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Shock-induced aggression in mice is modified by lithium

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ABSTRACT

Aggression is associated with numerous psychiatric disorders. Evidence suggests that lithium decreases aggression in humans and rats. The effects of lithium on aggression related behavior, and in particular shock-induced aggression, has not been as thoroughly explored in mice. Male mice were treated with lithium and tested in the shock-induced aggression and dominance tube tests. Mice treated with lithium were also assessed for thermal pain and shock sensitivity in the hot plate and jump-flinch tests. In the shock-induced aggression paradigm chronic lithium significantly decreased both the frequency and duration of attacks, without affecting social interaction or behavior in the dominance tube. Acute lithium significantly decreased the total duration of attacks and social interaction but did not affect behavior in the dominance tube test. Neither treatment regimen had an effect on temperature sensitivity in the hot plate test or on activity levels in the open field. However, chronic lithium modified the response of mice to shock in the jump-flinch test, but not at the shock level used in the aggression test. The results of this study indicate that lithium decreases shock-induced aggression in mice, but effects on baseline response to shock confound interpretation of this behavioral effect of lithium.

1. Introduction

Aggression is associated with numerous psychiatric disorders including autism, schizophrenia, affective disorders, and suicidal behavior (Fava, 1997; Joiner et al., 2005; Kovacsics et al., 2009; Riley et al., 1989; Swann, 1999). Aggressive symptoms can be reduced by treatment with pharmacological agents such as mood stabilizers (Fava, 1997). In particular, lithium is effective in reducing aggression in a variety of populations. The results of three double blind studies and one open clinical study indicate that four or more weeks of lithium treatment reduced aggression in children and adolescents with conduct disorder (Campbell et al., 1984, 1995; Malone et al., 1994, 2000). In three studies of long-term lithium treatment in prison populations, treatment significantly reduced aggressive behaviors exhibited by the prisoners (Sheard, 1971; Sheard et al., 1976; Tupin et al., 1973). Lithium has also been shown to be clinically effective in reducing aggression in females with psychiatric illness as well as in mentally handicapped patients (Craft et al., 1987; Worrall et al., 1975). Understanding the mechanism by which lithium acts to reduce human aggression would provide insight into novel treatment approaches.

Studies in both mice and rats have shown a decrease in aggressive behavior exhibited in the resident-intruder test when the isolated rodent is treated with lithium (Brain and Al-Maliki, 1979; Einat, 2007; Malick, 1978; Oehler et al., 1985; Sheard, 1973). Previous research has also demonstrated that both acute and chronic lithium treatments significantly reduce shock-induced aggression in rats (Eichelman et al., 1973; Marini et al., 1979; Mukherjee and Pradhan, 1976; Prasad and Sheard, 1982; Sheard, 1970). However, to date, the effects of lithium on shock-induced aggression have not been fully assessed in mice (O'Donnell and Gould, 2007). Only one study has assessed the effects of lithium on shock-induced aggression in mice (Nakao et al., 1985). A single injection of lithium carbonate was effective in reducing shock-induced aggression. However this study did not assess the possible confounding effects of lithium on activity or sensitivity to shock, nor did it assess the efficacy of long-term lithium treatment in reducing aggression. As mice are a genetically tractable species, future work to determine the molecular mechanisms underlying lithium's anti-aggressive effects may be aided by the use of genetically modified mice.

The present study was undertaken to assess the effects of both acute and chronic lithium treatments on shock-induced aggression and social behaviors in mice. As an additional measure of social behavior, we tested the effects of lithium on behavior in the dominance tube test. We also conducted several experiments (hot plate and jump-flinch tests) designed to determine the effect of lithium administration on sensitivity to pain and shock in CD-1 as well as C57BL/6J and FVB/NJ inbred strains. The open field test was performed to determine if lithium treatment altered activity levels.

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2. Methods

2.1. Mice

Male mice were obtained from outside breeding colonies. CD-1 (Charles River Laboratories Inc., Wilmington, Massachusetts), C57BL/6J and FVB/NJ (The Jackson Laboratories, Bar Harbor, Maine) mice were shipped to our vivarium and allowed to acclimate to the facility for at least 1 week before behavioral testing began. Mice to be tested in the dominance tube or shock-induced aggression tests were single housed upon arrival. Mice that were tested in the open field, hot plate, or jump-flinch tests were housed four mice per cage. All mice were housed in a room with constant temperature (~22 °C) and a 12 h light/dark cycle (lights on/off at 0700/1900) with free access to food and water. Mice were 8–12 weeks of age at the time of behavioral testing. For chronic lithium experiments, mice were tested in the dominance tube test and then 4 days later the same mice were tested in the shock-induced aggression test with different group-housed partners. CD-1 mice receiving chronic lithium treatment were tested in the jump-flinch test and 1 week later in the hot plate test. All experimental procedures were approved by the University of Maryland Animal Care and Use Committee, and were conducted in full accordance with the Guide for the Care and Use of Laboratory Animals.

