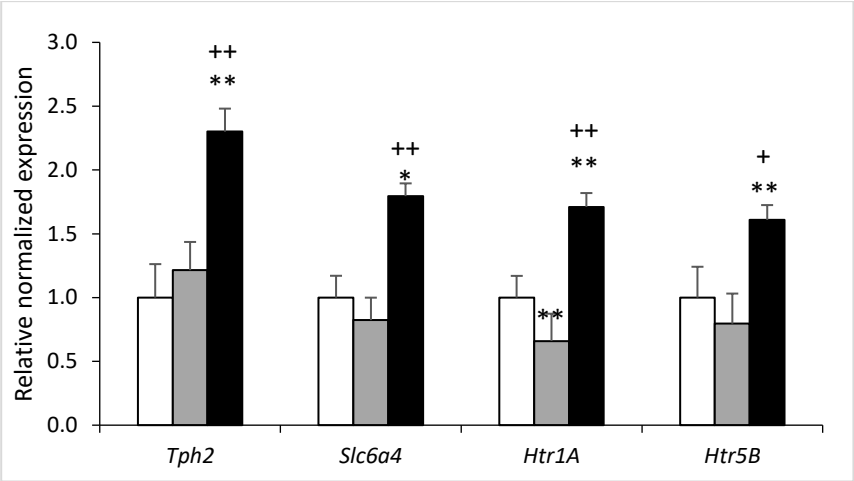

Figure 6. Influence of chronic LiCl treatment on serotonergic gene expression in the midbrain raphe nuclei of defeated mice. The measurement data is provided by the BioRad Amplifier software (USA). White columns – the control (N=9); grey columns – Sal-treated losers (N = 7); black columns – LiCl-treated losers (N=9); * – $P < 0.05$, ** – $P < 0.01$ *vs* controls; + – $P < 0.05$, ++ – $P < 0.01$*vs* Sal-treated losers, t-Student test. The data are reported as mean ± SEM.