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Patterns of ingestion of rats during chronic oral administration of lithium chloride

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ABSTRACT

Chronic lithium administration to rodents is used to explore the potential neural mechanisms of mood stabilization, as well as to model the side effects of chronic lithium on multiple organ systems. Oral administration of lithium in the maintenance diet or drinking water is convenient, but lithium can acutely affect intake and it can mediate acquisition of conditioned taste aversions (CTA). We compared ad libitum food and fluid intake by male rats with LiCl or NaCl solutions as their sole source of fluid across 20 days, with a commonly used dosage of LiCl (24 mM: 1 g / L LiCl). To quantify the pattern of intake, rats were housed in cages equipped with lickometers to detect licks and infrared photobeams to detect food access with 6-s resolution. To determine if rats formed a CTA to LiCl, they were subsequently tested with access to NaCl. Rats showed an immediate avoidance of the LiCl solution, as seen on the first day of access by an increased latency to initiate drinking and a decreased size of drinking bouts. Rats showed a differential response to LiCl vs. NaCl after as few as 5 licks. Chronic consumption of LiCl solution led to significantly decreased food and fluid intake compared to baseline, with concomitant weight loss. The decreased intake was realized by marked changes in the pattern of drinking and feeding bouts: a decrease in per-lick volume and a decrease in licks per drinking bout, and an increase in feeding bout duration resulting in an overall decrease in eating rate. Conversely, chronic NaCl access led to an increase in drinking bout number and licks/bout. The avoidance of LiCl was likely a combination of toxic effects of ingested LiCl and rapid acquisition of a learned aversion to the taste of LiCl, as shown by an extinguishable generalized aversion to NaCl solution during subsequent NaCl test days. The marked effect of chronic oral LiCl on ingestion may impact the oral dosing of lithium as well as the rat's metabolic status.

1. Introduction

Chronic lithium administration is the pre-eminent treatment for bipolar disorder in humans. In rodent models, Li is chronically administered to explore potential neural mechanisms of mood stabilization [1, 2], as well as to model the side effects of chronic Li on multiple organ systems.

Although human therapy employs a daily dose of Li_2CO_3 in extended-release form, in animal subjects Li is often administered as LiCl or Li_2CO_3 in the drinking water or mixed with the maintenance diet. While providing Li solution as the sole source of fluid is convenient, it means that the pattern of drinking determines the pattern of dosing. Because the plasma levels of Li peak shortly after bouts of intake, the pattern of intake reflects a pattern of self-administration of the drug. The physiological or neural sequelae of Li administration may therefore be yoked to the structure of Li-containing fluid or food intake.

Because Li has both taste and post-ingestive properties, the pattern of drinking Li solutions may diverge from the pattern of drinking plain water, as well as altering patterns of eating. Changes in meal patterns may reveal evidence of toxic effects, as a combination of acute unconditioned and learned conditioned effects, as rats readily learn a conditioned taste aversion (CTA) to Li solutions [3–7]. The pattern of chronic Li intake may have clinical relevance, if it reflects some of the side effects seen in humans on chronic Li, such as anorexia or weight gain, polydipsia and polyuria, and nausea and diarrhea [8].

Long-term changes in drinking might also reveal the emergence of compensatory behaviors, comparable to those seen in animals that must subsist on unpalatable or other toxic diets [9–15]. Animals might change

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the diurnal timing of intake, the number of intake bouts, or the size of intake bouts.

Previous ingestive studies have looked at changes in food and fluid intake and weight gain during chronic lithium administration in drinking water [16], or in maintenance chow [17], or via meal-initiated intraperitoneal infusions [18], or via osmotic mini-pump [19–21]. Not many, however, have looked at changes in meal patterns induced by chronic lithium [18].

There have also been several studies on the intake of LiCl solutions on ingestion rate and subsequent acquisition of CTA [5,6,22,23], including some of the earliest papers on CTA [3,24]. These studies have usually employed short-term access (e.g. 30 min or less) in water-restricted rats to encourage intake of the LiCl, which served as both taste conditioned stimulus (CS) and toxic unconditioned stimulus (US). There have also been studies on the acute effects of acute LiCl injection on subsequent food or fluid intake, which help distinguish acute toxic effects from emerging associative CTA effects on intake [12, 25–27]. There have not been studies, however, of differences in intake of Li vs. Na during long-term studies that might be affected by gustatory and associative effects.

In this study we compared chronic ad libitum intake by male rats of LiCl or NaCl solutions as their sole source of fluid across 20 days at concentrations based on a commonly used dosage of LiCl (24 mM: 1 g / L LiCl) [28–30]. The use of NaCl (1.4 g/L) allowed for both an osmotic control and a gustatory control, as there is much evidence for similarity in Na and Li taste transduction and perception by rodents [24,31]. To quantify the pattern of intake, rats were housed in cages equipped with lickometers to detect licks and infrared photobeams to detect food access with 6-s resolution. Daily body weight and intake was measured, and various feeding and drinking bout parameters were calculated. To determine if they formed a CTA to LiCl, after a brief recovery phase rats were subsequently tested with access to NaCl.

2. Material and methods

2.1. Subjects

Adult male Sprague-Dawley rats (Charles River Laboratories, Wilmington, MA) weighing between 216 and 386 g (mean 308 ± 18 g) at the beginning of the experiment were used. Rats were housed individually in special cages (described below) in a temperature-controlled colony room. Rats were maintained on a 12:12 light/dark cycle with lights on at approximately 0600 h, and data were collected for 23 h each day, allowing 60 min to weigh and replenish the food and fluid containers (usually between 0900 and 1000 h). All procedures were approved by the FSU Institutional Animal Care and Use Committee.

2.2. Apparatus

Rats were housed in standard polycarbonate wire-top cages measuring 27×48×20 cm modified with holes cut in the front and in the rear of the cage to accommodate one feeding compartment and two drinking ports [32]. A stainless-steel nest box was placed inside the cage. At the front of the cage a stainless-steel food compartment held a 4 oz glass food jar. When a rat entered the feeding compartment, its head broke an infrared beam located above the food jar so that every second during the 23 h period the rat had its head inside the compartment was recorded. Thus, time in the food compartment during a feeding episode, not amount of food intake, was measured.

A two-bottle stainless steel holder was located opposite the food compartment. In the present experiment, only one bottle was used: it was placed on the left side for the duration of the experiment. The sipper tube on the bottle was recessed just within tongue's reach behind a slot, allowing contact circuits to record each lick that the rat made during a 23 h period.

A computer recorded time spent in the food compartment and number of licks with 6 s resolution, meaning that after every 6 s bin, the number of seconds that the rat was in the food compartment and the number of licks on the sipper tube were recorded. This resulted in 13,800 6 s bins for every 23 h testing period during which the data were recorded. The computer was always started by 1000 h. A photo detector allowed the computer to record the time when the room lights went off and on during the 23 h period.

2.3. Procedure

Rats (n = 16) were run successively in 2 cohorts of 8 each. All rats were provided with ad libitum access for 23 h per day to powdered Purina rodent chow and a single bottle of fluid. Food and fluid intake were measured by weighing food jars and drinking bottles (± 0.1 g) before and after the 23 h recording period.

