BEHAVIORAL ASSESSMENT OF THE TIME COURSE AND RELATIVE INTENSITY OF ACUTE LITHIUM CHLORIDE TOXICOSIS IN ADULT AND WEANLING RATS

by

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Thesis submitted to the Graduate Faculty of the
Virginia Polytechnic Institute and State University
in partial fulfillment of the requirements for the degree of
MASTER OF SCIENCE
in
Psychology

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July, 1978

Blacksburg, Virginia

ACKNOWLEDGEMENTS

I wish to extend sincere appreciation to J. J. Franchina for his invaluable assistance in the production of this thesis. In addition, I wish to thank A. H. Schulman and J. F. Kehoe for their advisory assistance. Finally, the tireless help of is gratefully acknowledged.

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A number of authors have concluded that young rats are inferior to adult rats in the performance of passive avoidance tasks and in long-term retention tasks (Campbell & Coulter, 1976). For example, Riccio and Schulenburg (1969) using a "step off" task found learning deficits as a function of age in rats from 10 to 30 days old. Campbell, Misanin, White, and Lytle (1974) found passive avoidance learning deficits and retention deficits in 16 to 25 day old rats.

There have been two basic types of explanation for the performance deficits seen in young rats (Coulter, Collier, & Campbell, 1976). Neurological explanations stress factors such as response withholding deficits, lack of myelination and cortical development. Psychological explanations point to variables such as generalization decrement due to growth factors. Generally, it has been difficult to attribute response deficits in young animals to neurological factors because psychological explanations can not be ruled out.

For example, Campbell et al. (1974) have suggested the young rat's tendency toward higher activity levels than adults, interferes with the performance of the passive avoidance response. In studies of long term retention, growth factors produce other problems. As Coulter et al. (1976) point out significant growth during the retention interval will alter both the perceived appearance of the apparatus as well as the nature of the response to be performed. Coulter et al.'s solution to the growth factor problem was to use an off baseline CER conditioning paradigm to study long term retention in young rats. They maintained that as an internal response, the CER was not subject to change as the rats matured and thus rendered psychological determinants of forgetting inoperative.

The conditioned taste aversion paradigm is also attractive for use in developmental studies of learning and memory because, like the CER, it is based on internal cues (in this case gustatory) which do not seem readily susceptible to growth factors. Also, the development of consummatory behaviors in rat pups is relatively precocious (Hall, Cramer, & Blass, 1977; Tisher & Blass, 1976; and Wirth & Epstein, 1976) so rats can meet the response requirements of the paradigm at an early age.

Despite its apparent suitability for the study of passive avoidance learning and retention in infants, relatively few developmental studies of taste aversion have been reported. To complicate matters the results of these studies often conflict. It is apparent that young rats can exhibit taste aversion learning. Grote and Brown (1971a), using 22 day old subjects, found reliable taste aversion after one exposure to .12M LiCl solutions with a retention interval of three days. Ader and Peck (1977) showed the retention of a taste aversion to saccharin 60 days after one conditioning trial in weanling rats. However, the effect was only seen using a test procedure in which two rats had to compete with each other for access to the test bottle. Galef and Sherry (1973) demonstrated aversions to the diet of the nursing mother when conditioning occured 21 days post partum.

However, in direct comparisons with adults, young rats have sometimes shown inferior performance (Klein, Domato, Hallstead, Stephens, & Mikulka, 1975; Baker, Baker & Kesner, 1977; Klein, Barter, Murphy & Richardson, 1974) while other studies have shown equivalent performance in the two age groups (Grote & Brown, 1971; and Klein, Mikulka, Domato, & Hallstead, 1977). Inferior taste aversion learning in young rats can

often be traced to methodological problems. For example, a number of studies have shown that two bottle preference testing techniques show much stronger taste aversions in young subjects than do one bottle procedures (Grote & Brown, 1971b; Klein, Domato, Hallstead, Stephens and Mikulka, 1975; and Baker, Baker and Kesner, 1977). Baker, Baker, and Kesner found that the use of a long (60 min) CS exposure also degrades taste aversion performance in young rats, apparently because it causes a long effective CS-UCS interval. In addition, Baker et al. suggested that the illness inducing agents used in their study (apomorphine and lithium chloride [LiCl]) may have produced differing levels of illness in young and adult rats. Feigley and Spear (1970) found decreasing the intensity of the aversive stimulus in a passive avoidance task decreased the rate of learning equally for adult and infant rats. Thus, if young rats experience less severe illness than adult rats it could explain differences between them in taste aversion performance.

After finding that apomorphine and LiCl UCS's each produced equally poor taste aversion performance in young rats relative to adult rats, Baker et al. attempted to test the hypothesis of different levels of illness in pups and adults by measuring latency to drink starting 10 minutes after injection of 15 mg/kg of apomorphine. Young and adult rats had mean latencies to drink of 64 minutes and 122 minutes, respectively. This difference, while not statistically significant, is nonetheless quite large numerically, and is suggestive of a difference in drug effectiveness between the adults and pups. Furthermore, observations of 19 day old rats during the course of several unpublished taste aversion experiments conducted at Virginia Polytechnic Institute and

State University suggest that young rats showed fewer toxic symptoms of a LiCl UCS than the adult rats in the same treatment groups.

One purpose of the present research was to examine potential developmental differences in LiCl toxicity which might explain differences in taste aversion learning between young and adult rats. LiCl was used for the present study because it has been cited as the UCS in taste aversion experiments more often than any other substance (Riley & Baril, 1976) and since it is used so frequently, it must be considered the drug of choice for studies of learned aversion. In addition, because LiCl does not produce its effects via local irritation (Shou, 1957), it seems reasonable that the numerous neurological and physiological differences between adult and infant rats may produce differences in reactivity to LiCl.

The time course of LiCl toxicosis is another factor which is relevant to the proper design of taste aversion experiments. In studies of the effect of CS-UCS interval (Ahlers & Best, 1971; Kalat & Rozin, 1973; and Nachman, 1970), it is important to know how soon after injection the drug takes effect. In developmental studies of CS-UCS interval (e.g., Baker, Baker & Kesner, 1977) age dependent differences in the time course of the toxicosis could produce unequal effective CS-UCS intervals. The onset and duration of recovery from illness is also highly relevant in determining appropriate test intervals (Nachman, 1963), in studies which attempt to show a learned preference to a substance as a result of its being paired with recovery from illness (Green & Garcia, 1971; Zahorik & Bean, 1975; and Zahorik, Maier, & Pies, 1974).

A number of studies have attempted to measure recovery from illness in a taste aversion context. Best and Zuckerman (1971) and Kesner, Berman & Burton (1975) measured latency to drink in adult rats after 12 mg/kg or 15 mg/kg injections of apormorphine. They found latencies of 80 min and 90 min, respectively. Baker, Baker, and Kesner (1977) also using latency to drink, and 15 mg/kg apomorphine found latencies of 122 minutes for adults and 64 minutes for pups. Green and Garcia injected groups of adult rats during their daily fluid access period or 1, 2 or 3 hours prior to this period, and measured the number of subjects in each group which drank. All subjects injected during the access period stopped drinking in two minutes, and 3 out of 4 rats did not drink in the 1 hour group. In the 2 hour group all subjects drank but the amount of water consumed was less than noninjected controls. The 3 hour group showed consumption identical with controls. Nachman (1963) used amount drunk during ad libitum fluid access measured 24 and 48 hours after LiCl injections. He found reduced intake in the first 24 hours relative to saline controls but equal intake after 48 hours. The second purpose of the present research was to further elucidate the time course of LiCl toxicosis in the context of its use in a conditioned taste aversion paradigm.

This paper presents the results of two studies which were designed to be sensitive to the most frequently seen acute toxic symptoms of LiCl in order to measure their time course and assess developmental differences in symptom intensity. Symptoms which have been observed in rats include a period of inactivity, lying down (Nachman, 1963; Nachman & Ashe, 1973). In addition, Shou (1957) lists a number of gastro-

intestinal symptoms such as nausea, salivation, vomiting and diarrhea which have been observed in man and other animals.

The first study investigated the effect of acute LiCl administration on bar pressing behavior. It was thought that the bar pressing measure would be sensitive to changes in activity level as well as the gastrointestinal aspects of the illness since the behavior is food motivated. The second study looked at the effect of LiCl on open-field behavior in order to get a more global picture of the symptoms seen during toxicosis.

Experiment 1

Reports of the effect of lithium on bar pressing behavior are relatively rare. Crnic (1976) studied the effect of LiCl on responding to a multiple fixed ratio, fixed interval, time-out schedule. LiCl was administered via two divided i.p. injections each day. Testing was conducted 1 hour after injection. Analysis of the bar pressing behavior for both changes in rate and pattern of responding showed no significant effects of LiCl for the 1.5 and 2.5 mEq groups when subjects were compared to their baseline performance. However, subjects in the 3.0 mEq group developed longer post-reinforcement pauses and made fewer responses during the final quarter of the fixed interval. Considerable individual differences in the effect of LiCl on bar pressing behavior were found. However, the direction of the changes in rate were always downward regardless of their magnitude. Smith and Smith (1973) found that rats who were fed lithium adulterated diets (70 mM Li/kg of food) showed lower rates of bar pressing than non-lithium controls (training and reinforcement parameters were not reported). Edelson, Gottesfeld, Samuel, and Yuwiler (1976), using 2.0 mEq of LiCl and testing 10 minutes after i.p. injection observed a decrease in rates of bar-pressing maintained via response contingent stimulation of the median forebrain bundle.

