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Dose response effects of lithium chloride on conditioned place aversions and locomotor activity in rats

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Abstract

The present study examined the multi-variable locomotor activity effects of lithium chloride (LiCl) treatment in male rats. Of interest was a determination of which variables might show a dose–response relationship in LiCl-induced conditioned place aversions. Automated open-fields were partitioned into two chambers distinct in tactile and visual cues. A control group [$n=8$] received saline (NaCl; 0.15 M) paired with both chambers while three LiCl groups (0.15 M; 32 mg/kg [$n=7$], 95 mg/kg [$n=7$], 127 mg/kg [$n=7$]) received LiCl paired with the normally preferred chamber and saline paired with the non-preferred chamber. During extinction trials, rats were allowed to choose between the two chambers to provide an index of conditioned place aversions. Locomotor activity and its distribution within the chambers were also assessed during both conditioning and extinction trials. Dose-dependent decreases occurred in all measures of locomotor activity following LiCl administration during conditioning. During extinction trials, place aversions developed in animals conditioned with LiCl. LiCl-treated rats spent significantly less time in the LiCl-paired chamber relative to controls but not in a dose-dependent manner. Animals that had been conditioned with 95 or 127 but not 32 mg/kg LiCl, displayed significantly more vertical activity in the LiCl-paired chamber than controls during extinction trials. These findings indicate that, in addition to producing dose-dependent unconditioned effects on locomotor activity, LiCl also produces dose-dependent conditioned effects on vertical activity. These conditioned rearing response effects provide a valid measure of the conditioned avoidance response that provides evidence for dose-dependent LiCl-induced conditioned place aversions.

Keywords: Lithium chloride; Place conditioning; Biased conditioning apparatus; Nausea; Toxin; Long-Evans rat; Open-field

1. Introduction

Lithium chloride (LiCl) is a toxin that induces vomiting in species with an emetic reflex, including some primates, dogs, cats, and ferrets (Borison, 1989). Furthermore, LiCl produces symptoms consistent with visceral illness in species that do not vomit, such as rats and mice (Ossenkopp and Eckel, 1995; Parker, 2003). For example, LiCl administration causes a reduction in food intake (e.g., Curtis et al., 1994), decreases sodium consumption following sodium depletion (e.g., Chavez et al., 1995), and induces conditioned taste aversions to flavors paired with administration of the toxin (e.g., Eckel and Ossenkopp, 1996; Ossenkopp et al., 2003; Parker, 1982; Zalaquett and Parker, 1989; Nachman and Ashe, 1973; Nachman, 1970). In addition to producing conditioned taste aversions, LiCl also causes conditioned place aversions to environments previously paired with toxin administration (e.g., Frisch et al., 1995; Miller et al., 2000; Parker, 1992; Turenne et al., 1996; White and Carr, 1985).

LiCl-induced conditioned place aversions have been found following the pairing of specific environmental cues and a range of doses of the toxin. For example, Khroyan et al. (1995) demonstrated that rats spent significantly less time in a chamber paired with administration of 127 mg/kg LiCl than in a chamber paired with saline injections. Similarly, rats spent significantly less time in a chamber

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when it was paired with injections of 62 mg/kg (White and Carr, 1985) or 75 mg/kg LiCl (Parker and McDonald, 2000) relative to a saline-paired chamber. It also was demonstrated that injections of 50 mg/kg LiCl significantly reduced the amount of time spent in the drug-paired context (Turenne et al., 1996) as did doses of 40 and 20 mg/kg LiCl (Miller et al., 2000). However, administration of 10 mg/kg LiCl did not produce a significant decrease in the time spent in the toxin-paired chamber (Miller et al., 1999, 2000).

Despite much research on dose-related effects of LiCl-induced conditioned place aversion, no clear dose-dependent effects of LiCl on the strength of the conditioned avoidance response were found in these previous studies when time spent in the drug-paired chamber was used as the measure of aversion. Some studies demonstrated that increasing doses of LiCl decreased the amount of time spent in the toxin-paired chamber in a dose-related manner (Miller et al., 1999, 2000), while others indicated that no dose-dependent relationship exists (White and Carr, 1985).

It is known that LiCl administration tends to suppress locomotor activity in rats (e.g., Johnson, 1972, 1975; Ladowsky and Ossenkopp, 1986; Meachum and Bernstein, 1992; Parker et al., 1984). For example, injections of 95 mg/kg LiCl have been shown to significantly reduce ambulation in an open-field (Smith, 1981) and treatment with 64 mg/kg LiCl causes significant decreases in three measures of locomotor activity (Smith, 1983). However, few studies have examined the effects of LiCl on locomotor activity during both the conditioning and test (extinction) trials in the place aversion procedure. A detailed analysis of locomotor activity throughout the place conditioning procedure could provide additional indices of LiCl-induced conditioned place aversion and clarify a putative dose–response relationship.

Measuring activity during conditioning trials allows for the examination of the unconditioned (direct) effects of the toxin while measures of activity obtained during test (extinction) trials allow for the assessment of conditioned locomotor effects of the toxin. Conditioned effects are those effects that are elicited in an environment previously paired with toxin administration. One such study examining unconditioned and conditioned responses, observed that, during conditioning, the duration and frequency of rearing exhibited by rats decreased, along with crossings over gridlines within the chamber, following administration of 127 mg/kg LiCl (Parker et al., 1984). During test trials, these rats conditioned with 127 mg/kg LiCl displayed an increase in rearing duration and “limb flicks” (rapid shaking of the forepaws). In addition, Meachum and Bernstein (1992) recorded decreased grooming during conditioning in rats administered 127 mg/kg LiCl. During the test trial, these rats exhibited an increase in “freezing” behaviour as well as decreased grooming.