For each of the 2 runs, the experiment was divided into four testing phases: 1) baseline water, 2) experimental, 3) water recovery, and 4) NaCl test phase. In the baseline water phase, all rats were provided with single bottle of distilled water for 5 days. In the experimental phase, the rats (n = 8 / solution) were given either LiCl (1 g/L; 24 mM) or NaCl (1.4 g/L; 24 mM) dissolved in distilled water as their sole source of fluid for 20 days. In the water recovery phase, all rats were given distilled water for 5 days. Finally, in the NaCl taste test phase, all rats were given 24 mM NaCl for 4 days. Drinking solutions were prepared fresh and replaced each day.

2.4. Intake analysis

Any differences found in food and water intake during the experimental phase could be due to a change in a variety of factors: (1) the number of ingestive episodes (bouts), (2) the duration of these bouts, (3) the efficiency of feeding or drinking within the bouts, or (4) intake occurring outside of a bout (sampling). Feeding and drinking bouts were defined as follows:

For feeding, a bout started when a rat entered the food compartment for 3 s. The bout had to include at least 30 s with the rat within the food compartment (i.e. breaking the infrared beam) in order to be included as a valid bout. The bout ended when the rat left and did not re-enter the food compartment again for 50×6 s bins (5 min).

For drinking, a bout started when a rat made 3 licks. A drinking bout had to contain at least 30 licks to be included as a valid bout; previous work showed that this criterion included >99 % of licks in bouts [32]. The bout ended when the rat did not lick for 50×6 s bins (5 min). Licks that were recorded outside of defined bouts were designated as "sampling licks."

Using the daily food and fluid intake and the above bout criteria, 11 dependent variables were measured or calculated for each day (see Table 1.)

Table 1
Variables measured or calculated for each day of experimental phase for both food and fluid intake.

body weight (g)
intake (g) of food and fluid
number of bouts
number of daytime (light phase) bouts
number of nighttime (dark phase) bouts
mean bout size (licks / bout, or beam breaks / bout)
mean bout length (s)
bout efficiency = intake / (number of bouts x mean bout duration) (g/min)
volume per lick (μl) or intake per beam break (g)
number of licks or beam breaks included in all bouts ("within bouts")
number of licks or beam breaks NOT included in bouts (sampling licks "outside bouts")

2.5. Statistical analysis

All bout and intake data were analyzed using Statistica software (Statsoft, Tulsa, OK), using two-way analyses of variance with one repeated measure (NaCl-treated rats vs. LiCl-treated rats, with testing day as the repeated factor). Post-hoc comparisons were made by Neuman-Keuls test and were considered significant if $p<0.05$. Data are presented as mean ± *s*.e.m.

Minute-by-minute statistical comparison of cumulative lick curves were made by two-tailed *t*-test, applying a Bonferroni correction for 1380 comparisons to detect significance at an alpha level of 0.000362.

3. Results

Examples of patterns of drinking and feeding across the experiment for 2 individual rats are shown in Figs. 1 and 2, respectively. The F- and p-values of individual 2-way ANOVA comparisons for the ingestive variables measured (Table 1) are given in Table 2 (for feeding) and Table 3 (for drinking). The mean daily values of body weight and ingestive variables are plotted in Figs. 3–6. Cumulative fluid intake curves (showing cumulative licks per minute) are plotted in Figs. 7–9.

Two general phenomena were observed: a large difference in patterns of intake between rats with access only to LiCl vs. those with access only to NaCl, and 2) a transient aversion to drinking NaCl seen in LiCl-treated rats during the NaCl test phase. Thus, chronic LiCl access had a direct effect on ingestion and a learned effect resulting in a CTA against salt solutions.

3.1. Baseline water phase

There was no difference in body weight, feeding, or drinking between NaCl and LiCl groups in the baseline water phase (except for slightly but significantly longer feeding bouts in the LiCl group). Rats ate 27.3 ± 0.6 g of chow per day in an average of 15.9 ± 0.9 feeding bouts with an average bout length of 379.9 ± 20 s. Rats drank 43.0 ± 1.5 g of water per day in an average of 22.6 ± 1.5 drinking bouts with an average bout size of 369.0 ± 26.9 licks, with a lick volume of 5.5 ± 0.2 μl.

During the baseline water phase, the two groups of rats had nearly identical rates of water intake across the 23-h recording period, such that their cumulative intake curves were not significantly different (see Fig. 7A). Rats initiated drinking mostly towards the end of the lights-on period and drank 93 % of their daily water intake during the dark period.

3.2. Experimental NaCl or LiCl phase

3.2.1. Feeding

Rats given NaCl as the sole source of fluid showed no change from the baseline water phase in total food intake or any other feeding variable.

Rats given LiCl as their fluid source showed a significant drop in food intake that persisted across all days of LiCl treatment (see Fig. 4A). The number of feeding bouts did not change much during LiCl treatment (see Fig. 4B). However, the feeding-bout length and time spent in the food cup (seconds of beam break) increased above baseline during LiCl treatment and was significantly greater than the feeding bout length of NaCl-treated rats on a majority of days (see Fig. 4C). Because overall intake dropped while feeding bout length increased, there was a significant decrease in average feeding efficiency from 0.31 g/min in the baseline water phase to 0.19 g/min at the end of LiCl access.

3.2.2. Drinking

Compared to baseline water intake, rats with access to NaCl drank significantly more fluid per day (52 ± 0.3 g; see Fig. 5A). There were no significant changes in number of bouts, lick volume, or drinking outside

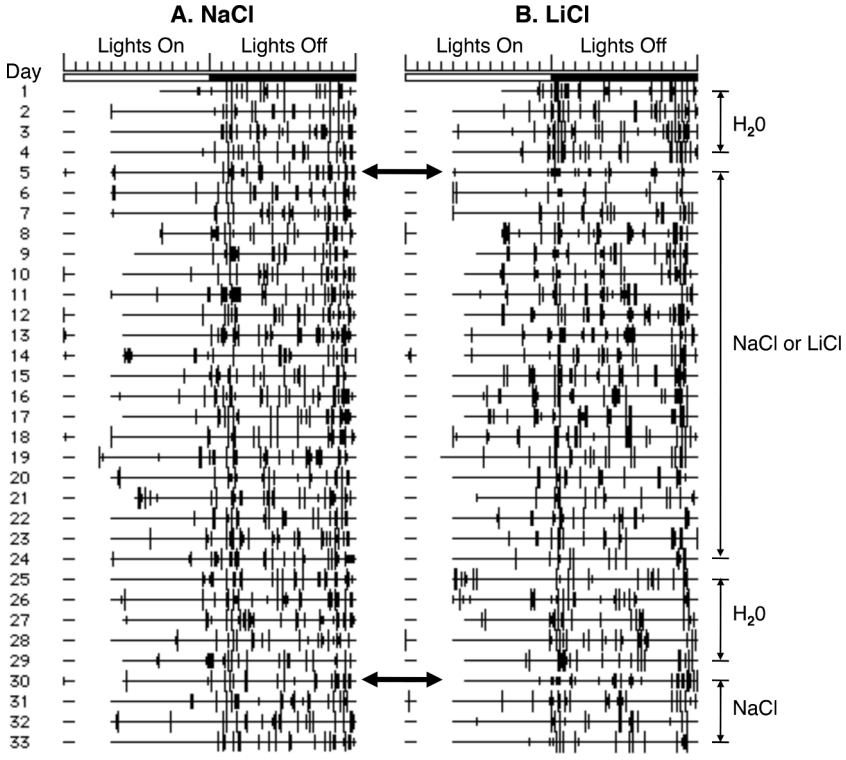

Fig. 1. Example graphs of licking patterns of individual rats drinking NaCl (A) or LiCl (B) during the experimental treatment phase. Horizontal lines represent individual days; the light cycle is indicated by the white/black bar at the top of each graph. Gaps in the record are the periods of daily maintenance. Vertical deflections from the horizontal indicate 1, 100, or 500 licks summed into 5-min bins. A decrease in licking by the LiCl-treated rat is evident on the first 3 days of LiCl access (upper arrow) and on the first NaCl test day (lower arrow).