The present study looked at the effect of LiCl on rates of food reinforced bar pressing in adult and weanling rats. The reinforcement schedule was a VI-60 selected because of its ability to produce relatively stable rates of responding (Mackintosh, 1974). The LiCl was delivered via a single i.p. injection just before the bar pressing test

session. It was expected that the administration of LiCl would reduce rates of bar pressing to the extent that the toxic symptoms of the drug interferred with the bar pressing response. Further, if weanlings were less affected by LiCl than adults, (which would explain their relatively poor taste aversion performance) they should show correspondingly smaller decreases in rate of bar pressing than adults.

Method

Subjects. Subjects were 24 adult and 24 weanling rats (Sprague-Dawley descendants) from the animal colony maintained by the Psychology Department at Virginia Polytechnic Institute and State University. Three to five days after parturition the litter size was culled to eight. Subjects in the adult groups remained with the dam until they were 25-27 days of age and were then housed in the animal colony until Day 1 of the experiment. The age of adult subjects on Day 1 of the experiment ranged from 84-86 days. Rat pups were removed from the dam at 15 days of age and began Day 1 of the experiment.

Apparatus. The apparatus consisted of two operant chambers (BRS/LVE) equipped with retractable levers. Programming and recording equipment was located in an adjacent room. Continuous white noise (70 db) was present at all times. Standard formula 20 mg food pellets (P. J. Noyes Co.) were used as reinformcements for bar pressing.

Design. A two-by-three factorial design was used in which two levels of age (weanling and adult) were crossed with three levels of injection condition (3.0 mEq .15 M LiCl, 1.2 mEq .15 M LiCl, and 0.9% normal saline 20 ml/kg). Eight subjects, four male and four female, were used in each cell of the design for a total of 48 subjects.

Sixteen additional subjects were run, four in each of the two operant chambers at each of the two age levels. These subjects were used to replace the four rats at each combination of box and age level which had the lowest baseline rates of responding. This was done to help prevent floor effects and to produce a more homogenous subject population in terms of initial rate of responding.

Preliminary Bar Press Training - Weanlings. On the morning[1] of Day 1 of the experiment pups were weighed and housed individually in hanging cages in a room with a 12 hr light/dark cycle and a temperature of 78° F. Subjects were provided with ad libitum food, in the form of dry ground lab chow (Purina Co.) and food pellets (P. J. Noyes Co.) as well as ad libitum tap water. On the morning of Day 2 all food was removed from the cages and until the evening of Day 5, when the test session was over, subjects received no food (other than the reinforcement earned during bar pressing) unless their weight, measured before the training session, dropped below 80%of theweight of free feeding control animals. If this occurred, the subjects were allowed access to food for 1 hour following the training session.

On the evening of Day 2 rats were hand shaped under a continuous reinforcement schedule. The shaping sessions generally lasted 30 minutes but could be extended or shortened at the discretion of the experimenter, depending on the progress of the subject. In any case, no animal was allowed to earn more than 50 reinforcements. On the morning of Day 3, rats received 1/2 hour of training under a VI-30 sec schedule.

Beginning on the evening of Day 3 all subjects began a squad training procedure. In this procedure, rats in groups of four, were transported by hand in and out of the operant chamber, round-robin, for a

series of short bar pressing trials. Rats were put into the box while the bar was retracted and the house lights were out. At the end of 10 seconds the house lights came on and the bar was extended. The subjects were allowed to bar press until the end of the trial (either 50 or 110 sec; see below). At the end of the trial the subject was removed and the next subject was placed in the box. When rats were not bar pressing in the operant chamber they were kept in their home cages which had been placed adjacent to the apparatus.

The first squad training session, on the evening of Day 3 used a 110 sec trial length and a VI-60 sec reinforcement schedule. The training session lasted a total of 2 hours, giving each subject 30 bar pressing trials. Subsequent training sessions were identical to the first, except for the use of a 50 sec trial length, and were conducted on the morning and evening of Day 4 and the morning of Day 5. Data from the last hour (15 trials per subject) of the Day 5 training session were recorded and served as the baseline rate of bar pressing. After the training session on the morning of Day 5, subjects were returned to the housing area with no fluid available until the evening test session.

Preliminary bar press training - adults. Pilot work indicated that when adult rats received the same training procedure as weanlings, they tended to become immobile and did not eat reinforcements when in the operant chambers. In order to get the adult rats to bar press it was necessary to incorporate a pre-training regimen which included food deprivation, handling, and pre-exposure to the apparatus. Thus, four days before the start of the experiment adult subjects were removed from the colony and housed individually with ad libitum water but no food. Each

evening, up to and including Day 1 of the experiment, the subjects were handled by picking them up and placing them on the experimenter's arm 20 times.

On Day 1, after being handled, subjects were given access to 1/2 teaspoon of food pellets in their home cages. On the morning of Day 2 subjects were placed two at a time in the operant chamber in which they were to be trained and a teaspoon of food pellets was placed in the food cup. Subjects remained in the chamber for 1 hour. Hereafter, subjects received no food other than that earned in bar pressing unless their weight dropped below 80% of their free-feeding body weight. If this occurred, the subject was allowed 1 hour access to food. Finally, for adult rats, all squads were made up of the same sex subjects, since odors from estrus females previously in an apparatus have been shown to effect the behavior of subsequent males (Walsh & Cummins, 1976).

Except for the pre-training regimen, all other aspects of the procedure were the same for adult and weanling subjects.

Testing. Testing was conducted on the evening of Day 5. Immediately prior to the time subjects were removed from the housing area for the test session they were given 15 minutes access to a 12% (w/v) sucrose solution in a bottle with a ballbearing drinking tube.[2] Exposure to the novel sucrose flavor was provided in order to assess the development of taste aversions as a result of the LiCl injections and to provide a salient stimulus with which to associate the LiCl illness. During the sucrose exposure all subjects were observed to assure they had tasted the sucrose solution. Any subject who had not tasted the solution at the end of the 15 minute exposure period was force-fed a small quantity of the fluid.

After the sucrose exposure subjects were removed to the testing room and were allowed to bar press using the same protocol as in the training sessions except that immediately (no more than one minute) prior to the first bar pressing trial subject's received an i.p. injection of .15 M LiCl, either 3.0 mEq or 1.2 mEq, or .9% normal saline (20 ml/kg). For each rat, a total of 21 post-injection 50 second bar pressing trials were conducted. A bar pressing trial occurred immediately after the injection, then one every four minutes for the next hour, and once every 15 minutes for the final hour and one half, for a total test period of two and one half hours. During each of the 50 second trials the total number of bar presses emitted by the subject was recorded automatically.

At the conclusion of the testing session subjects were returned to the housing area and received ad libitum food and tap water. After 30 minutes the water bottles were removed and weighed to measure the amount drunk. Then, the bottles were returned to the cage for another 11 1/2 hours. At the end of this time period, on the morning of Day 6, the water bottles were again removed and weighed in order to measure intake. The bottles were not returned to the cages until the evening of Day 6, at which time each rat received a test for sucrose taste aversion using a two bottle preference procedure. One bottle contained 12% sucrose; the other tap water. The bottles were available for 30 minutes, after which the bottles were removed and weighed to measure the intake of each fluid.

Results

Figure 1 presents mean number of bar presses across test trials (time for each dosage condition and age group. Figure 1 shows that, relative to the pre-test baselines, all groups show an initial decrement in bar-press responding immediately after the injection. For adult subjects this effect dissipates rapidly in the saline group, while in the LiCl groups, responding recovers only briefly and then declines further, the lowest response level occurring at 12 minutes. The 3.0 mEq group showed lower responding than the 1.2 mEq group. For the weanlings the initial decrement in responding for the saline group was not followed by the rapid rise in responding seen in the adult saline group. Further, weanlings in the saline group do not reach their former level of baseline responding until 28 minutes post-injection. Despite the weanlings' depressed performance in the saline group, the 3.0 mEq group shows even lower rates of responding, especially in the 20 to 28 minute time period. The performance of the weanling 1.2 mEq group during the early test trials is somewhat erratic but seems to follow the pattern of the 3.0 mEq group more closely than that of the saline group.