2.2. Drugs

Acute treatment consisted of a LiCl (Sigma, Saint Louis, Missouri; 300 mg/kg LiCl dissolved in 0.9% saline) or saline alone administered via an i.p. injection 5 h prior to behavioral testing. Unpublished studies in our laboratory have shown that this time point results in peak brain levels and relatively low serum levels.

Chronic drug treatments began when the mice were 8 weeks old and consisted of randomizing cages to either control or lithium chow. Lithium chow (lithium chloride; LiCl, 4.0 g/kg) was identical to control chow with the exception of the added LiCl. The lithium chow was custom ordered from Bio-Serv (Frenchtown, New Jersey) and the diet was maintained for 3–4 weeks. Both groups received an additional bottle of saline (0.9% NaCl) to prevent ion imbalances resulting from lithium administration.

2.3. Open field test

General activity was assessed in an open field measuring 50 × 50 cm. Mice were placed in the center of the arena and were videotaped with an overhead camera for 10 min. TopScan automated software (CleverSys Inc., Reston, VA) was used to determine the distance traveled by each mouse.

2.4. Dominance tube test

Our dominance tube apparatus is constructed out of plexiglass and consists of a 36 cm long tube with a diameter of 3.5 cm that is attached on either end to a start box (measuring 10 × 8 × 8 cm). At the center of the tube is a clear gate with perforations that allow for olfactory and visual investigation, but not physical contact. A single housed mouse and an unfamiliar group-housed mouse were placed in opposite start boxes and allowed to acclimate to the apparatus for 3 min (Messeri et al., 1975). When the animals met in the middle of the tube after the acclimation period the center gate was lifted. The diameter of the tube is such that the mice cannot move past one another; typically one mouse will force the other to back out of the tube and into their start box. The test is concluded once one mouse has forced the other back, and a blinded observer records this result live. The apparatus was cleaned between each trial.

2.5. Shock-induced aggression

A single housed male mouse and an unfamiliar group-housed partner were placed in the testing chamber (Colbourn Habitest Chamber, measuring 20 × 20 × 30 cm). The chamber is a clear box with a metal rod floor through which electrical shocks are delivered. Pairs of mice were allowed to acclimate to the chamber for 2 min and during this time social interactions were observed. Social interactions include sniffing, allogrooming, and following behaviors. After this initial two minute period, shocks were delivered for 5 min to the mice (5 shocks/second at 0.26 mA intensity). This shock intensity was chosen because pilot testing indicated that at lower intensities the mice did not show robust aggressive behavior, but at higher intensities the mice exhibited jumping. After this time period mice were observed for an additional 3 min during which shocks were not delivered. Aggressive behavior (attacks) was recorded using a stop watch and counter by a trained, blinded observer during the entire 10 min test. The chamber was cleaned after each trial.

2.6. Hot plate test

Animals were tested for their reaction to thermal pain in the hot plate test. The testing apparatus consists of a hot plate with an attached plexiglass box (with no bottom) that prevents the mice from leaving the hot plate surface. Mice were placed in the center of the hot plate and the time it took for a mouse to lick a rear paw was recorded in real-time by a blinded observer. The rear paw lick measurement was used because it has been suggested to be a more reliable indicator of discomfort than the front paw (Bannon and Malmberg, 2007). The maximum length of a trial was 30 s. In eight week old CD-1 mice treated acutely with lithium, the hot plate was set to 58 °C. Twelve week old CD-1 mice that were treated chronically with lithium were tested on the hot plate at a temperature of 55 °C. The younger mice were tested at higher temperatures because pilot experiments demonstrated that the majority of younger mice did not respond within 30 s to the 55° stimulus.

2.7. Jump-flinch test

Mice were tested for their sensitivity to shock in the jump-flinch test. This test is conducted in the same chamber used for shock-induced aggression (Colbourn Habitest Chamber). Mice were presented with a single shock (1 s in duration) every 30 s. The shocks began at 0.1 mA and increased in 0.05 mA intensity to a maximum of 0.80 mA. The intensity at which an animal exhibited a flinch response (noticeable physical reaction to the shock), vocalization response (audible vocalization in response to the shock), and jump response (the mouse jumped with all 4 paws leaving the grid floor) was recorded in real-time by a blinded observer.

2.8. Lithium concentration assay

Mouse brains were analyzed for lithium content after acute or chronic lithium treatment. Brains were rapidly dissected, frozen in isopentane chilled on dry ice, and then stored at −80 °C. Lithium concentrations were determined following polytron homogenization of the entire brain in 3 volumes of 0.5 N trichloroacetic acid, followed by centrifugation (Gould et al., 2007; Hamburger-Bar et al., 1986). Lithium assays were performed with a digital flame photometer (Cole-Palmer Model 2655-00, Chicago, Illinois).