The purpose of the present study was to examine activity variables for additional evidence of a dose–response relationship in LiCl-induced conditioned place aversion using a new, automated, two-chamber place conditioning apparatus that allowed for multi-variable assessments of locomotor activity. Locomotor activity was measured during both conditioning and extinction trials allowing for an examination of both the unconditioned and conditioned effects of LiCl.

2. Materials and methods

2.1. Subjects

The subjects were 29 male Long-Evans rats (Charles River, Canada) weighing between 290 and 350 g at the start of the experiment. The rats were housed in pairs in standard polypropylene cages ($45 \times 22 \times 20$ cm) in a temperature-controlled colony room (20 ± 1 °C) maintained on a 12:12 h light:dark cycle (lights on at 07:00) with ad libitum access to both food (Prolab RMH3000 lab chow) and tap water. All testing took place during the light phase of the light:dark cycle. All procedures were approved by the University of Western Ontario Animal Care Committee and were in accordance with the Canadian Council of Animal Care (CCAC) Guidelines.

2.2. Drug

Lithium chloride (Fisher Scientific, Toronto, ON) was dissolved in distilled water to a molarity of 0.15 M. Doses of LiCl were achieved by manipulating the volume of the 0.15 M solution and were administered at 32, 95 and 127 mg/kg intraperitoneally (i.p.). These doses of LiCl were chosen on the basis of past research demonstrating LiCl induced-conditioned place aversions with doses ranging from 20 mg/kg to 127 mg/kg (Khroyan et al., 1995; Miller et al., 2000; Parker, 2003). Isotonic (0.9%, 0.15 M NaCl; i.p.) saline was used both as the 0 mg/kg LiCl salt solution, administered at 5 ml/kg and as a control injection for LiCl, administered in the same volume as LiCl.

2.3. Apparatus

The two chamber, place-conditioning apparatus consisted of eight, modified Versamax Animal Activity Monitors (Accuscan Model RXYZCM-16, Columbus, OH). Each monitor consisted of a clear Plexiglas open-field ($40 \times 40 \times 30.5$ cm) covered by a Plexiglas lid with air holes. Infrared photobeams were located 2.54 cm apart and 5.7 cm above the floor along the perimeter of the box (16 beams per side). Two additional banks of 16 photobeams were located on opposite sides of the box, 2.54 cm apart and 16.4 cm above the floor. Beams breaks in lower and upper banks correspond to movements in the horizontal or vertical plane, respectively (Ossenkopp and Kavaliers, 1996). All activity monitors were connected to a Versamax data analyzer (Accuscan Model DCM-8, Columbus, OH), which then transmitted data to an IBM Pentium II computer for

further analysis. Locomotor activity and its distribution within the two chambers were quantified using the Versamax Software System (Version 2.60, Accuscan, Columbus, OH).

Each Animal Activity Monitor was divided into two equal sized chambers ($20 \times 40 \times 30.5$ cm) by a clear Plexiglas partition located parallel to the elevated photobeams so as not to interrupt these sensors. The two chambers differed in visual wall cues and tactile floor cues. One chamber contained a removable clear, smooth plastic floor while the other chamber contained a removable clear, rough Perspex floor. The outside wall of each chamber displayed either a solid gray or black (1.8 cm) and white (2 cm) striped pattern. During conditioning trials, a solid Plexiglas partition confined the animal to one chamber and the walls of both chambers displayed the same visual pattern. During extinction trials, a Plexiglas partition with a doorway (10×15 cm) allowed passage between the chambers with each chamber displaying one of the wall patterns. During both conditioning and extinction, the gray wall pattern was paired with the rough-textured floor and the black and white striped wall pattern was paired with the smooth floor. Average illumination in the rough floor-gray wall chamber was 1065.5 ± 209.6 lx (measured with Digital Illuminometer, Mitchell Instruments Model YF-1065F, San Marcos, CA) and average illumination in the smooth floor-striped wall chamber was 1084.0 ± 214.2 lx.

The place preference and activity variables were quantified directly by the Versamax analyzer for each of the two chambers. The place preference measure was Time (s) spent in each chamber during extinction trials. Horizontal activity measures included Total Horizontal Distance (cm), Horizontal Movement Time (s), and Number of Horizontal Movements. Vertical activity measures included Vertical Time (s) and Number of Vertical Movements.

2.4. Procedure

2.4.1. Conditioning

Rats arrived in the laboratory at least 1 week prior to the commencement of experimental manipulations. Animals were handled on each of 3 days before beginning conditioning. An overview of the temporal aspects of the conditioning procedure is presented in Fig. 1. Subjects received two conditioning cycles, each consisting of four, 30 min conditioning trials. Conditioning trials were 30 min in length because LiCl exerts maximal effects on behaviour 15–30 min following administration (e.g., Parker et al., 1984). The conditioning trials were separated by 24 h and alternated between drug and saline trials. Thus, each conditioning cycle was comprised of two drug and two saline trials. The two conditioning cycles were separated by 72 h. Prior to conditioning trials, animals were weighed and following administration of drug or saline were immediately placed in the apparatus. Each apparatus and all removable floors were cleansed with a mild detergent solution and rinsed with a baking soda solution after each conditioning and extinction trial.

On odd-numbered conditioning trials (Trials 1, 3, 5, 7), different groups of rats received injections (i.p.) of one of four doses of LiCl (0 mg/kg [$n=8$], 32 mg/kg [$n=7$], 95 mg/kg [$n=7$], and 127 mg/kg [$n=7$]) and were then confined to the rough floor-gray wall chamber for 30 min. Using a biased place conditioning procedure, injections of LiCl were always paired with the most-preferred rough floor-gray wall chamber as shown in previous studies (Ossenkopp et al., 2002). On even-numbered conditioning trials (Trials 2, 4, 6, 8), rats received control injections of 0.15 M NaCl in the same volume as the LiCl injection and were confined to the smooth floor-striped wall chamber for 30 min. Locomotor activity was measured during each of the conditioning trials.