Comparison of the upper and lower portions of Figure 1, which present the data for the weanling and adult groups, respectively, show that the time course of the drug effect is different for the two age groups. Weanling rats in the LiCl groups show a faster return to baseline levels of responding than adult rats. It can be seen that in the weanling groups the lines for the three dosage conditions converge at around 60 minutes and that for the final one and one-half hours of testing the

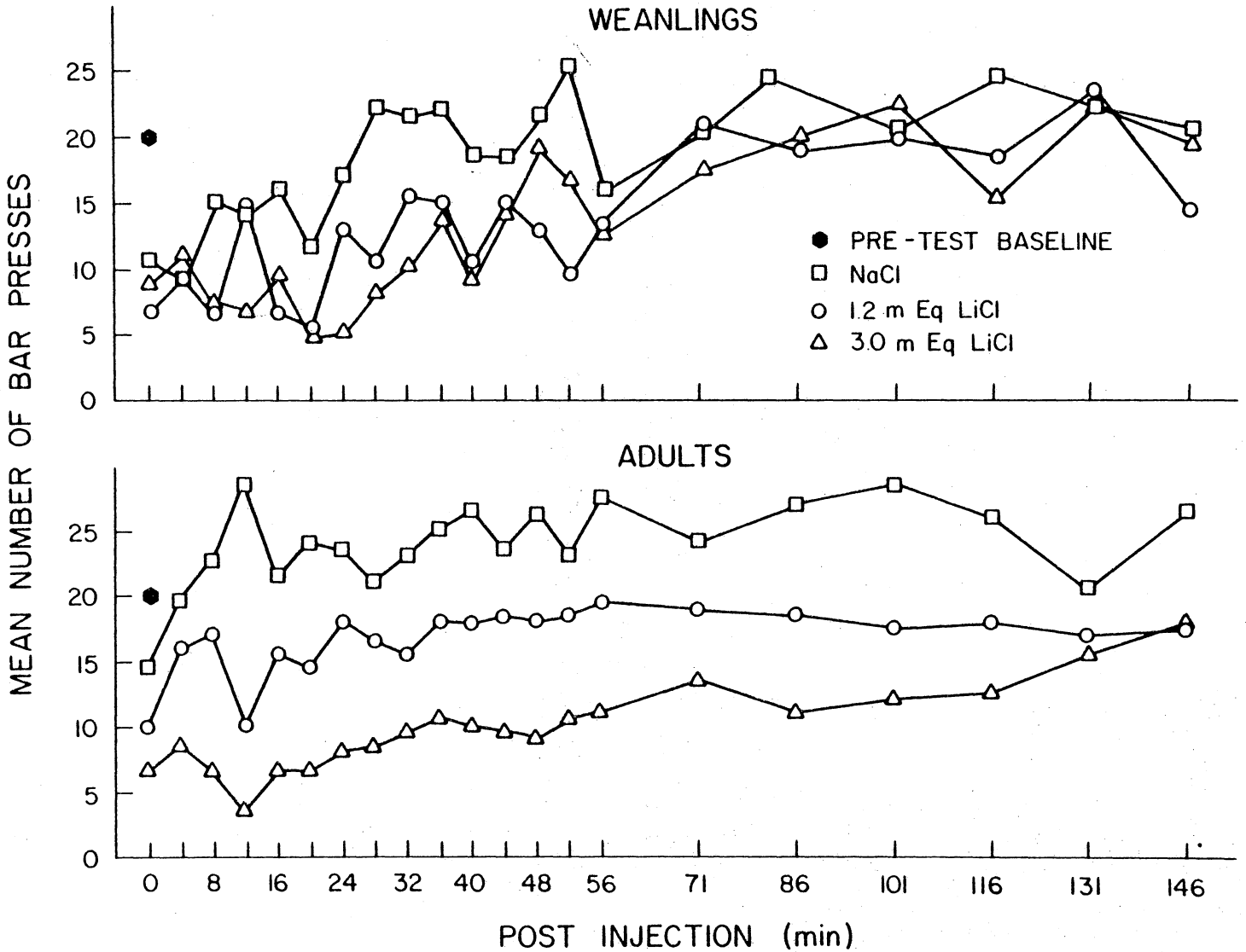

Figure 1. Mean number of bar presses as a function of minutes following the injection for weanlings and adult rats in LiCl and saline conditions.

lines for all three groups are intermeshed and relatively stable at the approximate level of the pre-test baseline. However, for the adults, all three dosage groups remain separated until the latter part of the observation period when the two LiCl groups show roughly equal rates of responding but are still somewhat below the saline group.

Analysis of variance[3] over all the data in Figure 1 yielded significant main effects of dose (F (2, 36) = 5.65, p = .007), box (F (1, 36)= 9.97. p = .003), time (F (20, 720) = 1.85, p = .01), and the age x time interaction (F (20, 720) = 1.85, p = .01). No other main effects or interactions reached significance at the .05 level. A Duncan's Range test across the main effect of dose revealed that the mean for the saline group, 21.16 responses, was significantly different ($p < .05$) from the means of the 1.2 and 3.0 mEq groups, which were 14.93 responses and 11.36 responses, respectively. However, the means for the two LiCl groups did not differ significantly from each other. Simple effects analysis of the age x time interaction showed significant time effects (adults, F (20, 360) = 2.90, $p < .001$; weanlings, F (20, 360) = 6.13, $p < .001$). Examination of the graphs in Table 1 suggest that the interaction was due to lower overall rates of responding in the weanling group during the early test trials.

In order to evaluate the time course of the drug effect, a trial by trial analysis of the data was conducted using a separate age x dose analysis of variance for each test trial. The results of those analyses are shown in Table 1 which presents the probability level of each effect which was significant at or beyond the .05 level. Table 1 shows that dose effect was reliable from 8 to 40-minutes post-injection; age

Table 1

Probability Values for the Effects of Age, Dose and Age x Dose from the Trial by Trial Analysis of Variance for Bar-Pressing Data

Minutes Post-Injection	Effect		
	Age	Dose	Age x Dose
0	N.S.	N.S.	N.S.
4	N.S.	N.S.	N.S.
8	N.S.	.006	N.S.
12	N.S.	.000	.030
16	N.S.	.005	N.S.
20	.005	.002	N.S.
24	N.S.	.006	N.S.
28	N.S.	.002	N.S.
32	N.S.	.030	N.S.
36	N.S.	.040	N.S.
40	N.S.	.010	N.S.
44	N.S.	N.S.	N.S.
48	N.S.	.040	N.S.
52	N.S.	.006	N.S.
56	N.S.	N.S.	N.S.
71	N.S.	N.S.	N.S.
86	N.S.	N.S.	N.S.
101	N.S.	N.S.	N.S.
116	N.S.	.040	N.S.
134	N.S.	N.S.	N.S.
146	N.S.	N.S.	N.S.

effects and age-dose interactions are essentially absent. A similar trial by trial analysis for each age group showed that adults exhibited a significant ($p < .05$) dose effect on test trials 12, 16, 20, 24 and 105 minutes post-injection; while weanlings showed a significant dose effect on fewer test trials, 28, 32, and 56 minutes. The finding of a different significance pattern for the adults and weanlings in the trial by trial analysis suggests the presence of an age x dose x time interaction which the overall analysis was too insensitive to uncover.

Table 2 shows the amount drunk during the water intake periods where the 1/2 hour columns refer to the amount drunk in the first 30 minutes after the end of testing, 2 1/2 to 3 hours post-injection. The "overnight" columns contain data from the period immediately following the 1/2 hour measurement until the morning after the test session (a period of 11 1/2 hours). The total column reflects the sum of 1/2 hour and overnight intakes. A separate analysis of variance was performed on each of the three measures.

The top portion of Table 2 shows that, in the first 30 minutes after testing, water intake declined as LiCl dosage increased, but that the effect was greater for the adults than for the weanlings. Analysis of variance over this data revealed significant effects of age (F (1, 42)= 94.79, $p < .001$), dose (F (2, 42) = 5.83, $p = .006$), and age x dose (F (2, 42) = 4.23, $p = .02$). Simple effects analysis revealed that the source of the interaction was the existence of a significant dose effect in the adult groups (F (2, 23) = 5.15, $p = .02$), but no significant dose effect for the weanling group (F (2, 23) = 1.07, $p > .05$). A Duncan's Range test across the simple effect of dose for the adult groups showed

Table 2

Mean Water Intake in Grams for Weanling and Adult Rats in LiCl and Saline Injection Conditions

	Age	
Dose	Weanling	Adult
	1/2 Hour	
NaCl	.84	7.61
1.2 mEq	.59	4.67
3.0 mEq	.56	4.05
	Overnight	
NaCl	6.01	19.37
1.2 mEq	6.16	23.35
3.0 mEq	4.40[a]	17.61
	Total	
NaCl	6.85	26.98
1.2 mEq	6.75	28.02
3.0 mEq	4.96[a]	21.66

[a]Data for this group contains only 7 observations due to a bottle which fell off the cage.

that the 1.2 mEq and 3.0 mEq both differed from the saline group ($p < .05$) but not from each other.

The middle section of Table 2 indicates a continued depression in the water consumption of the 3.0 mEq group relative to the saline controls during the overnight measurement. However, in the 1.2 mEq groups consumption is above that of the saline controls. The analysis of variance once again showed significant effects of age, dose, and age x dose (F (1, 41) = 310.07, F (2, 41) = 5.44, F (2, 41) = 4.07; $p < .001$, $p = .004$, $p = .02$, respectively). Simple effects analysis again showed that the source of the interaction was the presence of a significant dose effect in the adult groups (F (2, 23) = 5.38, $p = .01$) but no significant dose effect in the weanling groups (F (2, 22) = 2.38, $p > .05$). A Duncan's Range test across the simple effect of dose for the adult group indicated that the NaCl and 3.0 mEq groups were significantly different from the 1.2 mEq group ($p > .05$) but not different from each other.

Finally, the lower part of Table 2 shows that in terms of total intake saline and 1.2 mEq groups are roughly equivalent while 3.0 mEq groups showed a lower level of intake. Analysis of variance conducted on this data precluded a significant age effect (F (1, 41) = 3.64, $p < .001$), a dose effect (F (2, 41) = 4.76, $p = .01$) as well as a significant interaction between age and dose (F (2, 41) = 3.72, $p = .03$). As was true of the other water intake measures, simple effects analysis of the total intake showed no significant dose effect (F (2, 22) = 2.96, $p > .05$) for the weanlings while for the adults the dose effect reached significance (F (2, 22) = 4.52, $p = .02$). A Duncan's Range test over

the adult data showed that the 3.0 mEq group was significantly different from both the saline and 1.2 mEq groups ($p < .05$) which did not differ from each other.