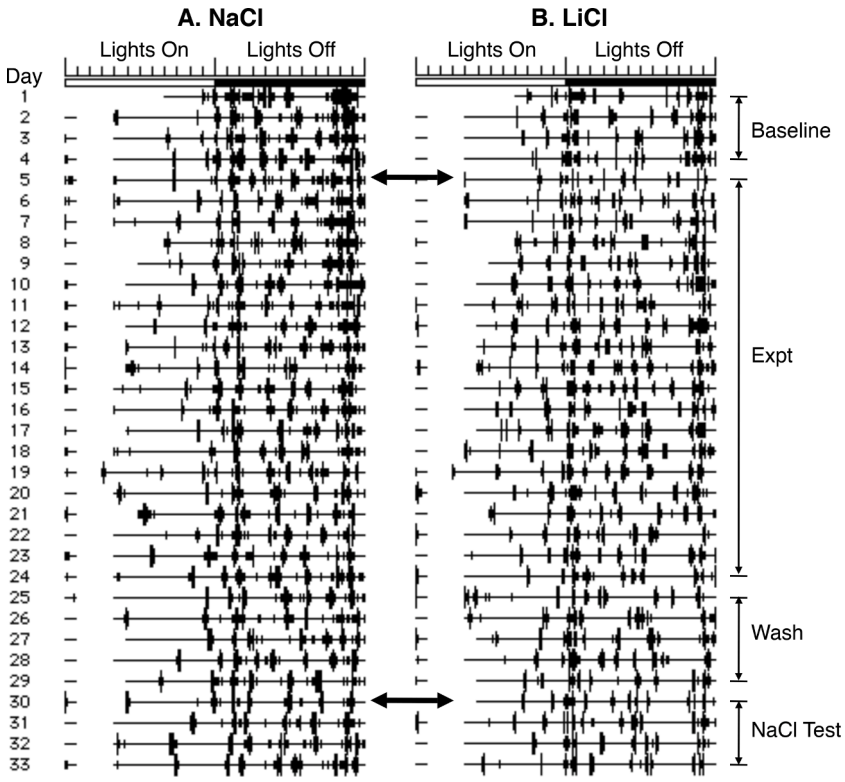

Fig. 2. Example graphs of feeding patterns of individual rats drinking NaCl (A) or LiCl (B) during the experimental treatment phase, plotted as in Fig. 1. Vertical deflections from the horizontal indicate 1, 100, or 200 s within the food port summed into 5-min bins.

Table 2
Results of 2-way repeated measures ANOVA of feeding variables.

Variable	Effect of LiCl Rx	Group F (1,13)	Test Day F (32,416)	Interaction F (32,416)
Chow Intake	LiCl < NaCl	**17.35***	**8.28***	**8.20***
# Bouts		0.44	1.11	1.23
Light bouts		0.79	**3.93***	1.26
Dark bouts		0.13	**2.14***	1.46
Bout size	NaCl < LiCl	0.96	**2.06***	**4.38***
Bout length	NaCl < LiCl	1.56	**1.66***	**4.03***
Bout efficiency	LiCl < NaCl	**17.05***	**10.32***	**8.76***
Intake / beam break	NaCl < LiCl (days 1–4) LiCl < NaCl (days 6–24)	**10.77***	**20.02***	**23.19***
# Beam breakswithin bouts	NaCl < LiCl	1.02	**3.92***	**5.76***
# Beam breaks outside bouts		0.76	**1.94***	0.85

* significant effect, $p < 0.05$.

Table 3
Results of 2-way repeated measures ANOVA of drinking variables.

Variable	Effect of LiCl Rx	Group F (1,13)	Test Day F (32,416)	Interaction F (32,416)
Fluid Intake	LiCl < NaCl	**53.53***	**5.97***	**15.90***
# Bouts	LiCl < NaCl	1.77	**4.70***	**3.55***
Light bouts	LiCl < NaCl	1.13	**4.89***	**2.69***
Dark bouts	LiCl < NaCl	1.73	**5.11***	**3.36***
Bout size	NaCl < LiCl	0.25	**4.36***	**5.71***
Bout length	NaCl < LiCl	1.55	**3.68***	**1.70***
Bout efficiency	LiCl < NaCl (days 8,14) NaCl < LiCl (days 29,33)	0.40	**8.06***	**7.36***
Lick volume	LiCl < NaCl	**8.55***	**6.47***	**5.23***
# Licks within bouts	LiCl < NaCl	**8.79***	**2.69***	**7.25***
# Licks outside bouts	NaCl < LiCl	**5.02***	**3.72***	**2.28***

* significant effect, $p < 0.05$.

of bouts, and only a non-significant increase in bout size (see Fig. 5B), which nonetheless accumulated to an increase in number of licks per day.

Rats with access only to LiCl showed a precipitous decrease in fluid intake to 15.3 ± 1.6 g on the first day of LiCl treatment, with a partial recovery to 25.4 ± 2.0 g within 3 days of treatment (see Fig. 5A). Although total intake remained low, the pattern of LiCl intake changed over the course of LiCl treatment (see Fig. 5B and 5C). In the first few days, LiCl-treated rats showed a significant decrease in the number of drinking bouts per day with little change in drinking bout size. Within 3 days, however, the number of drinking bouts per day had increased to baseline level with only a small drop in drinking bout size.

Thus, the number and size of drinking bouts returned to baseline levels while total fluid intake remained low. The continued low level of LiCl intake is explained, however, by a gradual and significant drop in the average lick volume (see Fig. 6A). As the number of bouts increased during the first few days of LiCl access, the average lick volume decreased from 5.8 ± 0.4 μl/lick in the baseline water phase to 4.0 ± 0.3 μl / lick after 13 days of drinking LiCl.

It is also notable that during the first 10 days of LiCl access, rats drank not only in bouts but also in many small bursts of "sample licking" that did not meet the formal criteria for a bout. Thus, compared to the baseline water phase or to NaCl-treated rats, on most days LiCl-treated rats emitted fewer licks within bouts (see Fig. 6B), and made more

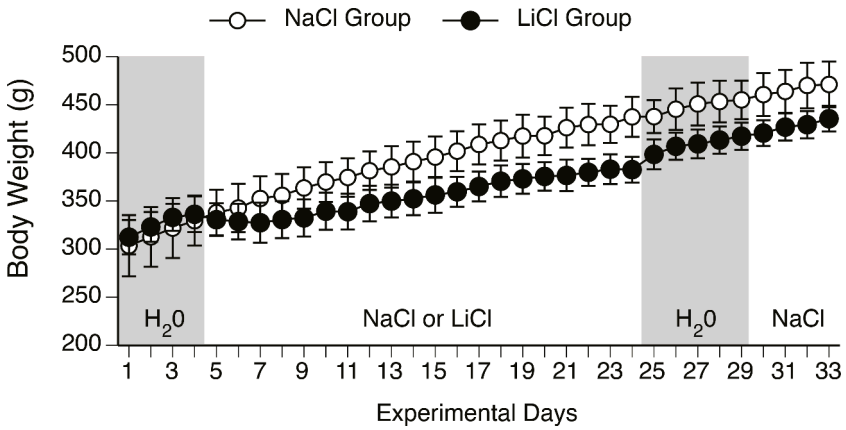

Fig. 3. Daily body weights (mean ± *s*.e.m.) of rats in NaCl (white circles) or LiCl (black circles) groups. During chronic access to LiCl solution, rats initially lost weight before starting to regain weight. but they failed to catch up with rats in the NaCl group.