2.4.2. Extinction

Seventy-two hours following the last conditioning trial, rats received four, 20 min extinction trials separated by 24 h. Extinction trials were 20 min in length to minimize the effects of habituation on locomotor activity which occur after 20 min (Engeland et al., 2003). Rats received no injections prior to the extinction trials. The animals were initially placed in the corner of the chamber previously paired with LiCl and allowed unrestricted access to both chambers. Locomotor activity and the spatial distribution of behaviour were assessed in each chamber.

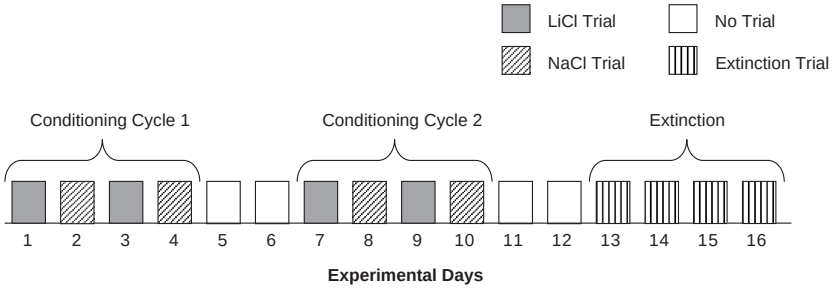

Fig. 1. An overview of the timeline (days) of the conditioning and extinction procedures. “LiCl Trial” indicates conditioning trials where rats received injections of one of four doses of LiCl and were then confined to the most-preferred chamber for 30 min. “NaCl Trial” indicates conditioning trials where rats received control injections of NaCl and were then confined to the less-preferred chamber for 30 min. “No Trial” indicates that no conditioning took place on these days. “Extinction Trial” indicates that rats received no injections but were allowed unrestricted access to both chambers for 20 min.

2.5. Statistical analyses

Behavioural data obtained during both conditioning and extinction trials were analyzed using a mixed design analysis of variance (ANOVA) for each measure. Post-hoc tests of significant main effects and interactions were conducted among groups using Tukey's HSD method. All hypothesis tests used $\alpha = .05$ to determine significance. All data were analyzed using SPSS 11.0 for Windows.

3. Results

3.1. Effects of lithium chloride during conditioning

The effects of LiCl on horizontal and vertical locomotor activity during conditioning are depicted in Figs. 2 and 3, respectively. These data were analyzed using a mixed-design ANOVA with a between-subjects factor of LiCl-Dose (4 levels: 0, 32, 95 and 127 mg/kg) and two within-subjects factors of Treatment (2 levels: LiCl and saline) and Trial (4 levels: 4 conditioning trials). LiCl administration significantly suppressed locomotor activity during conditioning as a significant main effect of LiCl-Dose, collapsed over both Trial and Treatment, was obtained for all activity variables including total horizontal distance [$F(3,25) = 10.15$, $P < 0.001$], horizontal movement time [$F(3,25) = 9.58$, $P < 0.001$], number of horizontal movements [$F(3,25) = 8.70$, $P < 0.001$], vertical time [$F(3,25) = 15.91$, $P < 0.001$], and number of vertical movements [$F(3,25) = 7.79$, $P < 0.01$]. Post-hoc tests were conducted for each conditioning trial to further analyze these significant LiCl-Dose effects on locomotor activity. Significant group differences are displayed in Figs. 2 and 3.

This analysis also revealed a significant effect of Treatment for horizontal movement time [$F(1,25) = 7.49$,