2.9. Statistical analysis

Statistical analysis was performed using GraphPad Prism Version 5 or SPSS Statistics 17.0. Fisher's exact test was used to analyze results from the dominance tube test. Un-paired *t*-test was used to compare

lithium and control groups in the open field, shock-induced aggression, hot plate, and jump-flinch tests. In the jump-flinch test, when one treatment group showed no variation in the intensity eliciting a flinch response, a one-sample *t*-test test was used. Jump-flinch test data at 0.25 mA was analyzed using Fisher's Exact test. For all analyses, $p \leq 0.05$ was considered significant. Data are presented as mean ± standard error. Animals that were more than 2 standard deviations above or below the group mean were deemed outliers and removed from the data set. This resulted in the removal of one animal from each treatment group in the analysis of the effect of acute lithium treatment on the frequency of aggressive behavior, but the statistical significance of this analysis was not altered. The remaining mice were removed from the jump-flinch test analyses. In 2 cases the statistical interpretation was altered. In chronically treated CD-1 mice, one mouse from each treatment group was removed from the vocalization analysis, and this resulted in an insignificant *p* value ($p = 0.11$) becoming significant. In the same cohort, one lithium treated mouse was removed from the jump analysis and this resulted in an insignificant *p* value ($p = 0.16$) becoming significant.

3. Results

Clinically, a response to lithium is typically not seen until after several weeks of treatment. However in animal tests of anti-depressant efficacy, such as the forced swim test, short term treatment with lithium results in a significant behavioral response (O'Donnell and Gould, 2007). Because of a range of temporal effects in preclinical models, in our studies we tested the effects of both acute and chronic lithium treatments on behavior. CD-1 mice were chosen for aggression testing because this strain typically displays aggressive behavior when isolated.

3.1. Open field, dominance tube, and shock-induced aggression tests: chronic lithium

In the open field test, chronic treatment with lithium did not significantly affect total distance (in mm) traveled ($t(22) = 1.66$, $p = 0.11$; mean ± SEM control, lithium: 37,107 ± 2138, 31,580 ± 2555). In the dominance tube test, 9 of 11 lithium treated and 10 of 13 saline treated single housed mice forced the group-housed partner to back out of the tube (Fig. 1A). There was no significant difference between the treatment groups in this test (Fisher's Exact Test, $p = 1.00$). Four days later the same mice were tested in the shock-induced aggression paradigm with an unfamiliar group-housed partner. During the initial two minute habituation period there was no significant difference between the treatment groups in the duration of time spent in social interactions (Fig. 1B; $t(25) = 0.50$, $p = 0.62$). During the entire 10 minute encounter lithium treated mice showed a significantly shorter duration of aggressive interactions (Fig. 1C; $t(25) = 2.43$, $p = 0.023$) and significantly fewer aggressive behaviors (Fig. 1D; $t(25) = 2.68$, $p = 0.013$) than control mice. At the time of testing the lithium treated mice weighed significantly less than the control mice ($t(25) = 3.85$, $p = 0.0007$). However, both the control and lithium treated groups were significantly lighter than their group-housed partners used for both behavioral tests ($t(26) = 2.32$, $p = 0.028$; $t(24) = 2.68$, $p = 0.013$). Thus, both treatment groups were tested against heavier partners in the dominance tube and aggression tests.

3.2. Open field, dominance tube, and shock-induced aggression tests: acute lithium

In the open field test, acute lithium treatment did not significantly alter the total distance traveled (in mm) in the open field ($t(22) = 0.54$, $p = 0.59$; mean ± SEM control, lithium: 38,781 ± 1412, 40,389 ± 2598). Dominance tube behavior was unaffected by lithium treatment; 6 of 8 control mice and 7 of 8 lithium treated mice forced the group-housed mice to back out of the tube (Fig. 2A; Fisher's exact test, $p = 1.00$). A separate cohort of CD-1 mice were administered a single i.p. injection of LiCl (300 mg/kg) and tested 5 h later in the shock-induced aggression test. During the initial acclimation period the lithium treated mice displayed a significantly shorter duration of social interaction than the control mice (Fig. 2B; $t(19) = 2.89$, $p = 0.0095$). Lithium significantly decreased the duration of aggression observed during the test (Fig. 2C; $t(19) = 2.18$, $p = 0.042$) but did not significantly modify the frequency of aggression, although a trend was observed for lithium treated animals to show fewer aggressive interactions (Fig. 2D; $t(17) = 2.059$, $p = 0.055$).