As a measure of taste aversion learning, sucrose and water consumption data from the two bottle preference test was converted to a percent of total fluid intake measure by dividing the amount of sucrose consumed by the sum of sucrose and water consumed (i.e., SUC/[SUC + HOH]). These data are shown in the upper portion of Table 3. They indicate that all the LiCl groups consume a relatively smaller amount of sucrose, indicating the presence of a sucrose taste aversion. However, the amount of suppression of sucrose intake is not as low for the weanling groups as for the adult groups. Analysis of variance on the data in Table 3 yielded significant main effects of age and dose (F (1, 42) = 16.58, $p < .001$; and F (2, 42) = 74.11, $p < .001$, respectively). The age x dose interaction was also significant (F (2, 42) = 9.34, $p < .01$). Simple effects analysis revealed no age effect between the saline injected groups ($F < 1.0$), but a highly significant age effect across the 1.2 mEq groups (F (1, 14) = 37.73, $p < .001$) and the 3.0 mEq groups (F (1, 14) = 17.54, $p < .001$).

The lower portion of Table 3 presents the absolute sucrose intake during the two bottle test. This data also shows strong taste aversions in the LiCl groups. However, in contrast to the percent of total fluid measure, the absolute intake is similar for both adults and weanlings. Analysis of variance conducted on the absolute intake data showed all effects were significant beyond the .001 level (age, F (1, 47) = 12.74; dose, F (2, 47) = 22.28, age x dose F (2, 47) = 12.08). Simple effects

Table 3

Mean Sucrose Preference and Mean Amount of Sucrose Intake for Weanling and Adult Rats in LiCl and Saline Injection Conditions

	Age	
Dose	Weanling	Adult
	Percent of Total Fluid Intake	
NaCl	65.0	72.0
1.2 mEq	37.4	11.1
3.0 mEq	34.8	11.6
	Absolute Sucrose (grams)	
NaCl	2.5	9.1
1.2 mEq	1.3	1.2
3.0 mEq	1.3	1.6

analysis revealed that the age effect was significant only in the saline groups (F (1, 15) = 14.98, p = .002). Neither of the LiCl groups showed a difference between the two age groups in the amount consumed (1.2 mEq, F (1, 15) < 1.00; 3.0 mEq, F (1, 15) < 1.00). Duncan's Range test across the dose effect showed that for both ages the saline group was significantly different (p < .05) from both LiCl groups. The LiCl groups did not differ.

Discussion

The present study examined the effect of a single i.p. injection of LiCl on bar pressing behavior in adult and weanling rats. Overall it was found that LiCl produced decrements in appetitively motivated bar pressing behavior. This agrees with other studies of appetitively motivated bar pressing (Crnic, 1976; Edelson, Gattesfeld, Samuel, and Yuwiler, 1976; Ramsey, Mendels, Hamilton, & Frazer, 1972; and Smith & Smith, 1973).

One purpose of the present study was to explore the possibility of differential reactivity to LiCl in adult and weanling rats. The data provide evidence both for and against this proposition.

The graphs in Figure 1 argue in favor of an age difference. It appears from these graphs that young rats recover from toxicosis faster than adult rats. This effect can be seen most clearly at the 56 minute point and beyond where the lines for the weanling groups have converged while the lines for the adult group remains separated. It can also be seen that the low point of responding in both LiCl groups occurs later for the weanlings than for the adults.

Statistical support for the numerical trends seen in Figure 1 is lacking. The age x dose x time interaction did not reach significance

in the overall analysis and essentially no age x dose interactions were found in the trial by trial analysis. The only hint of an age effect occurred when the dose effect was analyzed separately at each level of age and test trial. In this analysis, dose was consistently significant for more test trials and at earlier times for adults than for weanlings, a finding which corresponds to the pattern of results seen in Figure 1. However, without a significant age-dose-time interaction this analysis must be considered exploratory.

Visual inspection of the raw data suggests that large individual differences in the effect of LiCl may have caused the analysis of variance to be fairly insensitive. Table 4 presents the raw data from four subjects in the 3.0 mEq LiCl group. Adult subject number 41 and weanling subject number 128 are both examples of rats which show strong effects of LiCl. In both these subjects bar pressing was completely eliminated for a number of trials. Rats 63 and 163 on the other hand are examples of a weak response to LiCl. Neither rat stopped bar pressing entirely and the number of trials in which there is an obvious suppression of bar pressing is small. Similar extremes could be pointed out in the adult and weanling 1.2 mEq group and the weanling saline group. The presence of the responders and non-responders in the same group creates large within group variances and relatively large error terms.

Fluid intake data reported in Table 2 is supportive of the hypothesis of differential LiCl effects in adult and weanling rats. Numerically, lithium administration produced the same pattern of results for both age groups, however, the differences are significant only in the adult groups which suggest that the effects of LiCl are less intense for

Table 4

Number of Bar Presses on Each Trial for Weanling Rats #128 and 163 and for Adult Rats #41 and 63 in the 3.0 mEq LiCl Condition

Minutes post-Injection	Age			
	Adult		Weanling	
	#41	#63	#128	#163
0	16	6	11	1
4	2	16	10	18
8	1	10	7	27
12	0	6	1	6
16	0	8	1	12
20	0	16	2	8
24	0	20	0	3
28	0	13	0	24
32	0	28	0	28
36	7	32	0	39
40	3	28	0	33
44	4	18	10	29
48	9	21	27	22
52	11	14	20	8
56	0	17	9	1
71	15	21	24	12
86	1	20	22	1
101	3	12	19	14
116	11	17	29	9
131	12	26	24	34
146	42	15	25	10
Mean Rate of Bar Pressing During Baseline				
	30.33	21.40	31.53	16.80

weanling rats than for adult rats. It appears that in the present study lithium administrations reduced water intake 1/2 hour after the end of the test session. In the overnight period the intake of 1.2 mEq group appears elevated, however, examination of the total intake data shows that overall the intake of the 1.2 mEq group is similar to the saline group. This finding suggests that the elevated overnight intake in the 1.2 mEq group is a regulatory compensation for the reduced water intake in the first half hour post-testing. The 3.0 mEq does not compensate for its reduced water intake during the overnight period as the total intake figure for this group is below that of both the saline and 1.2 mEq group.

It appears from the present study that the time course of the toxicosis is different for adults than for weanlings. The graphs in Figure 1 suggest that adults begin to show the effect of the drug between 4 and 8 minutes post-injection and begin recovery after 12 minutes. Weanlings also show the first effects of the drug between 4 and 8 minutes, however, the onset of severe toxicosis appears to be somewhat slower as the lowest rates of responding do not occur until 20 minutes post-injection, after which the weanlings begin their recovery. It is noteworthy that, although weanlings show their strongest drug effects later than the adults, they apparently recover earlier. This is suggested both by the bar pressing data in Figure 1 and, by the fluid intake data in Table 1. Furthermore, although the bar pressing data suggest that the most intense period of lithium intoxication is over quite quickly, the water intake data show that effects of a 3.0 mEq dose of LiCl can be seen 14 1/2 hours after drug administration.

The data from the taste aversion test in Table 2 indicates that aversions were acquired by all LiCl groups. If one were to look only at the percent of total intake measure, one would conclude that adults show stronger aversions than weanlings. However, examination of the absolute intake data shows that both age groups consume roughly equal and very small amounts of sucrose. It seems reasonable to assume that the level of intake found in the LiCl graphs represents primarily measurement errors from spillage, dripping, etc., and that subjects showing intake of approximately one gram or less are in fact totally avoiding sucrose. This leads to the conclusion that the adults' lower scores on the percent intake measure are an artifact of their higher water intake and that both adult and weanling rats show equal performance in the taste aversion task.

Experiment 2

Experiment 2 examined the effect of LiCl on open-field behavior in adult and weanling rats to obtain a more comprehensive understanding of the behavioral manifestations of LiCl toxicosis. Because of the complex interactions between procedural and measurement factors in the open-field situation, the present study adapted a multi-measure approach, in which quantitative as well as qualitative measures of behavior were scored. Some of the problems affecting open-field measures in general and lithium's effect on activity in particular are discussed below.

Walsh and Cummins (1976) have cited a number of methodological variables which have been shown to alter activity levels in the open-field situation. Among these are size and shape of the apparatus; generally the larger the apparatus, the higher the activity level. In a square apparatus some subjects may tend to stay in the corners. Low levels of illumination tend to increase activity while high noise levels (both continuous and intermittent) tend to decrease it.