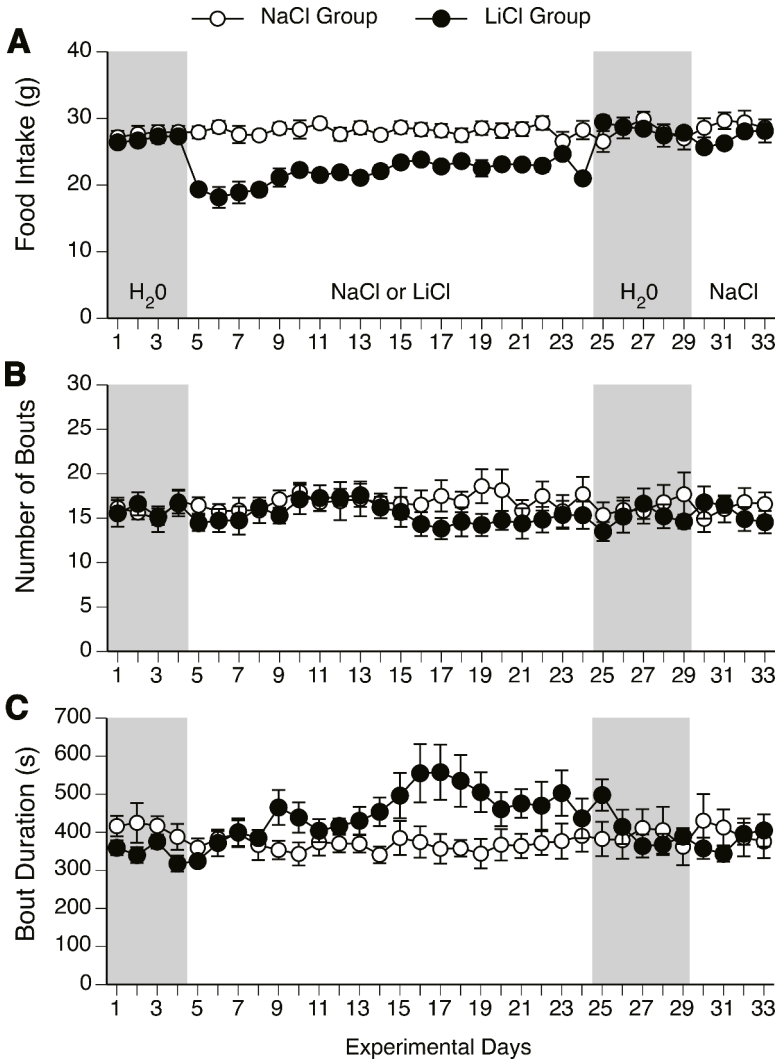

Fig. 4. Food intake variables (mean ± *s*.e.m.) of rats in NaCl (white circles) or LiCl (black circles) groups across the 4 phases of the experiment. A. Daily food intake (g) was significantly decreased in the LiCl group during LiCl access compared to their own baseline and to the NaCl group. B. Number of feeding bouts per day was not significantly different between the two groups. C. Mean bout size (number of beam breaks per bout) was significantly higher in rats with LiCl access.

sampling licks outside of bouts (see Fig. 6C).

3.2.3. Acquisition of aversion to LiCl

NaCl and LiCl have similar neural gustatory properties for rats [31], but LiCl has toxic effects once ingested [33,34]. So, it is likely that rats

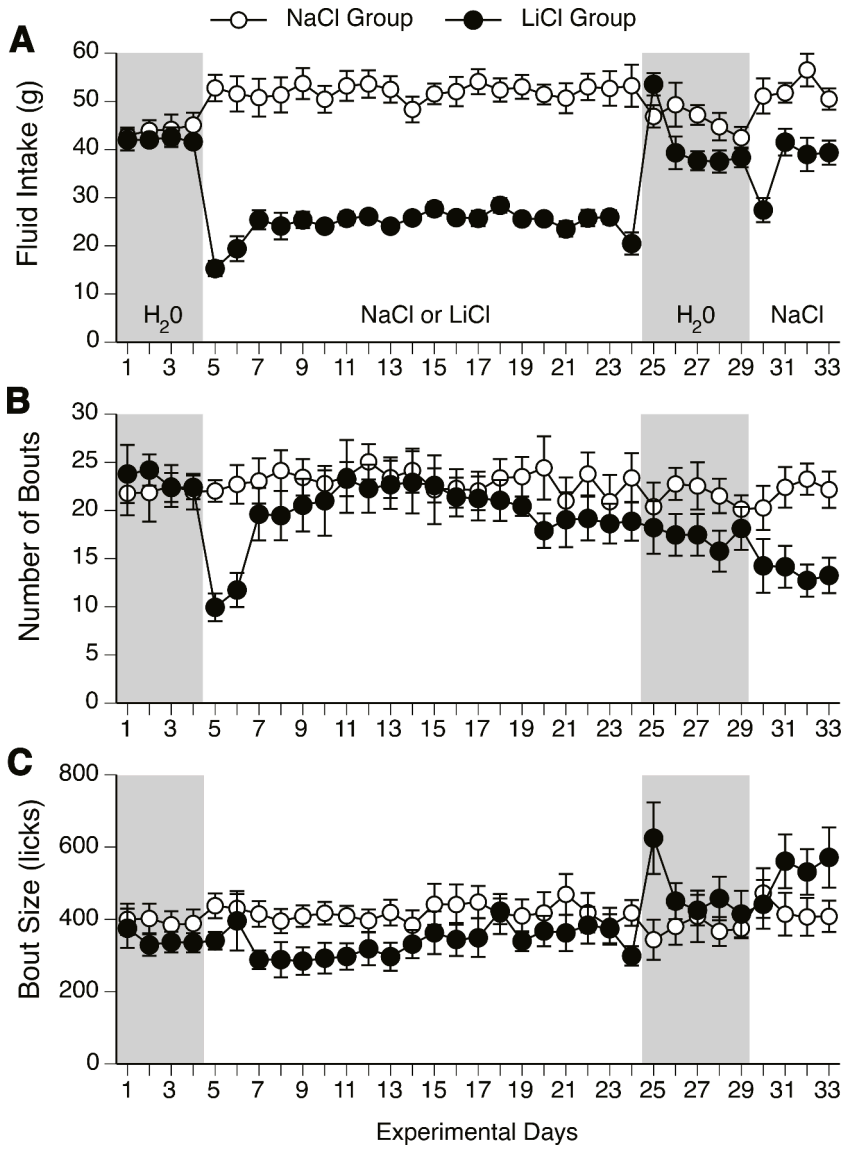

Fig. 5. Drinking variables (mean ± *s*.e.m.) of rats in NaCl (white circles) or LiCl (black circles) groups across the 4 phases of the experiment. A. Daily fluid intake (g) was significantly decreased in the LiCl group compared to their baseline intake during LiCl access and during the first day of the NaCl test phase. B. Number of bouts per day was significantly decreased in the LiCl group during LiCl access and during the NaCl test phase. C. Bout size (number of licks per bout) was decreased in LiCl group during LiCl access but elevated during water recovery and the tail of the NaCl test phase.

decreased their intake of LiCl at the start of the experimental phase not because of LiCl's taste per se, but due to post-ingestive toxicity, or the association of the taste of LiCl with its post-ingestive effects, or both. It was therefore of interest to examine more closely the pattern of ingestion on the first day of LiCl access, to determine when rats acquired their aversion to LiCl and began to decrease their intake. To this end we compared 1) the mean cumulative intake curves of water, NaCl, and LiCl and 2) the latency to initiate the first bout of drinking on the first day of NaCl or LiCl intake.