3.3. Hot plate test

A chronic dose of lithium administered to CD-1 mice did not significantly modify the latency of mice to lick their hind paw ($t(20) = 0.41$, $p = 0.69$; mean ± SEM control, lithium: 16.92 ± 2.93, 18.60 ± 2.79). Similarly, a single i.p. injection of lithium 5 h prior to hot plate testing did not significantly modify the latency to respond ($t(23) = 0.30$, $p = 0.77$; mean ± SEM control, lithium: 20.58 ± 1.55, 21.31 ± 1.87). Based on these results, it appears that lithium given either acutely or chronically does not modify sensitivity to thermal pain as measured by the hot plate test.

3.4. Jump-flinch test

CD-1 mice treated with a single injection of LiCl did not differ from control mice on the shock intensity that elicited a vocalization ($t(20) = 0.26$, $p = 0.80$) or jump response (Fig. 3A; $t(20) = 0.79$, $p = 0.44$). There was a trend for lithium to increase the flinch threshold (one-sample test, $t(9) = 2.090$, $p = 0.066$). These results suggest that acute lithium treatment had no effect on jump or vocalization response and a minimal (if any) effect on the flinch threshold. The same test was also conducted on a separate cohort of male CD-1 mice treated chronically with LiCl in the chow. A trend emerged for lithium to decrease the flinch threshold (Fig. 3B; $t(21) = 1.61$, $p = 0.079$). Lithium treated mice vocalized at a significantly higher shock intensity ($t(21) = 3.40$, $p = 0.0027$) and jumped at a significantly lower shock intensity ($t(22) = 2.076$, $p = 0.050$) compared to control mice (Fig. 3B). Thus, chronic lithium treatment has an effect on the animal's response to shock, but the direction of this effect is not clear as both a decrease (vocalization response) and an increase (jump response) in sensitivity was seen with lithium treatment. Weight may be a confound, as the lithium treated cohort weighed significantly less than control chow treated mice ($t(23) = 5.28$, $p < 0.0001$). We also examined the data to assess how lithium affected the reaction to shock at an intensity similar to that used in the shock-induced aggression paradigm, 0.25 mA (Fig. 3C). In CD-1 mice, chronic lithium treatment did not alter any of the responses (flinch: 11/11 control and 11/11 lithium mice flinched; vocalize: 3/11 control and 0/11 lithium mice vocalized, $p = 0.21$; jump: 1/11 control and 1/11 lithium mice jumped, $p = 1.00$) to this level of shock (Fig. 3C).

To assess if lithium-induced sensitivity to shock was strain dependent, and therefore that there may exist a better strain in which to conduct shock-induced aggression studies, we also tested C57BL/6J and FVB/NJ mice in the jump-flinch test after chronic lithium treatment (Fig. 4). Lithium treated C57BL/6J mice weighed significantly less than control mice ($t(22) = 7.026$, $p < 0.0001$), displayed no difference in flinch behavior (all mice flinched at the same intensity, 0.1 mA), vocalized at a significantly higher shock intensity ($t(22) = 2.089$, $p = 0.046$), and jumped at a significantly lower shock intensity ($t(21) = 4.75$, $p = 0.0001$) than control mice (Fig. 4A). It is possible that the effects of lithium on shock sensitivity may be influenced by weight, as chronic lithium treated CD-1 and C57Bl/6J mice lost weight while on the lithium diet. Therefore an