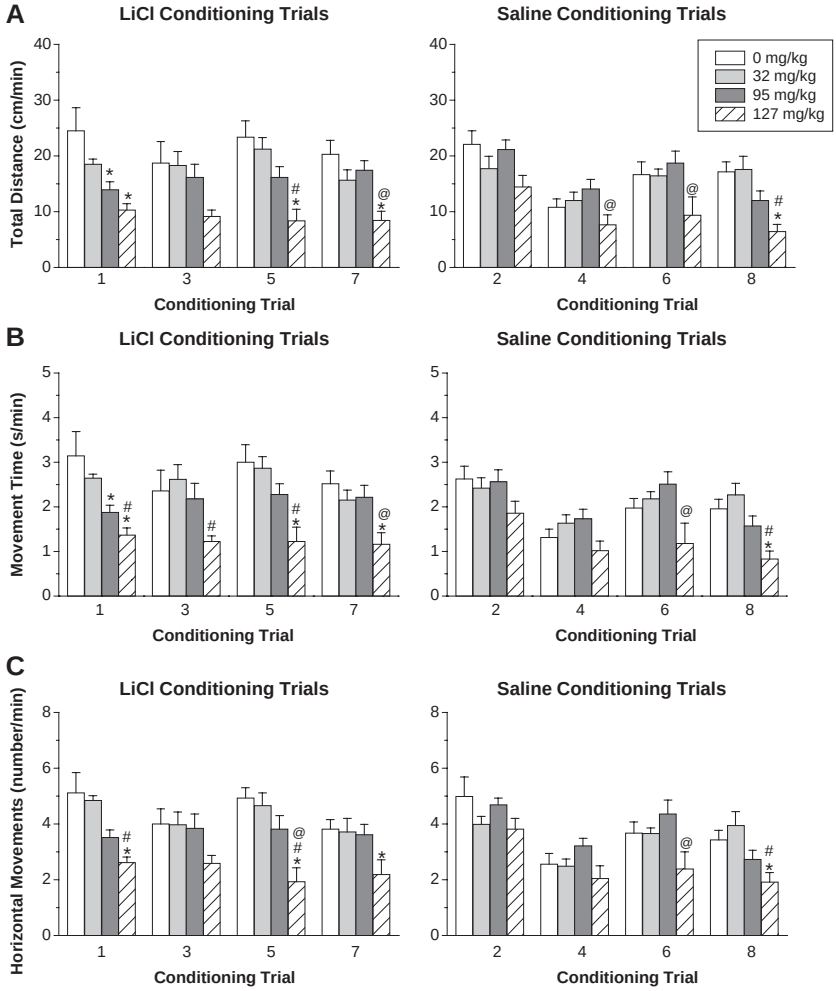

Fig. 2. Horizontal activity measures on each LiCl conditioning trial (left panel) and on each saline conditioning trial (right panel). (A) Group mean total distance per minute, (B) group mean movement time per minute, and (C) group mean number of horizontal movements per minute. *$P < 0.05$ mean of treatment differs significantly from 0 mg/kg LiCl treatment. #$P < 0.05$ mean of treatment differs significantly from 32 mg/kg LiCl treatment. @$P < 0.05$ mean of treatment differs significantly from 95 mg/kg LiCl treatment. Linear contrasts indicate that decreases in horizontal activity measures are significantly dose-related during LiCl-conditioning trials (P's < 0.05) but not during saline-conditioning trials (P's > 0.05). Error bars represent standard error of the mean (S.E.M.).

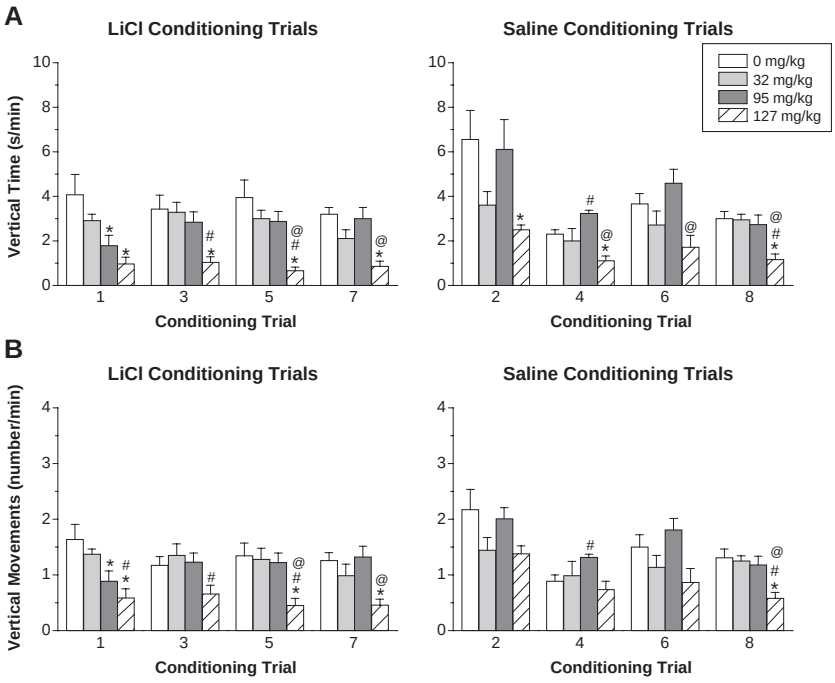

Fig. 3. Vertical activity measures on each LiCl conditioning trial (left panel) and on each saline conditioning trial (right panel). (A) Group mean vertical time per minute and (B) group mean number of vertical movements per minute. *$P<0.05$ mean of treatment differs significantly from 0 mg/kg LiCl treatment. #$P<0.05$ mean of treatment differs significantly from 32 mg/kg LiCl treatment. @$P<0.05$ mean of treatment differs significantly from 95 mg/kg LiCl treatment. Linear contrasts indicate that decreases in vertical activity measures are significantly dose-related during LiCl-conditioning trials (P's<0.05) but not during saline-conditioning trials (P's>0.05). Error bars represent S.E.M.

$P<0.05$], vertical time [$F(1,25)=8.94$, $P<0.01$], and number of vertical movements [$F(1,25)=11.61$, $P<0.01$]. Thus, these measures of activity differed following LiCl and saline administration when collapsed over conditioning trials, regardless of LiCl-Dose (see Figs. 2 and 3).

A significant Treatment × Trial interaction was found for all measures of activity including total horizontal distance [$F(3,75)=5.90$, $P<0.01$], horizontal movement time [$F(3,75)=4.99$, $P<0.01$], number of horizontal movements [$F(3,75)=6.24$, $P<0.01$], vertical time [$F(3,75)=10.47$, $P<0.001$], and number of vertical movements [$F(3,75)=9.43$, $P<0.001$]. This interaction indicated that locomotor activity changed differentially across LiCl conditioning trials compared with saline conditioning trials, irrespective of LiCl-Dose. Specifically, locomotor activity decreased significantly across saline conditioning trials but not across LiCl conditioning trials. This resulted from the fact that activity is high during the initial saline conditioning trials allowing for habituation-related decreases across the trials to be evident. However, LiCl administration suppressed activity during all conditioning trials, likely representing generalized suppression of behaviour.

Significant dose-dependent decreases occurred in all activity measures during LiCl conditioning trials as evidenced by significant linear contrasts (P's<0.05). No such relationship was evident during saline conditioning trials. However, animals in the 127 mg/kg LiCl group did exhibit significantly suppressed activity during the later saline control trials.

3.2. Conditioned place aversion

Time spent in the drug-paired chamber during extinction trials is depicted in Fig. 4. A mixed-design ANOVA with a between-subjects factor of LiCl (2 levels: yes or no) and a within-subjects factor of Trial (4 levels: 4 extinction trials) was conducted to evaluate these data. This analysis revealed a significant main effect of LiCl, collapsed over Trials, [$F(1,27)=8.46$, $P<0.05$]. This indicated that animals administered LiCl during conditioning spent significantly less time in the drug-paired chamber during extinction trials than the 0 mg/kg LiCl control group and demonstrated a LiCl-induced conditioned place aversion to the drug-paired chamber.