3.2.3.1. Cumulative intake. On the first day of the experimental phase, rats presented with NaCl began consumption almost immediately after presentation of the NaCl bottle (see Fig. 7B, thin line). Compared to their last baseline water day, licking by the NaCl group diverged significantly upward by minute 395 of access (372 ± 101 water licks vs. 980 ± 104 NaCl licks; paired *t*-test, $p = 0.00002$). Although the majority of fluid intake still occurred during the dark period, rats persisted in drinking ~16 % of NaCl during the lights-on period throughout the experimental phase (see Fig. 7C).

Rats presented with LiCl showed a decreased rate of drinking across the first day of access, consistent with their decreased fluid intake (see Fig. 7B, thick line). Compared to their last baseline water day, licking by the LiCl group diverged significantly downward by minute 636 of access (2352 ± 163 water licks vs. 924 ± 42 LiCl licks; paired *t*-test, $p = 0.00002$).

Fig. 8 shows the initial cumulative licking during the first hour of access to NaCl or LiCl, showing that there was substantial divergence in intake of the groups within ~10 min.

Relative to the intake of NaCl-treated rats, LiCl-treated rats decreased their rate of intake almost as soon as the experimental phase began, reaching significant divergence at minute 638 (2614 ± 275 NaCl licks vs. 924 ± 42 LiCl licks; *t*-test, $p = 0.00003$).

Although their overall intake increased across the course of the experimental phase, LiCl-treated rats continued to drink at a lower rate than NaCl-treated rats. Throughout the dark period of the last day of LiCl access, for example, rats drank LiCl at a lower rate than water during baseline; but across the lights-on period they drank LiCl at a higher rate than water during baseline and consumed 15 % of their daily fluid prior

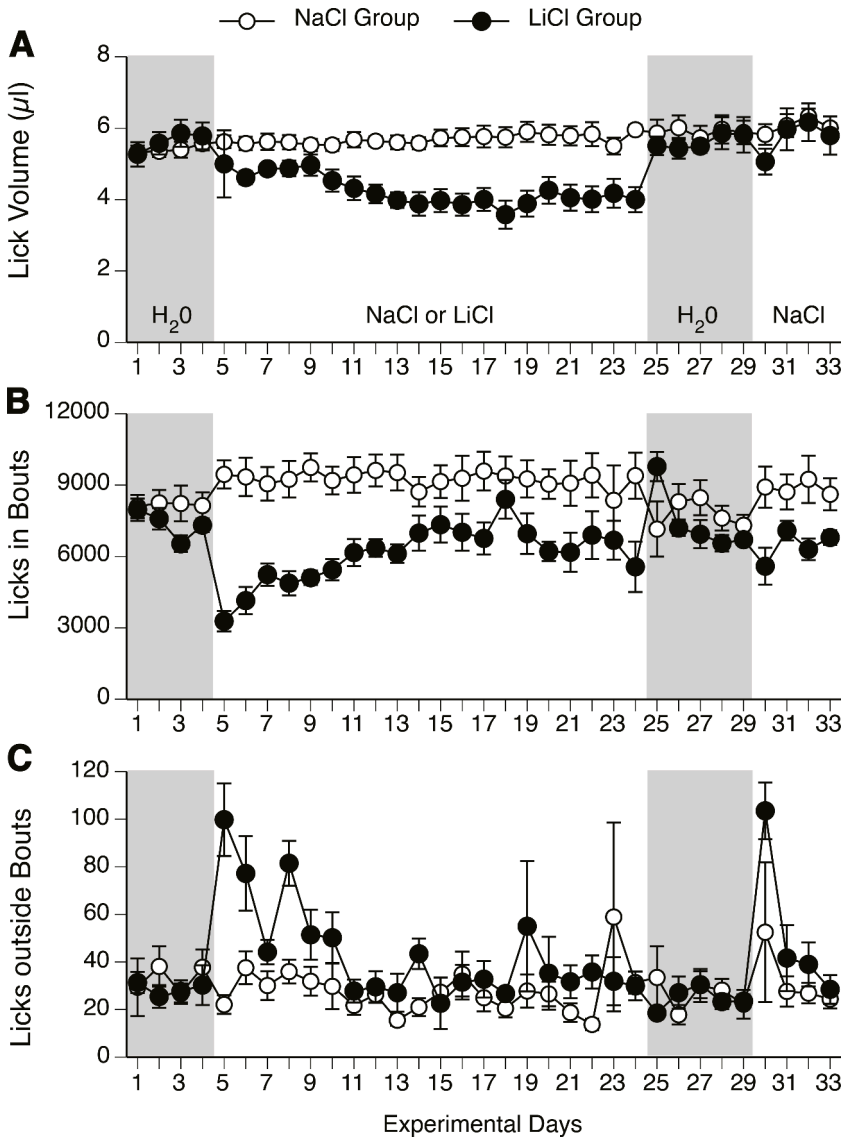

Fig. 6. Lick variables (mean ± *s*.e.m.) of rats in NaCl (white circles) or LiCl (black circles) groups across the 4 phases of the experiment. A. Volume per lick (μl), determined as (daily intake / licks per day), was significantly decreased in rats with LiCl access. B. Licks per day falling within bouts also decreased during LiCl access, consistent with the drop in licking bout size (Fig. 5C). C. Licks per day falling outside bouts (i.e., "sampling licks") were increased in the first days of LiCl access and the first day of NaCl test phase in the LiCl group.

to lights-off (see Fig. 7C, thick line). Thus LiCl-treated rats appeared to regulate their drinking by distributing intake across more of the entire day.

3.2.3.2. Latency to initiate licking. Closer examination of bout structure revealed that rats responded differentially to NaCl or LiCl almost immediately after the first presentation of the solutions on experimental phase day 1. Prior to initiating the first formal bout of licking, both NaCl- and LiCl-treated rats sampled the bottles with a small number (< 5) of sparsely distributed "sampling licks". There was no difference, however, in the number of pre-bout sampling licks between the two groups (see Fig. 10A).

After sampling, NaCl-treated rats initiated a bout of licking in 4-32 min after access to the bottle of NaCl. The range of latencies was wider in LiCl-treated rats (1-500 min), and on average LiCl-treated rats took longer to initiate licking in bouts vs. the NaCl-treated rats (155 ± 75 min vs 13 ± 4 min, $p < 0.05$). Also, the first bout of licking was significantly shorter for LiCl than NaCl (2.0 ± 0.1 min vs. 4.5 ± 0.5 min, $p < 0.05$; see Fig. 10B).