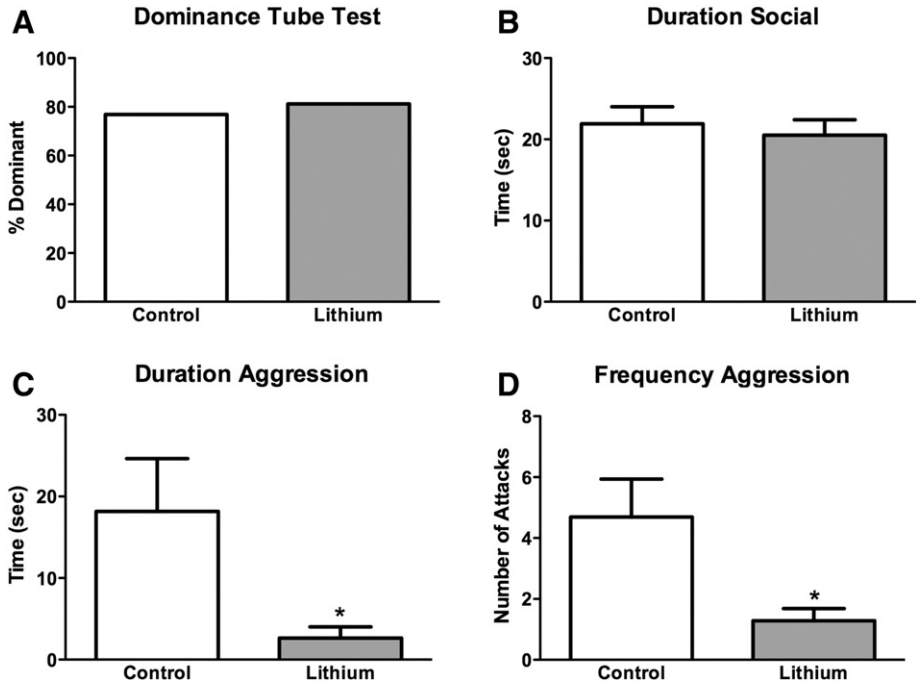

Fig. 1. Effects of chronic lithium in the dominance tube and shock-induced aggression tests. CD-1 mice received LiCl chronically in the chow (4 mg/kg). (A) Percentage of single housed mice in each group that forced the group-housed partner to retreat. (B) Duration of social behaviors exhibited during the initial two minute acclimation period of the shock-induced aggression test. (C) Duration of aggression observed over the entire shock-induced aggression testing session. (D) Frequency of aggression observed in the shock-induced aggression test. * $p<0.05$ (11–14 mice per group).

additional strain which does not lose weight with lithium treatment was also tested. FVB/NJ mice treated chronically with lithium did not differ in weight compared to the control chow treated mice ($t(22)=0.87$, $p=0.39$). Lithium treated FVB/NJ mice did not differ from controls on the intensity of shock eliciting a flinch response (Fig. 4B; $t(22)=1.44$, $p=0.16$). When compared to control chow treated mice, the lithium treated FVB/NJ mice vocalized at a significantly higher intensity ($t=4.78$, $d=20$, $p=0.0001$) and jumped at a significantly lower intensity than control mice (Fig. 4B; $t(20)=2.32$, $p=0.030$). The combined results of these studies suggest an effect of lithium on the sensitivity to shock that appears to be independent of strain or weight effects.

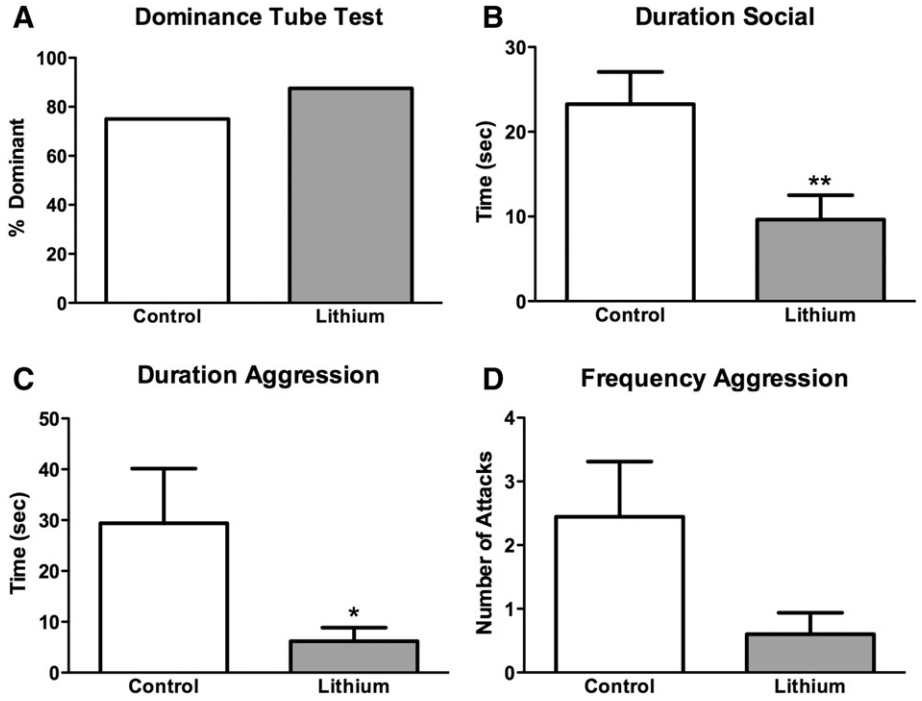

Fig. 2. Effects of acute lithium in the dominance tube and shock-induced aggression tests. CD-1 mice were administered a single i.p. injection of LiCl (300 mg/kg) or saline and tested 5 h later in the shock-induced aggression test. (A) Percentage of single housed mice in each group that forced the group-housed partner to retreat. (B) Duration of social behaviors exhibited during the initial two minute acclimation period of the shock-induced aggression test. (C) Duration of aggression observed over the entire shock-induced aggression testing session. (D) Frequency of aggression observed during the entire session. * $p<0.05$; ** $p<0.01$ (10–11 mice per group).