Additional analysis was conducted to evaluate dose-dependent effects on LiCl-induced conditioned place aversion. A mixed-design ANOVA with a between-subjects factor of LiCl-Dose (4 levels: 0, 32, 95 and 127 mg/kg) and a within-subjects factor of Trial (4 levels: 4 extinction trials levels) was performed to examine the time spent in the drug-paired chamber. This analysis did not reveal any significant effects of LiCl-Dose, [$F(1,25)=2.76$, n.s.] and non-significant linear contrasts (P's>0.05) indicated that there were no dose-dependent effects on LiCl-induced conditioned place aversion. Similarly, there was no significant effect of Trial [$F(3,75)=1.84$, n.s.] indicating that there were no differences in the duration of the conditioned avoidance response across trials among the groups.

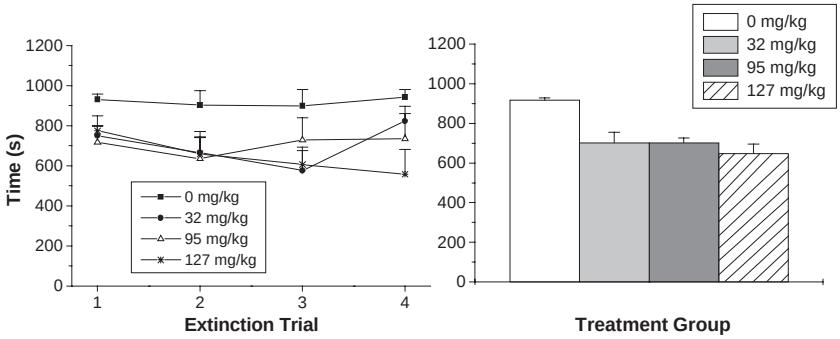

Fig. 4. Group mean time spent in the drug-paired chamber during each extinction trial (left panel) and averaged over extinction trials (right panel). Linear contrasts indicate that time spent in the drug-paired chamber is not significantly dose-related (P's > 0.05). Error bars represent S.E.M.

3.3. Locomotor activity during extinction trials

Measurements of locomotor activity obtained in each chamber during conditioning trials were corrected for the unequal amounts of time spent in each chamber. This correction entailed dividing values of each locomotor activity variable in a given chamber by the amount of time spent in that chamber and expressing these values as units of activity per minute. This correction allowed for a direct comparison of locomotor activity among groups as different groups spent different amounts of time in the chambers during extinction trials. These locomotor activity scores across extinction trials are depicted in Fig. 5 (Vertical Time), Fig. 6 (Vertical Movements), Fig. 7 (Horizontal Movement Time) and Fig. 8 (Total Horizontal Distance). These data were analyzed using a mixed design ANOVA with a between-subjects factor of LiCl-Dose (4 levels: 0, 32, 95 and 127 mg/kg) and two within-subjects factors of Chamber (2 levels: drug-paired and saline-paired) and Trial (4 levels: 4 extinction trials). This analysis revealed a significant main effect of LiCl-Dose, collapsed over Trial and Chamber, for the locomotor activity variables of vertical time [$F(3,25) = 11.52$, $P < 0.001$] and number of vertical movements [$F(3,25) = 5.26$, $P < 0.01$].

Further analysis was conducted to explore the nature of this dose relationship in each of the chambers and evaluate these activity variables for conditioned place aversion-related dose effects. This analysis revealed that this effect

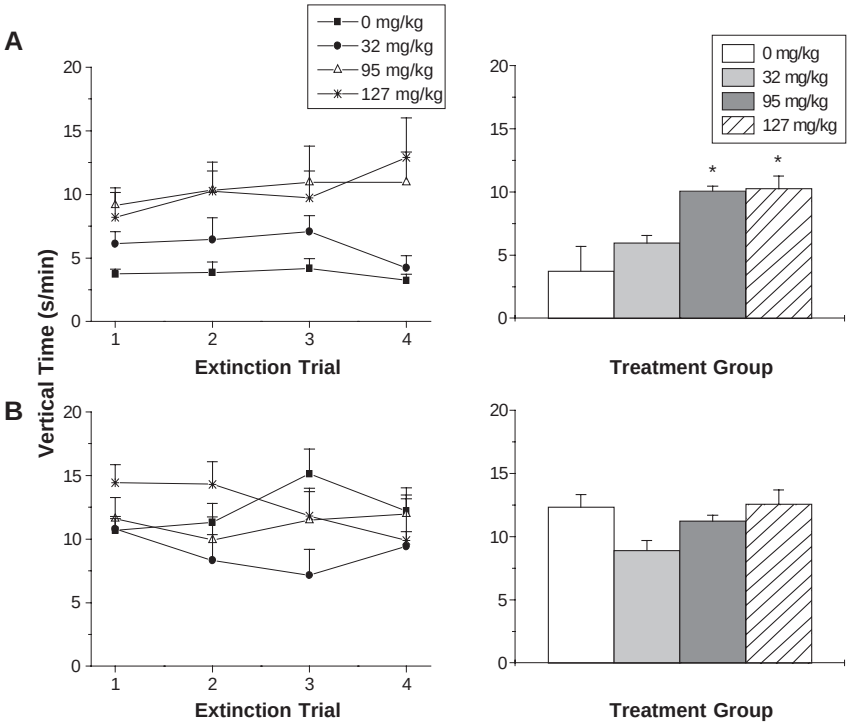

Fig. 5. Group mean vertical time per minute during each extinction trial (left panel) and averaged over extinction trials (right panel) in (A) the drug-paired chamber and in (B) the saline-paired chamber. *$P < 0.05$ group mean differs significantly from 0 mg/kg LiCl group mean. Linear contrasts indicate that vertical time per minute is significantly dose-related in the drug-paired chamber (P's < 0.05) but not in the saline-paired chamber (P's > 0.05). Error bars represent S.E.M.