There are also problems with choosing appropriate dependent measures. A particular measure may not be sensitive to a particular drug effect, or may not represent the underlying construct which the researcher thinks he is measuring. For example, both Delbarre, Dumas and Guionnier (1970) and Johnson (1972) observed a disassociation between rearing and horizontal ambulation, both of which have been used as measures of exploratory behavior (Walsh & Cummings, 1976). What is crucial is that this disassociation occurs only for particular drugs and for particular dosage levels. The Johnson study is especially interesting. He compared eight dosages of LiCl from 2.0 to 16.0 mEq injected i.p. with eight doses of

phenobarbitone. The effect of LiCl on ambulation was seen only in the highest dosage groups; rearing, however, was depressed even at the low dosage levels. On the other hand, phenobarbitone affected both components of behavior equally. In a similar vein, Denenberg (1969) conducted a factor analytic study of a large number of open-field behavioral measures. Previously, activity and defecation had both been used as measures of "emotionality", i.e., low activity and high defecation were thought to be indicative of emotionality. Denenberg found that while defecation was consistently related to emotionality, activity measures were positively related to a so-called exploration factor, except for the first exposure to the open-field, where a weak positive correlation was found between activity and emotionality. This indicates that the measurement characteristics of activity scoring vary with the number of exposures to the test situation and points out the danger of *a priori* assumptions about the meaning of behaviors seen in the open-field.

In addition to the measurement and interpretational difficulties encountered in open-field studies in general, studies of lithium in particular have been problematic. There has been little systematic observation of the effects of lithium on activity (Johnson, 1976). Furthermore, a number of reviewers have commented that the data which do exist often show conflicting results (Johnson, 1975; Samuel & Gottesfeld, 1973; and Small & Small, 1973).

For example, Johnson and Wormington (1972) recorded rearing behavior 5, 20, and 60 minutes after subcutaneous injections of 2.0, 4.0, or 8.0 mEq of LiCl and found reductions in the frequency but not the height of rearing behaviors. The maximum reduction was at 20 minutes post-

injection. Smith (1976) found that stomach loads of 1.5 mEq of LiCl given twice a day for 10 days decreased rearing frequency only during the first five minutes of a 15 minute test period. Johnson (1975) reports that Männistö and Saarnivaars (1972) found increased levels of activity in mice given LiCl for 17 days, while Smith and Smith (1973) found that relative to non-LiCl controls rats fed a lithium adulterated diet for one week moved around less in jiggle cages. In the open-field, Smith and Smith found the lithium treated rats crossed fewer lines and showed a longer latency to move from the center of the field to the wall.

Although the immediate source of the above conflicts is not clear, it is apparent that a wide variety of variables can alter the behavior of subjects following lithium administration. For example, Syme and Syme (1973) found that rats tested in pairs showed less activity 20 minutes after an i.p. injection of 3.0 mEq LiCl than rats tested alone. Other studies (Cox, Harrison-Read, Steinberg, & Tomkiewicz, 1971; Flemenbaum, 1975) have found that lithium reduces activity only when hyperactivity is induced via other drug treatments, e.g., amphetamines. Cain and Baenninger (1977) found that initial reductions in activity caused by LiCl intubation were attenuated after repeated doses, and after three successive poisonings (48 hours apart) adult rats showed no reduction in activity when compared with saline controls.

Because the effect of lithium on freely-occurring behavior in the open-field is so uncertain, the present study adopted a scoring procedure which accounted for a variety of behaviors. Ambulatory behavior, as measured by number of lines crossed, was scored continuously. In addition, qualitative scoring of the type of behavior occurring (grooming, rearing, etc.) was made on a time-sampled basis.

To avoid *a priori* assumptions about the meaning of the behaviors scored in the open-field the present study used factor analysis to identify groups of behavior which tended to have common sources of variance. These groups of variables, or factors, were then combined into a single composite variable, or factor score, which represents the underlying dimension along which the variables vary. Using this technique the data themselves define the measures, not the *a priori* expectations of the experimenter.

Method

Subjects. Subjects were 30 adult and 30 weanling rats (Sprague-Dawley descendants) from the animal colony maintained by the Psychology Department at Virginia Polytechnic Institute and State University. Three to five days after parturition, the size of each litter was culled to eight pups. Rats in the adult groups were separated from their dams when they were 25-27 days of age and group housed in the animal colony until Day 1 of the experiment when they were 83-90 days old. Rats in the weanling groups were separated from their dams at 15 days of age and immediately began Day 1 of the experiment.

Apparatus. Two open-field apparatuses were constructed, one for adults and one for weanlings. Each field was a circular round enclosure, 20-in high for adults, 8-in high for the weanlings, formed by aluminum flashing painted black, and resting on the concrete floor of the experimental room. Lines were painted on the floor in white paint as shown in Figure 2. The open-fields were dimly illuminated by a 7.5 watt bulb suspended seven feet above the center of the apparatus.

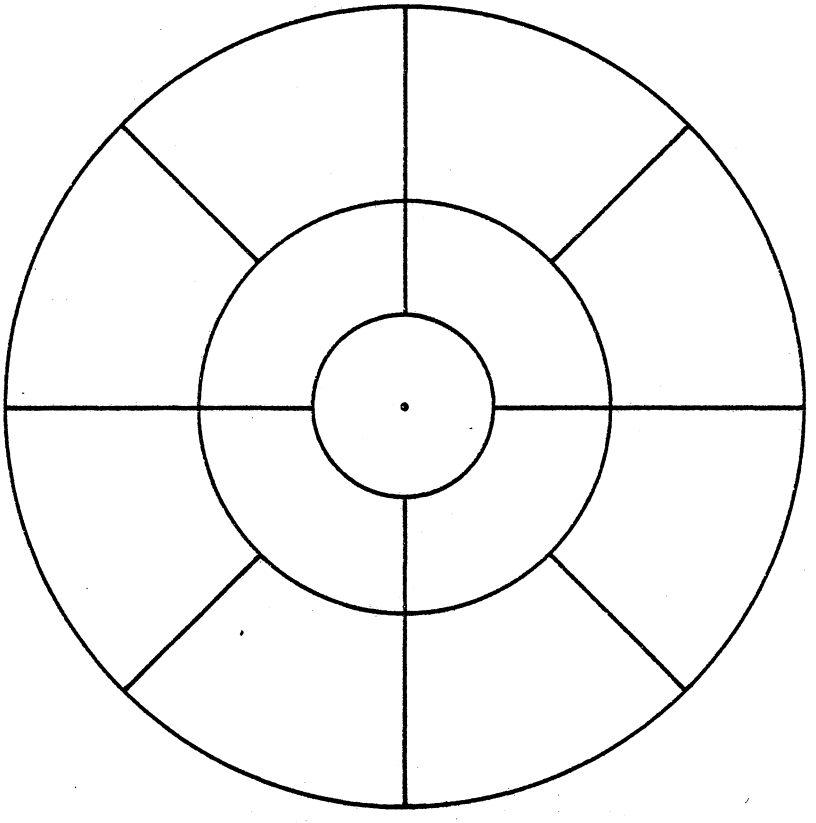

Figure 2. Diagram of the open-field apparatus.

Because size of the open-field has an effect on activity levels (Walsh & Cummins, 1976), an attempt was made to compensate for the size difference between adult and weanling rats. Thus, the open-field used for adults was 36-in in diameter, the field for weanlings was 25.5-in in diameter, which enclosed half the area of the larger field. The scaling criterion used was body length.

Two interval timers (Lafayette Instrument Co. Model 5400A) were used to time the test sessions.

Design. The experimental design was a 2 x 3 factorial which combined two age levels (weanling and adult) with three injection conditions (3.0 mEq .15 M LiCl, 1.2 mEq .15 M LiCl, and .9% isotonic saline, 20ml/kg). There were 10 subjects, five male and five female, in each cell of the design.

Procedure. On the morning of Day 1 of the experiment each subject was weighed and housed individually in hanging cages. The housing area was maintained at a temperature of 78° F with a 12 hour light/dark cycle. Subjects had ad libitum access to tap water and food in the form of dry lab chow (Purina Co.) in a mixture of both ground chow and biscuits. Beginning on Day 2, each rat was placed on a regimen of food deprivation to increase the level of activity in the open-field and to increase the similarity between the open-field and bar press studies. Accordingly, all food was removed from the cages on the morning of Day 2 and rats were given access to 1/2 teaspoon of dry ground lab chow twice a day (once in the morning and once in the evening, see footnote 1). Food deprivation continued until the end of open-field testing on the evening of Day 5 when food was once again available ad libitum.

Open-field observations were conducted on Day 5. Each subject underwent a baseline session on the morning of Day 5 and test session on the evening of Day 5. Each observation session consisted of a series of 50-sec observation trials. During the 50 second observations the subject's behavior was scored every 5 seconds, for a total of 10 scorings. Timing of the 5 second scoring periods was provided by one of the interval timers which was set to cycle every 5 seconds. The clicks produced by the timer as it cycled served as the observer's cue to score the rat's behavior in the open-field. Each of the scorings consisted of recording the number of lines crossed and the behavior exhibited by the subject immediately before the timing click. For recording purposes the subject's behavior was classified in one of the following categories:

1. Lying - Subject not moving, ventral aspect of body touching the floor;

2. Lying with head movements - Subject moving only head, ventral aspect of body touching the floor;

3. Inactive - Subject standing but not moving any part of body;

4. Head movements - Subject standing, body immobile, head moving, (for example, to look about, sniff, or chew) but head not in contact with other parts of the body;

5. Grooming - mouth or paw movements in which other parts of the body are licked, scratched, or stroked;

6. Rearing - Any behavior other than grooming in which the subject stands on its hind legs;

7. Activity - Any body movements which are not grooming or rearing (such as walking, running, circling, jumping or scratching).