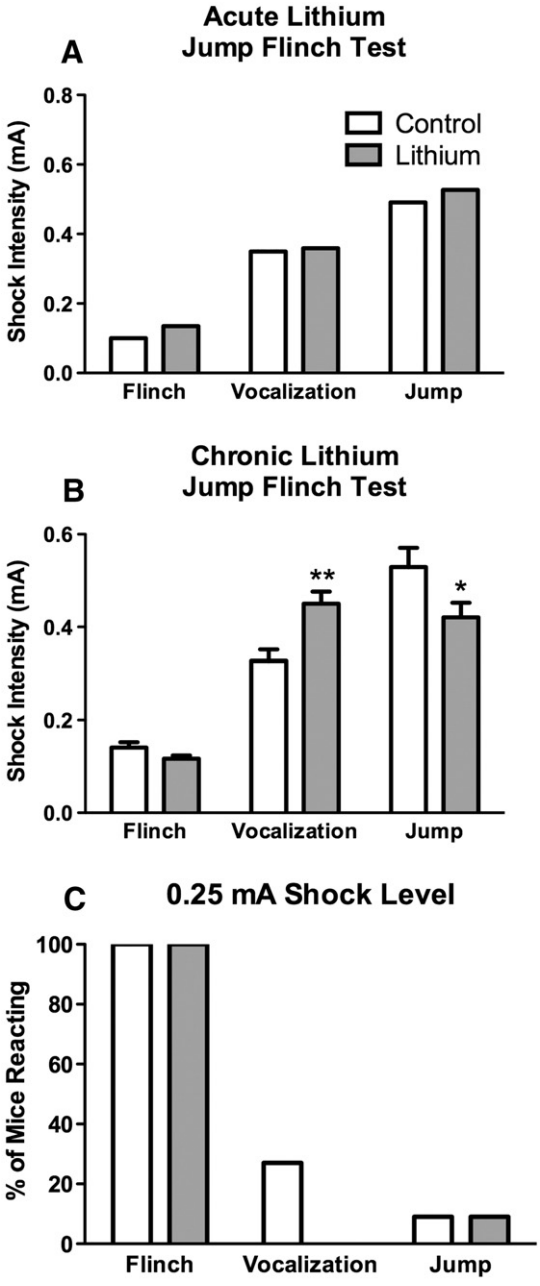

Fig. 3. Shock intensity at which CD-1 mice exhibited their first flinch, vocalization, or jump response. (A) CD-1 mice were given a single i.p. injection of LiCl (300 mg/kg) or saline and tested in the flinch jump test 5 h later. (B) CD-1 mice received LiCl chronically in the chow (4 mg/kg) and were tested in the jump-flinch test. (C) Percentage of chronically treated CD-1 mice responding to a 0.25 mA shock in the jump-flinch test. * $p<0.05$; ** $p<0.01$ (10–11 mice per group).

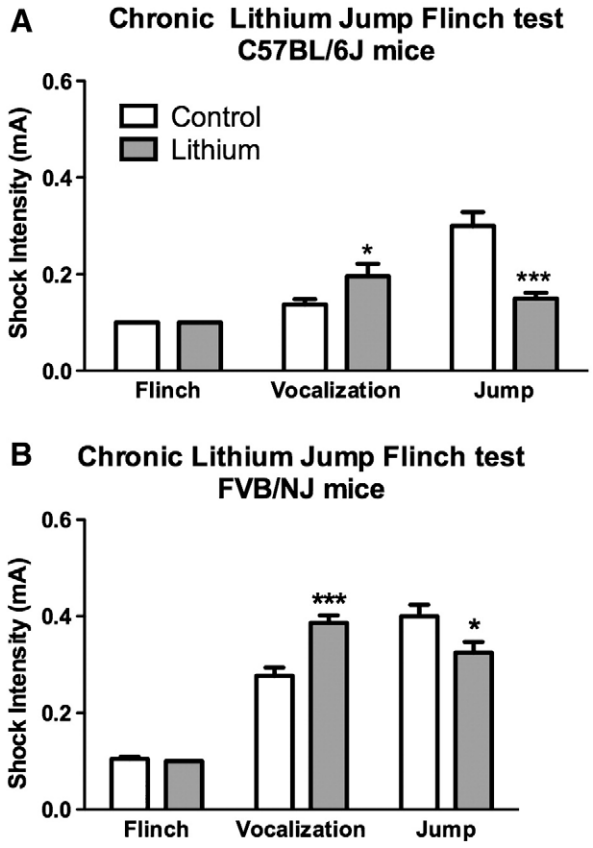

Fig. 4. Shock intensity at which C57BL/6J and FVB/NJ mice exhibited their first flinch, vocalization, or jump response. Mice received LiCl chronically in the chow (4 mg/kg). (A) Jump-flinch test results for C57Bl/6J mice. (B) Jump-flinch test results for FVB/NJ mice. * $p<0.05$; *** $p<0.001$ (10–12 mice per group).