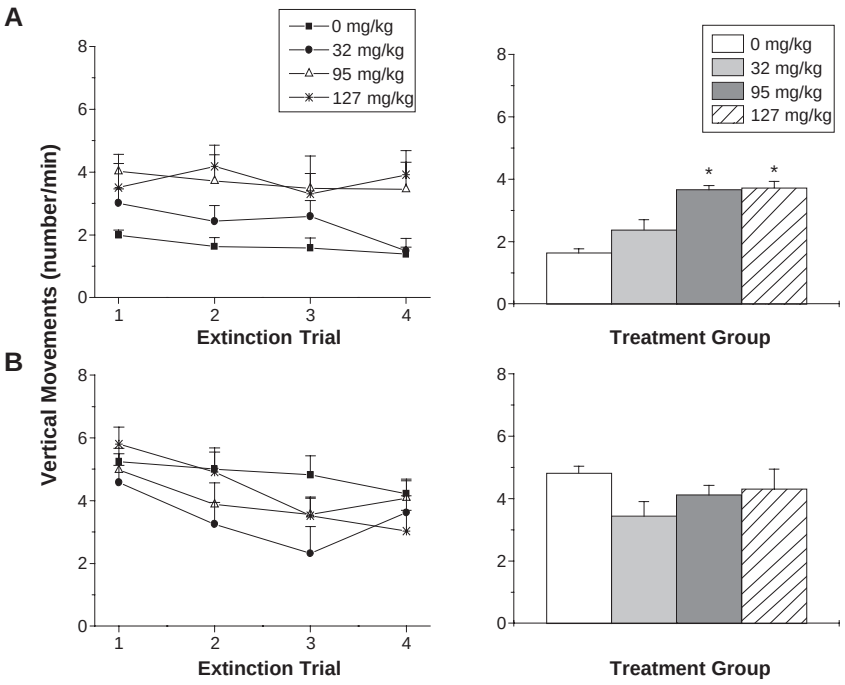

Fig. 6. Group mean number of vertical movements per minute during each extinction trial (left panel) and averaged over extinction trials (right panel) in (A) the drug-paired chamber and in (B) the saline-paired chamber. *$P<0.05$ group mean differs significantly from 0 mg/kg LiCl group mean. Linear contrasts indicate that number of vertical movements per minute is significantly dose-related in the drug-paired chamber (P's<0.05) but not in the saline-paired chamber (P's>0.05). Error bars represent S.E.M.

of LiCl-Dose on vertical locomotor activity was significant only in the drug-paired chamber. The effect of LiCl-Dose was significant for both vertical time [$F(3,25)=4.82$, $P<0.01$] and number of vertical movements [$F(3,25)=4.05$, $P<0.05$] in the drug-paired chamber. The effect of LiCl-Dose was not significant for either of these measures in the saline-paired chamber. Post-hoc tests indicated that animals that received either 95 mg/kg or 127 mg/kg during

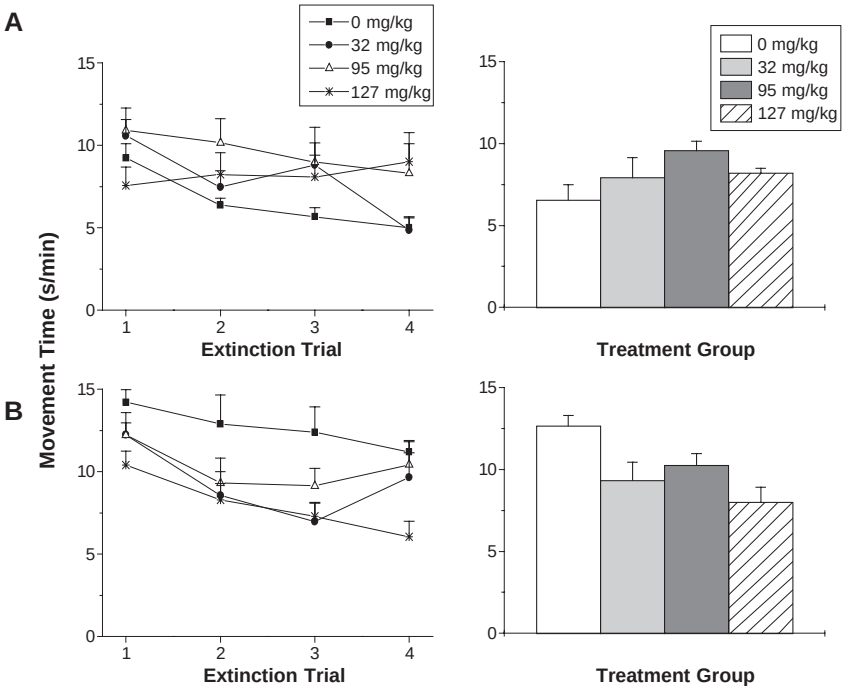

Fig. 7. Group mean movement time in the horizontal plane per minute during each extinction trial (left panel) and averaged over extinction trials (right panel) in (A) the drug-paired chamber and in (B) the saline-paired chamber. There are no significant differences among the groups. Error bars represent S.E.M.