An observation trial itself was conducted as follows: Five seconds before the observation trial was to start an indicator light, controlled by the other interval timer, went on. This was the experimenter's cue to remove the animal from its cage. At the sound of the first timing click, the rat was placed, by the base on its tail, into the center of the open-field, facing away from the experimenter. Scoring commenced at the next timing click. At the end of the observation trial the indicator light went out, the subject was removed to his home cage, and any urination or defecation deposited in the open-field was cleaned up with a dry paper towel. During the observation sessions rats were run in squads of two or three. For adults the squads always consisted of subjects of the same sex.

For the baseline session on the morning of Day 5 water bottles were removed and subjects were moved from the housing area to the testing room in their home cages where they remained except when they were in the open-fields. During the baseline session subjects received an observation trial every 4 minutes for one hour. No injections were given at this time. The purpose of the baseline session was to adapt the subjects to the test procedure and familiarize them with the open-field apparatus. After the baseline session the rats were returned to the housing area and were given their regular morning feeding. However, water bottles were not returned to the cages and no fluids were available until the start of the test session.

Immediately prior to the evening test session each rat received 12 minutes access to a 12% (w/v) sucrose solution. Exposure to the sucrose flavor was provided in order to assess the development of taste aversions

as a result of the LiCl injection. During sucrose exposure all rats were observed to ensure that they had tasted the sucrose solution. Any subject who had not tasted the solution at the end of 15 minutes was force-fed a small quantity of the fluid.

After the sucrose exposure period rats were brought to the testing room and received an i.p. injection of either 3.0 mEq LiCl, 1.2 mEq LiCl or normal saline immediately (no more than 1 minute) prior to their first observation trial. The experimenter conducting the observations was blind as to the content of the injection.

For each rat, a total of 21 post-injection observation trials were conducted. Observations were started immediately after the injection and occurred every four minutes for the next hour, and once every 15 minutes for the final hour and one half, producing a total observation period of two and one half hours.

After the testing session subjects were returned to the housing area and received access to ad libitum food and to 1/2 hour of tap water. After the water access period the bottle was removed and weighed in order to measure the amount drunk, then returned to the cage for another 11 1/2 hours. At the end of this time period, on the morning of Day 6, the water bottles were again removed and weighed in order to measure intake. The bottles were not returned to the cages until the evening of Day 6, at which time each rat received a test for sucrose taste aversion using a two bottle preference procedure. One bottle contained 12% sucrose; the other tap water. The bottles were available for 30 minutes, after which the bottles were removed and weighed to measure the intake of each fluid.

Over the course of the experiment two different experimenters were used to conduct the open-field observations, but only one observer was present at any particular time. In all cases if an observer conducted a subject's baseline session, (s)he also conducted the subject's test session.

Results

Descriptive analysis of raw open-field measures. Over all test trials adults crossed an average of 4.47 lines while weanlings crossed an average of 11.47 lines. Of the behaviors scored, activity occured most often (M = 2.23, SD = 2.01). The other behaviors had means and standard deviations as follows: inactivity, M = 1.89, SD = 2.42; rearing, M = 1.05, SD = 1.52; grooming, M = 0.46, SD = 1.23; lying, M = 0.10, SD = 0.82; lying with head movements, M = 0.01, SD = 0.18. Besides the above behaviors, which were scored directly in the open-field, a measure of latency to leave the center circle of the apparatus was calculated. The latency measure was calculated by counting the number of 5 second observation periods which elapsed before the subject crossed one line. The mean latency was 2.30 with an SD of 2.44.

In the open-field, active animals were most often involved in exploring the apparatus, although occasionally subjects would be involved in other activities such as circling, jumping or stretching. Stretching, the most frequent of these behaviors, occurred at two times; immediately after injection in the LiCl groups and in all groups during the every-15-minute observations when subjects apparently fell asleep during the ITI. Scoring of head movements generally represented looking about, sometimes in combination with intermittent activity. However, more

often, especially in adults, this pattern of behavior was maintained in a stationary position for relatively long periods of time. This was also true of grooming. Usually once an animal started to groom, it would continue to do so throughout the trial. Rearing tended to occur interspersed with brief periods of activity. Subjects would be active briefly, usually moving to another part of the field, and after rearing, would move and rear again. The lying behaviors occurred infrequently and only in the LiCl groups.

Factor analysis. The latency measure, along with the total number of each of the seven scored behaviors, and the $\log_{10}$ of total number of lines crossed for each test trial were subjected to factor analysis, using a principle factor solution with iteration and varimax rotation (Harman, 1976). The factor analysis resulted in four factors which together accounted for 100% of the estimated common variance in the data.

Table 5 presents the rotated factor matrix which contains the correlations between each of the variables and each of the factors. Factor 1 has high positive loadings on lines and activity and a high negative loading on inactivity and might reasonably be labeled "locomotion." Factor 2 loads highly only on the two lying behaviors and seems to represent "severe illness." Factor 3 has a high negative loading on head movements and a moderately high positive loading on rearing, suggesting that this factor represents behavior which is active but stationary. Finally, Factor 4 represents grooming and loads highly only on that behavior. The percent of variance accounted for by each of the four factors was 42.8%, 24.2%, 16.7% and 16.2%, respectively.

Table 5

Rotated Factor Matrix for the Variables in the Factor Analysis of Open-Field Behavior

Variable	Factor 1	2	3	4
Log_{10} lines	0.70	-0.20	0.42	-0.04
Latency	-0.25	0.35	-0.22	-0.01
Activity	0.82	-0.15	0.26	-0.13
Head movements	-0.07	-0.06	-0.91	-0.15
Grooming	0.02	-0.03	0.04	0.99
Rearing	0.23	-0.07	0.66	-0.09
Inactivity	-0.94	-0.10	0.04	-0.19
Lying (Head movements)	-0.01	0.84	0.03	-0.02
Lying	-0.03	0.88	0.01	-0.00

Analysis of the factor scores. Using the results of the factor analysis, factor scores for each of the four factors were calculated for each 50 second observation period. Calculation of inter-observer reliability from baseline observations on three adult rats produced correlations of .94, .82, .88 and .94 for each of the four factors, respectively.

Because of baseline differences between adult and weanling rats,[4] each subject's factor scores in testing was expressed as a proportion of their respective mean baseline factor score (i.e., test trial factor score divided by mean baseline factor score).

Since the four factors are independent four separate age x dose x test trial (time) analyses of variance were conducted. Factor 2 produced significant main effects of dose (F (1, 54) = 3.24, p = .05) and time (F (20, 1040) = 1.60, p = .05). No other main effects or interactions were significant (p > .05). A Duncan's Range test across the Factor 2 main effect of dose showed that the 3.0 mEq group and the saline group were significantly different (p < .05). The 1.2 mEq was not significantly different from the saline or 3.0 mEq group.

Figure 3 presents the proportion of baseline scores for Factor 2. The lower portion of Figure 3 shows that for adult subjects all three dosage groups show essentially identical performance except during the period between 8 and 32 minutes in which the 3.0 mEq group drops well below its baseline performance. The upper part of Figure 3 indicates a slight depression in the 1.2 mEq group between 8 and 28 minutes post-injection. The 3.0 mEq groups show a slightly deeper depression between 4 and 16 minutes. Visually these data suggest that LiCl had a stronger