Thus, rats sampled novel NaCl and LiCl solutions to the same extent, but the NaCl group began rapid, sustained consumption of NaCl soon thereafter while the LiCl group postponed LiCl intake for several hours. In other words, rats determined NaCl to be attractive and LiCl to be aversive within 15 min and after only 5 licks.

3.3. Recovery water phase

When water was returned to the rats, NaCl-treated rats decreased their fluid intake to baseline levels (see Fig. 5A and Fig. 6B); their food intake remained constant (see Fig. 4). LiCl-treated rats increased their daily food intake and decreased the length of their feeding bouts to baseline levels (see Fig. 4). On the first day of access, water intake and drinking bout size sharply increased above LiCl levels, before returning to baseline water values on the second day of water access (see Fig. 5A and C). Lick volume increased to 5.8 ± 0.3 μl/lick on the first day of water access (see Fig. 6A).

Cumulative fluid intake on the first day of the recovery phase showed a rapid rate of water intake in the LiCl-treated rats in the light period (i.

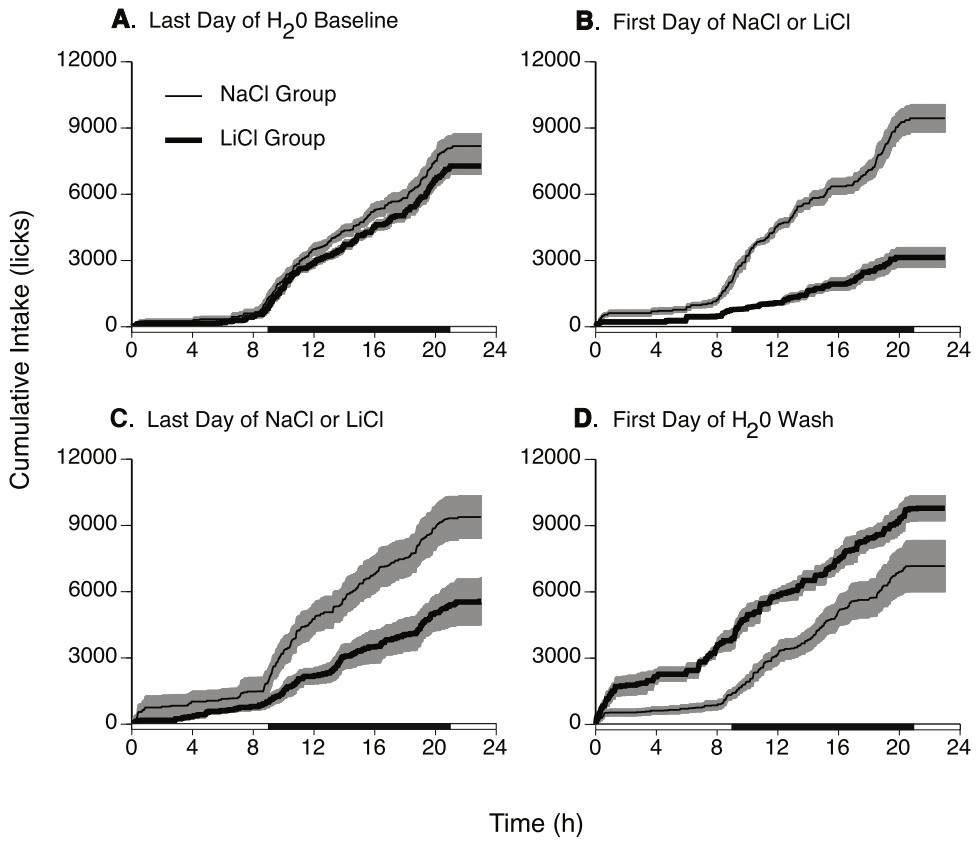

Fig. 7. Cumulative licks per minute (mean ± *s*.e.m.) across specific days for NaCl group (thin line) vs. LiCl group (thick line). Fresh bottles were provided at 0 h; lights went off at 9 h. A. Last day of the baseline water phase. There was little difference between groups in cumulative intake of water. B. First day of the experimental phase. The NaCl group increased intake, including during the end of the lights-on period; the LiCl group decreased intake. C. Last day of the experimental phase. The NaCl group continued to show increased intake. The LiCl group showed an increase in intake, including during the lights on period. D. First day of water recovery phase. The intake of the NaCl group returned close to baseline rate, while the LiCl group showed increased rate of water intake compared to baseline, beginning as soon as the bottle was presented.

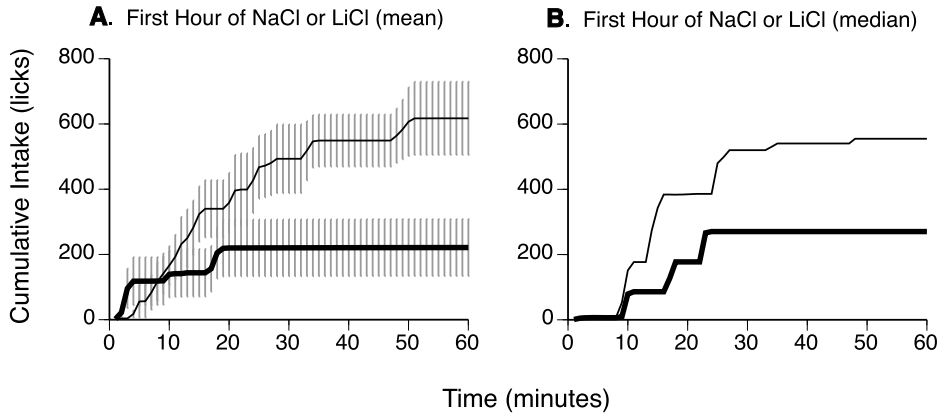

Fig. 8. Cumulative licks per minute during the first hour of NaCl or LiCl access shown as (A) mean ± *s*.e.m. or (B) median across the NaCl group (thin line) vs. LiCl group (thick line). After a low level of sampling by both groups during the first ~10 min of access, intake diverged with more and consistent consumption by NaCl rats compared to LiCl rats.

e., as soon as water was returned; see Fig. 7D). By the start of the dark period, the rate of drinking water by both groups was similar.

3.4. NaCl test phase

When returned to NaCl as a fluid source, NaCl-treated rats responded similarly to their first exposure to NaCl: total fluid intake and size of drinking bouts were again increased compared to water values (see Fig. 5A and C).