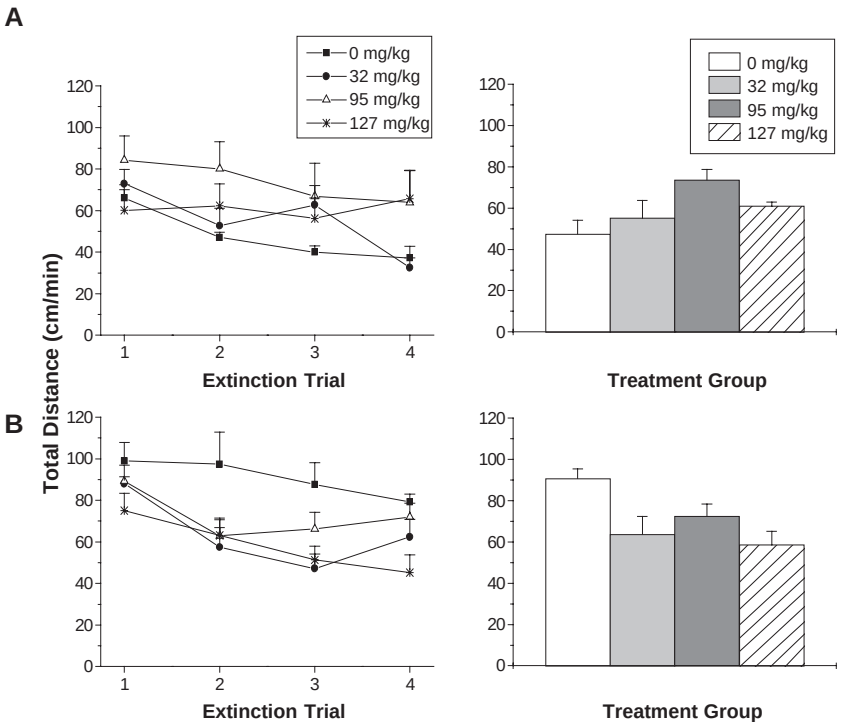

Fig. 8. Group mean total distance traveled per minute during each extinction trial (left panel) and averaged over extinction trials (right panel) in (A) the drug-paired chamber and in (B) the saline-paired chamber. There are no significant differences among the groups. Error bars represent S.E.M.

conditioning spent significantly more time in vertical activity in the drug-paired chamber during extinction trials than the 0 mg/kg LiCl control group (P's<0.05). Similarly, animals that were administered 95 mg/kg or 127 mg/kg LiCl during conditioning exhibited a significantly higher number of vertical movements in the drug-paired chamber during extinction trials relative to animals that received injections of 0 mg/kg LiCl (P's<0.05). Animals that received 32 mg/kg LiCl during conditioning did not differ significantly from the 0 mg/kg LiCl controls on either measure during extinction trials. However, measures of vertical time and number of vertical movements in the drug-paired chamber during extinction trials were shown to be significantly dose-related in all groups by significant linear contrasts (P's<0.05).

In addition, a number of measures of locomotor activity differed between the drug-paired and vehicle-paired chamber when collapsed over extinction trials, irrespective of LiCl-Dose, as a significant effect of Chamber was demonstrated for horizontal movement time [$F(1,25)=5.23$, $P<0.05$], vertical time [$F(1,25)=8.37$, $P<0.01$], and number of vertical movements [$F(1,25)=9.35$, $P<0.01$]. The ANOVA also revealed a significant Chamber × LiCl-Dose interaction for total horizontal distance [$F(3,75)=3.31$, $P<0.05$] indicating that some groups exhibited differences in distance traveled within each of the two chambers while others did not when collapsed over trials. Specifically, total horizontal distance traveled by the control group was significantly greater in the saline-paired chamber than in the drug-paired chamber. Animals conditioned with LiCl, however, did not exhibit this difference in total horizontal distance traveled in each chamber.

A significant three-way interaction of Chamber × Trial × LiCl-Dose was also obtained for total horizontal distance [$F(9,250)=2.52$, $P<0.05$] and horizontal movement time [$F(9,250)=2.46$, $P<0.05$]. Thus, the amount of total horizontal distance and horizontal movement time in each of the two chambers differentially varied across extinction trials among groups. Further analysis revealed that only animals conditioned with 32 mg/kg LiCl exhibited significantly different patterns of change across extinction trials in total horizontal distance and horizontal movement time between the drug-paired and saline-paired chamber. All other groups of animals displayed similar patterns of change across extinction trials between the two chambers.

4. Discussion

The present study provides the first multi-variable assessment of conditioned place aversion and both the unconditioned (following toxin administration) and conditioned (effects elicited in a context previously paired with the toxin) effects of LiCl on locomotor activity. Dose-dependent unconditioned effects of LiCl were evidenced by dose-dependent decreases in all of the measures of locomotor activity following LiCl administration during conditioning trials. Furthermore, even though a conditioned place aversion to the drug-paired chamber developed following conditioning with LiCl toxin, dose had no

differential effect on the amount of time spent in the drug-paired chamber. However, LiCl did produce dose-dependent conditioned effects on measures of vertical activity. This was shown by dose-dependent relative increases in vertical activity exhibited during extinction trials in the chamber previously paired with toxin administration.

4.1. Unconditioned effects of LiCl

The three doses of LiCl administered during conditioning produced a suppression of locomotor activity on all of the measured activity variables. These findings agree with previous research showing that LiCl administration results in decreases in both vertical and horizontal activity. For example, 127 mg/kg LiCl significantly decreased gridline crossings and rearing frequency in a conditioning chamber (Parker et al., 1984). Similarly, 95 mg/kg LiCl significantly decreased gridline crossings in an open-field (Smith, 1981) and injections of 85 mg/kg LiCl significantly reduced open-field ambulation and rearing (Johnson, 1975). Furthermore, administration of 64 mg/kg LiCl also produced significant suppression of gridline crossings, rearing frequency and time to reach a wall in an open-field arena (Smith, 1983). Moreover, dose-related decreases in all of the measures of locomotor activity occurred following LiCl administration. These dose-dependent decreases were most evident on the first conditioning trial because habituation caused a decrease in locomotor activity in the 0 mg/kg LiCl control group across conditioning trials. The multivariable assessment of the unconditioned effects of LiCl in the present study provides further evidence that the toxicity of LiCl can be evaluated by measuring locomotor activity following treatment.