3.5. Brain lithium levels

Brain lithium levels were assessed in a subset of mice of each strain tested. Chronic administration of LiCl in the chow of CD-1 mice ($n=8$) resulted in brain lithium levels of 1.04 ± 0.05 mmol/kg wet weight. Chronic administration of LiCl in the chow of C57BL/6J mice ($n=8$) resulted in brain lithium levels of 0.90 ± 0.02 mmol/kg wet weight. Chronic administration of LiCl in the chow of FVB/NJ mice ($n=12$) resulted in brain lithium levels of 0.64 ± 0.02 mmol/kg wet weight. Acute administration of LiCl via a single i.p. injection (300 mg/kg) to CD-1 mice ($n=8$) resulted in brain lithium levels of 1.14 ± 0.05 mmol/kg wet weight 5 h following administration. Thus, all brain lithium levels were within the range observed in the blood of patients undergoing lithium therapy (0.6 to 1.3 mM).

4. Discussion

Chronic treatment of CD-1 mice with lithium decreased the number and duration of aggressive interactions without modifying activity levels and social interaction in the shock-induced aggression test, or behavior in the dominance tube. Similarly, acute lithium treatment did not significantly modify activity levels or dominance tube behavior. Acute treatment with lithium decreased the duration of aggression, but also decreased the duration of social interaction in the shock-induced aggression test. These data from the aggression testing are similar to what has been previously shown in rats, where lithium reduced shock-induced aggression (Eichelman et al., 1973; Marini et al., 1979; Mukherjee and Pradhan, 1976; Prasad and Sheard, 1982; Sheard, 1970). Our results indicate that chronic lithium treatment reduces aggressive behavior in the shock-induced aggression test, and while a similar effect is seen with acute treatment these data are confounded by a significant decrease in social behavior with lithium treatment.

It is possible that the decrease in aggression seen in the shock-induced aggression test could be due to an effect of lithium to alter pain sensitivity. Some clinical evidence suggests that lithium may decrease an individual's sensitivity to pain (Fontrier, 2004; Goldstein, 1985; Tosca et al., 1981). We observed mice in both the hot plate and jump-flinch tests. Neither acute nor chronic lithium resulted in a change in hot plate sensitivity, suggesting that lithium does not alter sensitivity to thermal pain. Our results differ from some previously published reports examining the effect of lithium on this test in mice.

In mice, a single injection or 10 days of lithium treatment in the chow was shown to decrease pain sensitivity in the hot plate test (Amir and Simantov, 1981; Saarnivaara and Mannisto, 1976). In rats, two studies found that lithium treatment decreased the sensitivity to thermal pain in the hot plate test (Staunton et al., 1982; Yirmiya et al., 1988). However, another study in rats showed that acute treatment with lithium did not alter responses in the hot plate test, which is similar to our findings in mice treated acutely and chronically with lithium (McNally and Westbrook, 1998).

We found that chronic, but not acute, treatment with lithium significantly modified sensitivity to shock, and this effect was seen in three different mouse strains. Chronic lithium treatment resulted in an increased threshold for a vocalization response, as treated mice vocalized at a significantly higher shock intensity. However the lithium treated mice jumped at a lower shock intensity than the control group, suggesting an increased sensitivity for this response. This apparent discrepancy could be related to an inadequacy of the measure used for vocalization. The current study only assessed audible vocalizations, but it is possible that lithium may also alter ultrasonic vocalizations. An additional measure to assess ultrasonic vocalization was not possible as part of this study, but the results of using such an approach could possibly reconcile the discrepancy between vocalization and jump thresholds. Chronic lithium administration produced similar results in the jump-flinch test in CD-1, C57BL/6J, and FVB/NJ mouse strains, which suggests that this effect of lithium is independent of strain. This finding also suggests that this effect is independent of weight, since mice that either lose weight while on lithium (CD-1, C57BL/6J) or maintain their weight while on lithium (FVB/NJ) both showed similar changes in shock sensitivity. To our knowledge, no previous work has assessed lithium's effects on the jump-flinch test in mice. A study where lithium was administered to rats for 2 weeks found no effect of the treatment on the flinch or vocalization response, but found that lithium decreased the threshold which elicited a jump response (Harrison-Read and Steinberg, 1971). A separate study with rats found no difference between control and lithium treated rats (LiCl injections i.p. for 5 days) in flinch or jump response to electrical shock (Sheard, 1970). We further examined the jump-flinch data in CD-1 mice by assessing responses to a 0.25 mA shock, which is similar to the 0.26 mA shock delivered during the shock-induced aggression test. The control and lithium groups did not differ significantly in any of their three responses to this shock intensity. These data suggest that in CD-1 mice at this particular shock intensity, chronic lithium does not alter sensitivity to a shock stimulus and thus may not have confounded the interpretation of our results from the shock-induced aggression test.