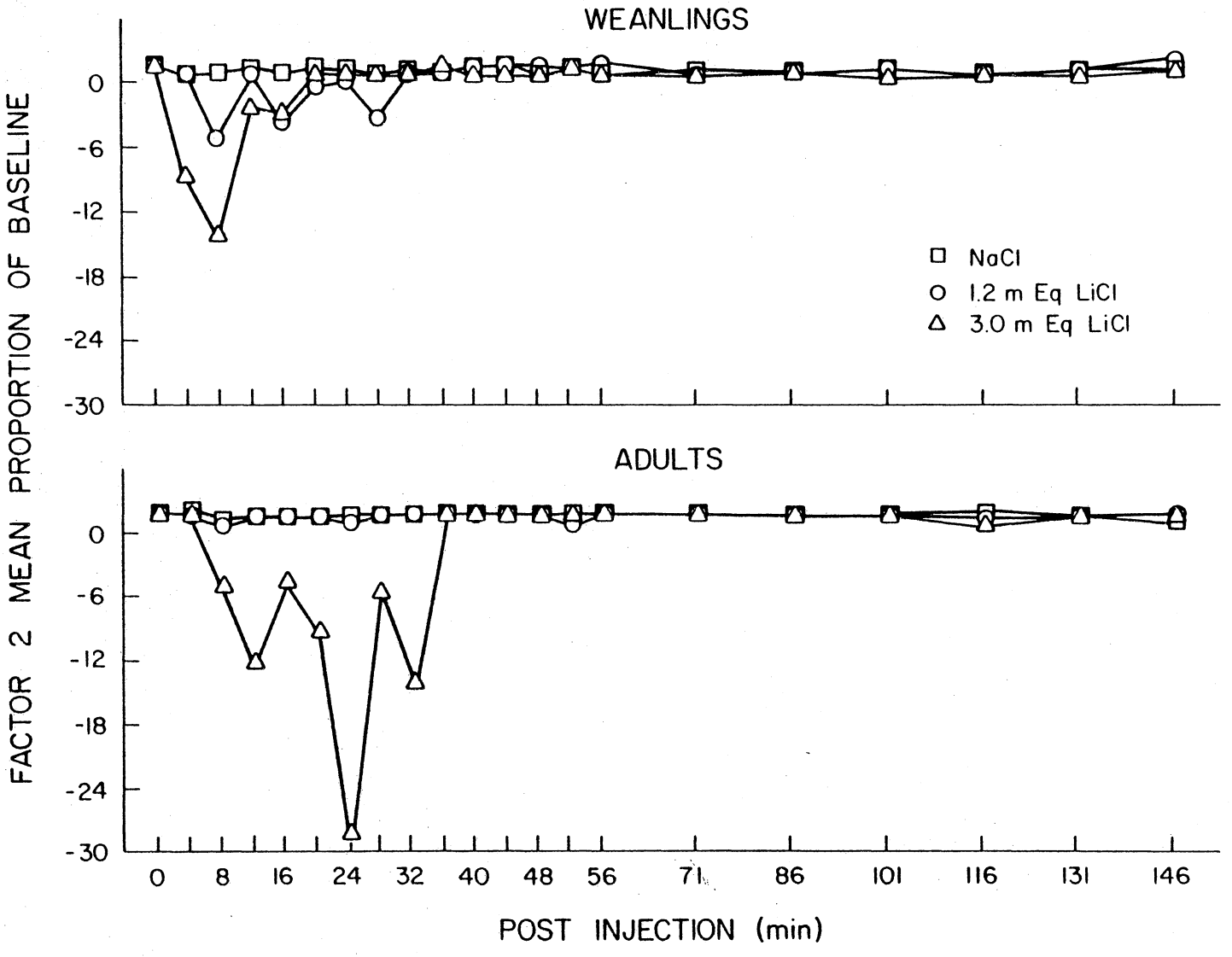

Figure 3. Factor 2 mean proportion of baseline activity as a function of minutes following an injection of LiCl or saline for weanling and adult rats.

effect on open-field behavior in adult rats than in weanling rats although statistical support for the visual trend is lacking in the analysis.

Table 6 shows the amount drunk during the measured water intake periods. The 1/2 hour column refers to the amount drunk in the first 30 minutes after the end of testing, 2 1/2 to 3 hours post-injection. The "overnight" columns contain data from the period immediately following the 1/2 hour measurement until the morning after the test session (a period of 11 1/2 hours). The total column reflects the sum of 1/2 hour and overnight intakes. A separate analysis of variance was performed on each of the three measures.

The top portion of Table 6 shows that in the first 30 minutes after testing, water intake is depressed in the LiCl groups. Analysis of variance over these data showed that all factors were significant (age, F (2, 54) = 7.73, p = .008; dose, F (2, 54) = 5.10, p = .009; age x dose, F (2, 54) = 3.23, p = .05). Simple effects analysis showed that the dose effect was significant for adults (F (2, 29) = 4.31, p = .02) but not for weanlings (F (2, 29) = 1.37, p > .05). A Duncan's range test showed that for adults the saline group consumed significantly more water than either of the LiCl groups (p < .05). The LiCl groups did not differ from each other.

The middle portion of Table 6 indicates that for adults water intake decreases as LiCl dosage increases. Weanling show a roughly equal water intake in the LiCl groups. Analysis of variance on these date produced a significant age effect (F (1, 54) = 168.91, p < .001) and a significant age x dose interaction (F (2, 54) = 3.43, p = .04). Simple

Table 6

Mean Water Intake in Grams for Weanling and Adult Rats in LiCl and Saline Injection Conditions

	Age	
Dose	Weanling	Adult
NaCl	1.17	4.06
1.2 mEq	0.90	1.10
3.0 mEq	0.77	1.50
	Overnight	
NaCl	5.99	25.74
1.2 mEq	7.10	23.98
3.0 mEq	7.08	18.95
	Total	
NaCl	7.16	29.80
1.2 mEq	8.00	25.08
3.0 mEq	7.85	20.45

effects analysis showed no significant dose effect for the weanlings (F (2, 27) = 1.41, p = .26). For the adults the dose effect just misses an acceptable level of significance (F (2, 27) = 2.85, p = 0.75).

Finally, the lower portion of Table 6 shows decreasing intake as a function of increasing dose in adults and roughly equal intakes across dose for the weanlings. Analysis of variance showed age, dose, and age x dose to be significant (F (1, 54) = 158.46, p < .001; F (2, 54) = 3.27, p = .05; and F (2, 54) = 4.39, p = .02, respectively). Simple effect analysis showed no significant dose effect for weanlings (F (2, 29) < 1.0) while the dose effect for adults was significant at the .02 level (F (2, 29) = 4.31). Duncan's Range test on the adult dose effect showed that only the difference between the saline and 3.0 mEq groups was significant at the .05 level.

Table 7 presents the mean percent sucrose intake (SUC/[SUC + HOH]) during the two bottle aversion test. It shows taste aversion effects for both the adults and weanlings; however, for the adults the mean percent sucrose consumed in the LiCl groups is somewhat less than for the weanlings. Analysis of variance on the data from Table 7 showed a significant dose effect (F (2, 54) = 64.01, p < .001). The age x dose interaction was not significant (F (2, 59) > 1.0). A Duncan's Range test showed that the two LiCl groups were significantly different (p < .05) from the saline groups but not from each other.

The lower portion of Table 7 presents the absolute sucrose intake during the two bottle test. As in Experiment 1, the absolute sucrose intake in Experiment 2 contradicts the percent of total fluid intake measure by showing that both adults and weanlings in the LiCl groups

Table 7

Mean Sucrose Preference and Mean Amount of Sucrose Intake for Weanling and Adult Rats in LiCl and Saline Injection Conditions

Dose	Age	
	Weanling	Adult
	Percent of Total Fluid Intake	
NaCl	72.9	69.20
1.2 mEq	27.8	18.70
3.0 mEq	33.5	18.90
	Absolute Sucrose Intake (grams)	
NaCl	2.74	5.86
1.2 mEq	1.00	1.11
3.0 mEq	1.29	1.62

drink approximately equal and very small amounts of sucrose. Analysis of variance over the absolute intake data indicated significant effects of age (F (1, 54) = 10.82, p = .002), dose (F (2, 54) = 32.06, p < .001) and age x dose (F (2, 54) = 7.21, p = .002). Simple effects analysis showed that age was significant only in the saline groups (F (1, 18) = 13.29, p = .002). F values for the age effect at 1.2 mEq and 3.0 mEq were both less than 1.0. Duncan's range test across the dose effect showed that the two LiCl groups differed from the saline group but not from each other (p > .05).

Discussion

Experiment 2 examined the effect of a single i.p. injection of LiCl on open-field behavior in adult and weanling rats. A variety of behaviors were scored and a factor analysis of the various measures produced four factors; locomotion, severe illness, stationary activity, and grooming, which were used in subsequent analysis. Analysis of variance on each of the four factors showed significant drug effects for only the severe illness factor. Initially, it is somewhat surprising that only one of the four factors showed significant drug effects, but this is probably due to the fact that the factors are arbitrarily defined and constructed so as to be independent. Thus, since one of the factors is related to the drug effect, the other factors would not be because they share no common variance.

It appears that overall, lithium administration reduced activity levels in the open-field. Analysis of the illness factor showed that rats in the LiCl groups scored higher on this factor. Since the illness factor showed a high positive correlation with lying and a negative

correlation with lines crossed and activity, it seems reasonable to conclude that this factor is indicative of low levels of activity. This finding agrees with the overall trend in the literature of lithium induced reductions in activity level (Johnson, 1975).

In regards to developmental differences the data shown in Figure 3 numerically suggest that the weanling rats were not as sick as the adult rats. This effect is seen as a deeper descent in the curve for the adult 3.0 mEq groups. It is also interesting to note the depression in the weanling 1.2 mEq group, when there is no depression seen in the adult 1.2 mEq group. This corresponds to a trend seen in the data from Experiment 1 in which the curves for the weanling 1.2 mEq and 3.0 mEq groups seem closer together than the curves for the same two adult groups.

Also, as in Experiment 1, the statistical analysis failed to confirm the visual trends seen in the data by indicating a nonsignificant age x dose x time interaction. The same problems with individual differences in the response to LiCl which were discussed in Experiment 1, were seen in Experiment 2. For example, of the 20 rats given 3.0 mEq LiCl only five (3 adults and 2 weanlings) showed toxic effects severe enough to cause them to lie down in the open-field, other rats in the same group showed no visually obvious effects of the injection.

As in Experiment 1, the data of Experiment 2 suggest a different time course of the illness for adults and weanlings. However, the two age groups show a pattern of results opposite that found in Experiment 1. In Experiment 2 it appears that weanlings experience the most intense drug effects sooner than the adults. The weanlings show their maximum

drug effect at 8 minutes post-injection while for adults the maximum effect does not occur until 24 minutes post-injection. The difference between the two studies may have occurred because bar pressing and open-field behavior measure different components of the illness, or the slightly different feeding regimens or pre-testing procedures may have been factors. It is also possible that sampling error produced the difference, especially since the illness effects seen in the data seem to result from a fairly small number of strong responders to lithium.

The post-test fluid intake data shown in Table 5, generally follows the pattern seen in Experiment 1 with LiCl causing a decrease in water consumption which is greater for the adults than for the weanlings. Adult subjects in the present study's 1.2 mEq group did not completely compensate for their initial reduced water intake by drinking more during the overnight period as the adult subjects in Experiment 1 did.