4.2. Conditioned effects of LiCl

4.2.1. LiCl-induced conditioned place aversion

Conditioned place aversion (less time in the toxin-paired chamber) developed following administration of LiCl during conditioning. This finding is consistent with previous research showing that LiCl produces conditioned place aversions with doses ranging from 20 mg/kg (Miller et al., 2000) to 127 mg/kg (Khroyan et al., 1995). However, in the present study, there was no dose-dependent effect on the strength of the conditioned place aversion. Previous research provides conflicting evidence for possible dose effects of LiCl on the strength of conditioned place aversions. While there is limited evidence that LiCl has dose-related effects on the strength of the conditioned avoidance response (Miller et al., 1999, 2000), other research indicates that no dose-dependent relationship exists (White and Carr, 1985).

Some of the findings in the present study conflict with the results of previous research. White and Carr (1985) observed that although 62 mg/kg LiCl produced conditioned place aversions to the drug-paired chamber, contrary to the results of the current study, 32 mg/kg LiCl did not. The inconsistency, with respect to this dose of LiCl, may be the result of differences in conditioning procedures and/or the choice of apparatus. White and Carr used an unbiased conditioning procedure, where the baseline preference of the animals for the two contexts is equal prior to conditioning and evaluated conditioned place aversion using within-group comparisons and compared time in the drug-paired chamber with time in the saline-paired chamber for each group. The present study, however, employed a biased conditioning procedure, in which the animals exhibited an unequal preference for the two chambers prior to conditioning and assessed conditioned place aversion using between-group comparisons by comparing time spent in the drug-paired chamber by each experimental group to time spent in this chamber by the controls. Thus, the unbiased vs. biased nature of the conditioning procedure used may influence the development and/or expression of conditioned place aversion in the same way the unbiased vs. biased nature of the conditioning procedure has influenced conditioned place preference (for brief review see, Cunningham, 2003).

4.2.2. Conditioned effects of LiCl on locomotor activity

LiCl administration during conditioning trials resulted in the development of conditioned effects on vertical locomotor activity during extinction trials. Specifically, previous LiCl administration elicited dose-dependent relative increases in vertical activity time and number of vertical movements in the chamber previously paired with the toxin. This dose-response relationship was indicated by significant linear contrasts in the chamber previously paired with toxin administration while no such relationship was displayed in the chamber previously paired with saline administration.

Previous studies have also demonstrated increases in rearing behaviour in contexts previously paired with LiCl (Johnson, 1972; Parker et al., 1984). Thus, the present finding, that 95 mg/kg and 127 mg/kg LiCl during conditioning produced significant increases in vertical activity relative to 0 mg/kg LiCl controls during extinction trials, while 32 mg/kg did not, agrees with past research. It has been shown that 127 mg/kg LiCl caused significantly more rearing during a test trial while 51 mg/kg and 13 mg/kg had no effect (Parker et al., 1984). In addition, administration of 254 mg/kg LiCl elicited increased rearing behaviour in the LiCl-paired context when drug administration was stopped (Johnson, 1972). These previous studies, together with the current results, suggest that only higher doses of LiCl administered during conditioning produce significant conditioned effects. Taken together, this is suggestive of a dose-dependent relationship on the conditioned vertical activity effects of LiCl. However, the fact that there was no difference between groups administered 95 or 127 mg/kg LiCl during conditioning suggests that there may be a ceiling effect with respect to dose-dependent conditioned increases in vertical activity.

Contrary to the findings of the present study and other past research, LiCl also has been shown to cause suppression of activity as a conditioned response (Meachum and Bernstein, 1992). Specifically, rats conditioned with 127 mg/kg LiCl exhibited decreases in general activity levels and spent significantly more time in "freezing" behaviour in the LiCl-paired context during the test trial (Meachum and Bernstein, 1992). It is possible that the conditioned effects obtained in the present study differ from those in the Meachum and Bernstein (1992) study because of procedural dissimilarities. In particular, Meachum and Bernstein subjected the animals to seven LiCl-context pairings whereas Parker et al. (1984) employed three LiCl conditioning trials, as did Johnson (1972). The present study used four LiCl conditioning trials. Thus, number of conditioning trials may influence the nature of the conditioned behavioural responses.

The vertical activity measured in the LiCl-paired context during extinction in the present study may be interpreted as a component of escape behaviour. Previous studies have suggested that vertical locomotor activity represents a measure of escape and not exploratory behaviour (Exner and Clark, 1993). In addition, increased rearing behaviour has been shown to correlate with measures of active avoidance (Wiersma et al., 1997). Higher levels of vertical activity during extinction trials in the present study correspond with larger decreases in locomotor activity during conditioning trials. This suggests that animals subjected to greater LiCl toxicity may be more motivated to display escape behaviour in the context previously paired with the toxin.

The evaluation of conditioned effects of LiCl on multiple measures of locomotor activity during extinction trials provides a richer description of LiCl-induced conditioned place aversion than exists to date by revealing additional indices of the conditioned avoidance response (Ossenkopp and Mazmanian, 1985). Although no dose-dependent effects of the toxin are exhibited in the amount of time spent in the drug-paired context, the conditioned effects on vertical activity display a clear dose-dependent relationship. This suggests that the place aversion and conditioned locomotor activity effects of LiCl are dissociable and provides evidence for a dose-response component to LiCl-induced conditioned place aversion that is not clearly evident if one solely uses time in the drug-paired context to evaluate place aversion. Quantifying vertical activity during extinction (or test) trials is a measure of LiCl-induced conditioned place aversion that appears to be more sensitive than the standard evaluation of proportion of time spent in the toxin-paired context.

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