Lithium is often used in clinical practice as an adjunct antidepressant agent. In mouse studies, some antidepressants of the selective serotonin re-uptake inhibitor class (SSRIs) are effective in modifying aggression. In mice, fluoxetine, sertraline, and fluvoxamine significantly reduced aggression in the resident-intruder paradigm (Matsumoto et al., 2007; Sanchez and Hyttel, 1994). Citalopram and paroxetine do not appear to be effective in reducing baseline aggression, but chronic treatment with citalopram reduces alcohol-heightened aggression in mice (Caldwell and Miczek, 2008; Sanchez and Hyttel, 1994). In rats, the effects of SSRIs on aggression appear to be dependent on the time course of administration. Acute doses of fluoxetine, citalopram, and paroxetine decrease resident-intruder and shock-induced aggression in rats (Datla et al., 1991; Mitchell and Redfern, 1992; Mitchell and Fletcher, 1993). However, chronic doses of fluoxetine and paroxetine increase resident-intruder aggression in rats (Mitchell and Redfern, 1992, 1997).

Lithium has a clinical profile similar to other mood stabilizers such as valproate and carbamazepine. In rats, valproate was reported to have no effect on shock-induced aggression (Rodgers and Depaulis, 1982). In REM sleep deprived rats, acute carbamazepine increased aggression and chronic carbamazepine decreased aggression (Tannhauser et al., 1984). When given both acutely and chronically to mice, valproate significantly reduces aggression in the resident-intruder test (Einat, 2007; Krsiak et al., 1981; Simler et al., 1982, 1983). Both valproate and carbamazepine have been shown to significantly reduce aggression in the shock-induced aggression paradigm in mice (Nakao et al., 1985; Puglisi-Allegra et al., 1981). Drug induced aggression in mice is also reduced by these mood stabilizers: valproate reduced apomorphine induced aggression and carbamazepine reduced clonidine induced aggression (Allegra et al., 1979; Fujiwara et al., 1988).

Experiments in the current study did not assess the mechanisms by which lithium may decrease aggressive behavior in mice. Previous work has suggested that, among many neurotransmitter systems, dysregulation of the serotonin system has been most consistently implicated in aggressive behavior in both humans and rodents. Low levels of the main serotonin metabolite, 5-hydroxyindolacetic acid (5-HIAA) in cerebrospinal fluid (CSF) have been associated with aggression in humans (Brown et al., 1982; Stanley et al., 2000). In rodents, serotonin levels, turnover, and 5-HIAA levels are negatively correlated with aggressive behavior (van Erp and Miczek, 2000). Additionally, administration of para-chlorophenylalanine (pCPA), a potent tryptophan hydroxylase inhibitor, increases aggression in both mice and rats (Chiavegatto et al., 2001; Vergnes et al., 1986). Mouse genetic knockout approaches that decrease serotonin levels also increase aggression (Beaulieu et al., 2008; Hendricks et al., 2003).

Emerging evidence indicates that serotonin neurotransmission may modify the activity of glycogen synthase kinase 3β (GSK-3β), an enzyme which has been implicated in the pathophysiology of numerous psychiatric disorders and is directly inhibited by lithium (Gould and Manji, 2005; Klein and Melton, 1996). Increasing the brain levels of serotonin in mice has been shown to increase inhibition of GSK-3β (Li et al., 2004). Previous work reported that knockin mice expressing a mutant form of the tryptophan hydroxylase 2 gene exhibit an 80% loss of serotonin in the brain and display increased aggression (Beaulieu et al., 2008). When these mice receive treatment with a GSK-3 inhibitor (TDZD-8), or lack one copy of GSK-3β, the aggression is attenuated (Beaulieu et al., 2008). A decrease in serotonergic neurotransmission thus appears to increase activity of GSK-3β. As decreased serotonin is associated with aggression in rodents, it is possible that lithium's ability to decrease aggression is based upon the drug's ability to inhibit GSK-3β. Future work will require additional genetic and pharmacological tools to investigate the relevance of serotonin and GSK-3β in the anti-aggressive effect of lithium.

In addition to being effective for the treatment of mood disorders, lithium has shown efficacy in reducing aggression in a variety of human populations (Campbell et al., 1984, 1995; Craft et al., 1987; Malone et al., 1994, 2000; Sheard, 1971; Sheard et al., 1976; Tupin et al., 1973; Worrall et al., 1975). Numerous studies have demonstrated that lithium decreases suicidal behavior (for meta-analysis and review see: (Baldessarini et al., 2006; Kovacsics et al., 2009)). The anti-aggressive property of lithium may be crucial to its additional clinical efficacy in reducing suicidal behaviors where aggression is hypothesized to play a role. Determining the molecular mechanisms underlying lithium's anti-aggressive effects may suggest new approaches for the treatment of aggression.

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