The initial response of LiCl-treated rats on the first day of access to NaCl was very similar to their response to LiCl during the experimental phase. Compared to water, fluid intake, number of drinking bouts and lick volume of LiCl dropped significantly (see Figs. 5A, 5B, and 6A). There was also a large increase in sampling licks (Fig. 6C). These changes were transient, however. By the second day of the NaCl test, the total fluid intake of LiCl-treated rats had returned to baseline water

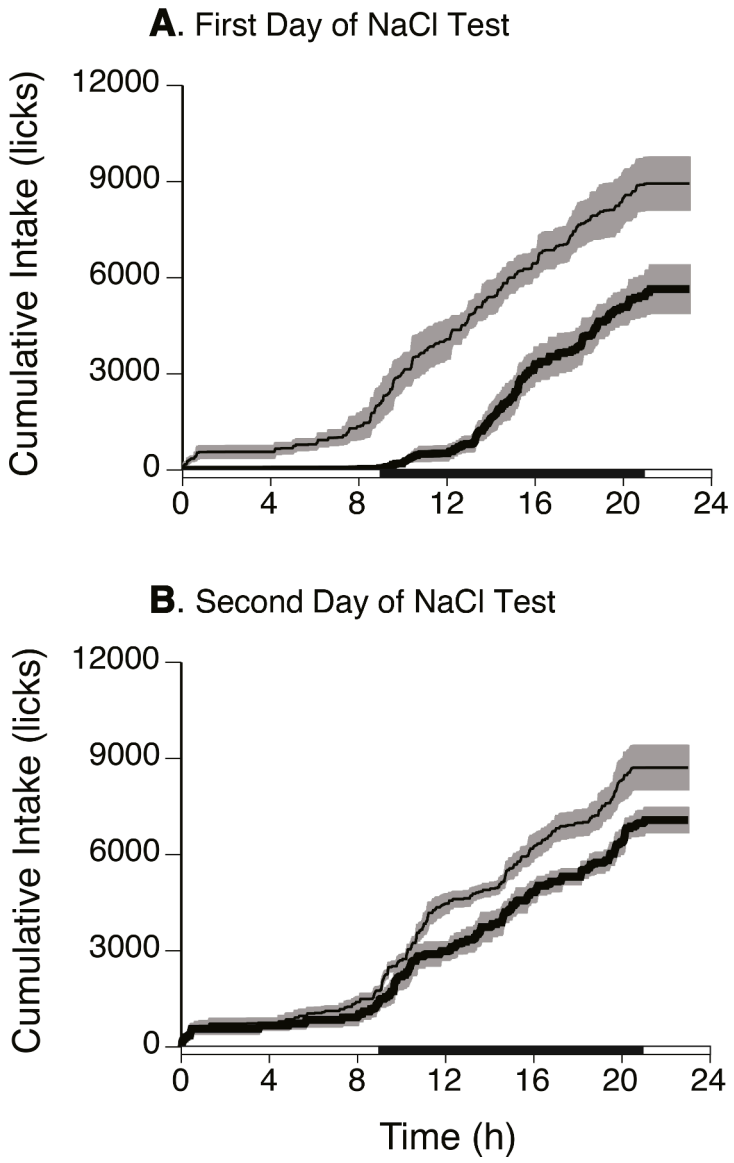

Fig. 9. Cumulative licks per minute (mean ± *s*.e.m.) during the first (A) and second (B) days of the NaCl test for NaCl group (thin line) vs. LiCl group (thick line). Fresh bottles were provided at 0 h; lights went off at 9 h. A. The NaCl group showed a greater rate of NaCl intake compared to the LiCl group, which showed very low intake until ~11 h into the test. B. On the second day of the NaCl test phase, the rate of intake of NaCl by the LiCl group was much closer to the intake of the NaCl group.

level, although it remained significantly lower than the intake of NaCl-treated rats. Increased NaCl intake by the LiCl-treated rats on the second day was accompanied by an increase in the size of drinking bouts, but not in the number of drinking bouts.

3.4.1. Cumulative licking

The decreased NaCl intake of the LiCl-treated rats indicated that they had acquired a CTA to LiCl that generalized to NaCl. Their decreased intake was transient, however. Because NaCl elicits similar taste responses as LiCl but without toxic post-ingestive effects, the pattern of licking to NaCl by the LiCl-treated rats shows the time course of extinction of the LiCl aversion.

When NaCl was returned to NaCl-treated rats, they drank the solution at a high rate parallel to their intake of NaCl during the experimental phase (see Fig. 9A, thin line). LiCl-treated rats, however, showed a very low rate of licking for the first 10–12 h of NaCl access; the cumulative intake curves diverged significantly by minute 730 (4215 ± 537 vs. 584 ± 245 licks, *t*-test, $p = 0.00002$; see Fig. 9, thick line). Although in the latter half of the day LiCl-treated rats consumed NaCl at a rate parallel to that of NaCl-treated rats, the total number of licks was nearly identical to the number of licks on their last LiCl access day.

On the second day of NaCl access, NaCl- and LiCl-treated rats had very parallel rates of intake across the day, and while intake was slightly lower for LiCl-treated rats the cumulative licks were not significantly different from NaCl-treated rats at any time point (see Fig. 9B).

3.4.2. Latency to initiate licking

The slow initial rate of NaCl intake by LiCl-treated rats during the NaCl test days was accompanied by a long latency to initiate the first bout of licking compared to NaCl-treated rats (621 ± 42 min vs. 40 ± 30 min, $p < 0.05$; see Fig. 10B). Prior to bout initiation, however, LiCl-treated rats frequently sampled the NaCl (53 ± 15 licks; see Fig. 10C). Once the LiCl-treated rats initiated a sustained bout of licking, the duration of their first bout was not significantly different than that of the NaCl-treated rats (2.8 ± 1.0 min vs 4.9 ± 0.9 min).

Thus, although LiCl-treated rat initially avoided sustained intake of NaCl, they began to consume NaCl at a rate parallel to that of NaCl-treated rats during the first dark period with NaCl access. In other

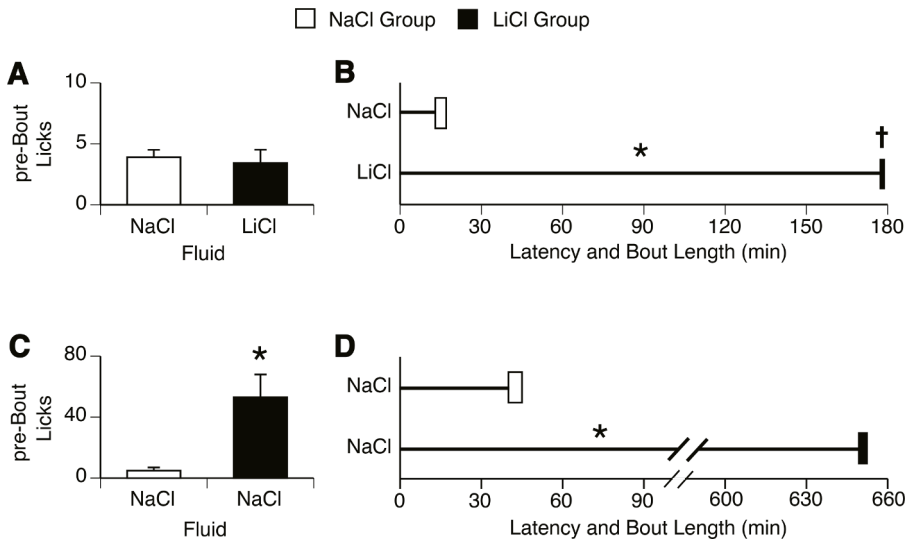

Fig. 10. Acquisition and expression of LiCl aversion measured by sampling and latency to initiate bouts of drinking in NaCl-treated (white bars) or LiCl-treated rats (black bars) on the first day of experimental and NaCl test phases. Left graphs (A, C): sampling as the number of isolated licks after bottle presentation but before initiation of a drinking bout. Right graphs (B, D): latency to initiate the first bout of drinking after bottle presentation (horizontal line) and the duration of first bout (box). A. Rats sampled NaCl and LiCl identically on first exposure. B. LiCl-treated rats had increased latency to initiate a bout of drinking, and a significantly shorter first bout of LiCl licking than NaCl-treated rats. C. At the start of the non-toxic NaCl test phase, LiCl-treated rats sampled significantly more than NaCl-treated rats. D. LiCl-treated rats had an increased latency to initiate drinking NaCl in bouts; however, the first bout of NaCl licking was not significantly different between groups.

words, the generalized aversion from LiCl to NaCl began to extinguish after only ~50 licks of NaCl and was largely extinguished within 10–12 h of NaCl access.