The taste aversion data shown in Table 6 is also similar to that found in Experiment 1. The percent of total intake measure suggests that weanling rats show weaker taste aversions than adults. However, examination of the absolute sucrose intake shows that in fact, adults and weanlings avoid sucrose equally well. The weanlings higher percent intake scores appear to be an artifact of floor effects in the measure of sucrose intake, and the larger water intake of the adults.

Finally, analysis of the levels of baseline responding in the open-field indicated that adjusting the size of the apparatus was not successful in equating levels of activity across age groups. Weanlings showed significantly higher activity levels than adults on three of the four factors. Given the tendency of smaller open-fields to decrease activity

levels (Walsh & Cummins, 1976), it must be assumed that weanling rats have a higher activity level than adults. This conflicts with Candland and Nagy (1969) who found that young Wistar rats had lower activity levels than adults up until approximately 40 or 50 days of age. This conflict may be due to strain differences between the Wistar rats used by Candland and Nagy and the Sprague-Dawley decendents used in the present study.

General Discussion

This paper has presented the results of two studies which sought to examine the toxic effects of lithium chloride within the context of its use as an aversive agent in conditioning experiments. Two different behavioral measures of the drug effect were used, open-field behavior and appetitively motivated bar pressing behavior. In addition, the studies examined the effect of LiCl on fluid intake 3 hours and 12 hours after drug administration.

Both the present studies provide some evidence of a developmental difference in the toxic response to LiCl. The numeric trend of the bar pressing data and scores on the sickness factor from the open-field data both suggest differences between adults and weanlings. The nature of this difference is somewhat obscured by the different time courses seen in the two studies. The bar pressing data suggests that weanlings recover faster than adults while the open-field study suggests the difference is more in the intensity of the drug effect. To this author's knowledge there are no other developmental studies of lithium toxicosis which could clarify these issues.

Statistical support for these numeric trends was found to be lacking in both studies. This was presumed to be because of the large individual differences in the response to lithium. Such differences have been observed before. Crnic (1976) found that only three out of five rats given daily doses of 3.0 mEq LiCl developed severe toxic symptoms. Of the two animals that tolerated the lithium administration only one showed a significant change in bar pressing behavior.

There are a number of steps which might be taken to increase the sensitivity of subsequent research in this area. Crnic suggests the use of serum lithium levels (rather than dose) in assigning subjects to groups since as she points out, lithium levels can vary as much as 100 to 200 percent from one subject to another after the administration of equal doses. Another apparent problem in the present study was the use of a relatively short 50 second test trial. This observation period was too short to produce a stable measure of behavior (see Table 1). Longer training sessions and allowing the rats to remain in the operant chambers throughout the test session might also help to stabilize rates of responding. In addition, subsequent pilot work for a study of LiCl's effect on food reinforced drinking behavior indicated that the use of a variable ratio rather than a variable interval schedule produced more constant rates of responding.

The water intake data from both studies support the contention of developmental differences in response to LiCl. In both studies, changes in water intake after LiCl injections were significant for adults but not for weanlings.

The overall pattern of results in the water intake measure suggests that LiCl administration decreases water intake. This finding conflicts with Crnic (1976) who found no change in water intake in the first 24 hours of LiCl administration (3.0 mEq in two divided doses a day) and an increase in consumption after three days. Smith and Balagura (1972) found increases in water consumption three hours after intubation of 10 ml of .12 M LiCl.

Other studies have found LiCl related decreases in water intake. Barker and Smith (1974) found that injections of 3.0 mEq LiCl reduced water intake 21 minutes and 50 minutes post-injection. Nachman (1963) observed reductions in total intake in the first 24 hours after rats drank an average of 9 ml of .12 M LiCl. As in the present experiment Nachman found that lithium treated rats compensated for their initial reduced intake and by the end of 48 hours the total intake of the lithium group and the distilled water controls were identical. Nachman's finding that LiCl treated rats compensate for their decreased water intake corresponds to a similar finding in Experiment 1 of the present study. The fact that Nachman's results did not show compensation until 48 hours (vs. 12 hours in the present study) is probably due to the much larger amounts of lithium ingested by his subjects. Crnic's failure to find differences in water intake at dosage levels comparable to the present study was probably due to the relatively long (24 hour) test interval used. Smith and Balagura's finding of increased water intake may be peculiar to their intubation procedure.

The data from the taste aversion tests in Experiments 1 and 2 show strong taste aversions to sucrose in both the 1.2 mEq and 3.0 mEq LiCl groups. Examination of the absolute intake data shows that all groups exhibit virtually total avoidance of sucrose. This finding is in conflict with Nachman and Ashe (1974) who found stronger taste aversions in adult rats given 3.0 mEq LiCl than 1.2 mEq. This conflict probably stems from differences in the fluid deprivation regimens used in the two studies. Nachman and Ashe used a deprivation schedule consisting of a single daily 10 minute fluid exposure for 4 days, and a one bottle

test which would produce a strong deprivational challenge. The present study used a single 8 hour deprivation period and a two bottle test, which would present a mild deprivational challenge. Frumkin (1975) has shown that deprivational states can overcome taste aversions. It's likely that the mild deprivation conditions used in the present study were not sufficient to induce subjects to drink sucrose in spite of their taste aversion.

It is interesting to speculate that the differences in sensitivity between one and two bottle testing procedures may be due to the different deprivational challenges they present. In a two bottle test it is not necessary for the subject to expose itself to the aversive flavor in order to meet its fluid needs. The same phenomenon may underlie differences sometimes found between young and adult rats in taste aversion performance. Campbell, Tehgtsoonian and Williams (1961) found that adult rats tolerated food deprivation longer than young rats. If the same were true of water, after a fixed period of deprivation, young rats would be in a greater need state than the adults, thus producing a higher effective level of deprivation. This greater deprivational state would tend to produce poor taste aversion performance in young rats when compared to adults.

Finally, the differences between the percent of total fluid intake and absolute sucrose intake measures of taste aversion, point out certain difficulties of interpretation which can be encountered. Developmental studies which look only at the percent of total fluid intake without regard to the absolute intake data (e.g., Barker, Baker, & Kesner, 1977) could be led to the false conclusion that the performance

of the young rats was inferior to the adult rats. The present study showed that even though young rats showed poorer performance on a percent intake measure, in terms of absolute intake both age groups show equivalent and very strong taste aversions.

Footnotes

[1]"Morning" sessions were conducted two hours after the start of the light cycle. "Evening" sessions were conducted immediately following the end of the light cycle.

[2]Ballbearing drinking tubes were used to improve accuracy whenever fluid intake was measured.

[3]In order to evaluate the two counter-balancing factors in the design, sex and box, for possible inclusion in the analysis as blocking variables, correlations were calculated between them and the dependent variable, number of bar presses. The absolute correlation between sex and bar presses was .13, while box and bar pressing yielded a correlation of .28. In accordance to the criteria suggested by Keppel (1973) box was included as a blocking factor because its correlation with the dependent variable exceeded .20. An analysis of variance on the mean number of bar presses during the last 15 trials of training on Day 5 showed no significant main effect of age (F (1, 36) = 2.83, $p > .05$) indicating that both adults and weanlings achieved similar levels of baseline performance on the bar pressing task. There was, however, a significant box effect (F (1, 36) = 8.45, $p = .006$) and a significant age-by-box interaction (F (1, 36) = 5.30, $p = .027$). Inspection of the group means indicated that one box produced lower rates of responding than the other and that the difference in rate of responding was less for pups than for adults.

[4]Analysis of variance on mean factor scores calculated from the baseline data revealed significant age effects for factors 1, 2 and 3 (p's $< .001$). Weanlings showed higher levels of activity on all three factors. Factor 4 produced no significant effects.

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BEHAVIORAL ASSESSMENT OF THE TIME COURSE AND RELATIVE INTENSITY OF ACUTE LITHIUM CHLORIDE TOXICOSIS IN ADULT AND WEANLING RATS

by

H. Alan Griesemer

(ABSTRACT)

Adult and weanling rats were used in two studies which investigated the time course of and developmental differences in the toxic effects of a single i.p. injection of LiCl. The studies examined LiCl's effect on food reinforced bar pressing, open-field behavior, water intake, and taste aversion learning. Both experiments used a 2 x 3 x 21 mixed design which provided for the factorial combination of adult and weanling age groups with three injection conditions, 1.2 mEq LiCl, 3.0 mEq LiCl, and isotonic saline, and 21 test trials over a period of 2 1/2 hours. Experiment 1 demonstrated that LiCl reduced rates of bar pressing. Numerically, weanlings showed the maximum toxic effects later and recovered earlier than adults, but the age difference was not significant. Data from the open-field observations were factor analyzed; analysis of variance on the resultant factor scores showed significant drug effects for only one of four common factors. The factor showing significant results correlated most highly with lying down in the open-field and numerically but not statistically indicated that weanlings exhibit an earlier but less intense effect of LiCl injection relative to adults.

Overall the behavioral effects of LiCl were strongest from 8 to 28 minutes post injection and were essentially absent after 2 1/2 hours.

LiCl was shown to reduce fluid intake with the reduction being less for weanlings than for adults. LiCl injection produced taste aversions in all groups, both ages showed equilivant levels of aversion.