4. Discussion

During chronic ad libitum access to LiCl as their only source of fluid, rats showed an immediate avoidance of the LiCl solution, as seen in an increased latency to initiate drinking bouts and a decreased size of drinking bouts on the first day of access. Chronic consumption of LiCl solution led to significantly decreased food and fluid intake compared to baseline, with concomitant weight loss. The decreased intake was realized by marked changes in the pattern of drinking and feeding bouts: a decrease in per-lick volume and a decrease in licks per drinking bout, resulting in a decrease in fluid intake; and an increase in feeding bout duration resulting in an overall decrease in eating rate. Conversely, chronic NaCl access led to an increase in drinking bout number and licks/bout. The avoidance of LiCl was likely a combination of toxic effects of ingested LiCl and rapid acquisition of a learned aversion to the taste of LiCl, as shown by an extinguishable generalized aversion to NaCl solution during subsequent NaCl test days.

4.1. Immediacy of LiCl avoidance

The response of rats to LiCl diverged from the response of rats to NaCl within minutes after access, in that rats with NaCl access quickly initiated large bouts of licking while rats with LiCl access postponed sustained drinking. It has long been known that rats which have an initial avidity to drink LiCl (e.g. water-restricted rats, or adrenalectomized rats that have a strong salt preference) will stop drinking LiCl in single or 2-bottle tests within 5–15 min after ingesting only a few milliliters [3,4,6,16,24].

Although individual rats in this study only received single-bottle access to LiCl, and so did not have the opportunity to directly compare LiCl and NaCl, the divergent responses across rats show their ability to distinguish the 2 salt solutions. The differential response is unlikely to be purely taste-driven, i.e., the lower short-term intake to LiCl is unlikely due to reduced palatability of LiCl vs. NaCl. LiCl and NaCl elicit comparable taste responses in naive rats at initial contact, with nearly identical behavioral and gustatory nerve electrophysiological responses [24,31,35].

However, after experience with both LiCl and NaCl solutions, rats are subsequently able to discriminate between LiCl and NaCl in 2-bottle preference tests [16,35,36], or to show differential intake in one-bottle tests [37]. This discrimination is not dependent on olfaction [36], and LiCl detection is not masked by admixture with NaCl [37]. The discrimination is made with so little intake of LiCl (e.g., in the first minute of a 10-min 2-bottle test [36]) that toxic post-ingestive effects as a basis for initial discrimination seem implausible.

Likewise, in the present study, rats with NaCl access initiated a sustained bout of drinking within minutes after only 5 initial sampling licks. Rats with LiCl access also made 5 sampling licks, but they then postponed initiating a bout of LiCl drinking for hours. It is hard to explain the differential effect of, and response to, such small quantities of Li and Na (i.e., 7 μmoles, or 5 μg and 17 μg, respectively).

4.2. Rapid onset of Li-induced CTA

CTA can be acquired within minutes, and so a learned association of LiCl with toxic consequences can explain the rapid decrease in consumption in the first hours of intake. As demonstrated using intraoral catheters, pairing a sucrose CS with LiCl injection results in CTA within 15–20 min [38–40]. Brief ad libitum ingestion of LiCl (in which the LiCl serves as both CS and US) by water-restricted rats results in a potent CTA after only a single trial [3,23], that readily generalizes to NaCl [3,4].

The amount of LiCl consumed in the first sustained bout of drinking in this study was consistent with the range of systemic intraperitoneal (i.p.) doses sufficient to induce CTA. Cumulative licking of LiCl on the first day of access significantly diverged from NaCl licking at ~900 licks, or ~5.6 ml at 6 μl/lick; at 24 mM, this corresponds to a dose of ~19 mg/kg LiCl. Earlier studies of ad libitum LiCl intake reported slow-downs of intake after 61 to 170 mg/kg LiCl had been ingested [4,5,22,24]. Nachman and Ashe [41] early on developed a dose-response curve for sucrose CTA induced by i.p. injection of LiCl in rats, as measured in single-bottle intake by water-restricted rats, with a significant CTA induced at 12.7 mg/kg (2 ml/kg of 0.15 M LiCl) up to a maximum CS suppression at 76 mg/kg (12 ml/kg of 0.15 M LiCl) and above.

The induction of a LiCl CTA in this study was confirmed during the

test days with NaCl access, when the LiCl-treated rats generalized their avoidance response to NaCl. The rapid extinction within a day without the post-ingestive consequences of LiCl intake supports an associative mechanism.

An alternative explanation to the reduced intake of NaCl by LiCl-treated rats is a neophobia to the novel taste of NaCl after prolonged toxicosis. Acute exposure to ionizing radiation or LiCl immediately prior to novel solution access enhances neophobia (i.e., decreased first-time intake of the solution) [42]. It has also been shown that repeated pairings of novel flavors with LiCl leads to a reduced intake of different novel flavors alone [43,44]. These effects might be unconditioned responses to toxin, or they might reflect conditioned novelty aversion. Given their similar gustatory properties, however, the salience of NaCl novelty vis-à-vis LiCl should be minimal.

4.3. Decreased food intake

Decreased food intake might have been due to an anorectic effect of Li toxicity, or it might be secondary to the decreased fluid intake. LiCl has an acute anorexic effect, although at higher doses than required for CTA induction. Acute injections of 60–120 mg/kg LiCl cause an acute decrease in food intake [25–27]. Meal-initiated i.p. infusions of 3.8 mg/meal LiCl lowered food intake by decreasing meal number but not meal size, presumably by postponing the start of the next meal [18]. High doses of LiCl can also increase acute food intake, but this may be an expression of pica or sickness-induced non-nutritive feeding using chow as the substrate [25,26]. There may also be a contribution of dehydration anorexia from the decreased fluid intake [45].

4.4. Regulation of LiCl intake

Rats initially modulated LiCl intake by decreasing the number of bouts and increasing the number of sampling licks made outside of bouts. Within a few days of chronic access, the number of bouts returned close to baseline, and LiCl intake per day doubled, although it remained at less than half of baseline water intake. An initial depression of intake, followed by gradually increased intake over days or weeks is typical when rats are presented with access to unpalatable (e.g. quinine- or tannic-acid- adulterated chow [9,11]) or toxic fluids (e.g. heavy metal solutions of cadmium [13] or lead [14]), or plant secondary metabolites [10,15].

Although the number of drinking bouts increased and the absolute amount of LiCl ingested increased over days, rats sustained a low intake of LiCl compared to baseline water by decreasing the size of the bouts and even decreasing the volume of individual licks. The ability to down-regulate the volume of individual licks has been reported before, for example to a liquid diet accompanying post-ingestive satiety [46], or to quinine solutions [47], or to LiCl solutions [4]. Affecting a decrease in toxin intake by decreasing the bout size but not the number of bouts is also found, e.g., in koalas ingesting formylated phloroglucinol compounds [15], or in rats ingesting lead acetate solutions [14] or tannic acid-adulterated chow [9].

4.5. Toxin effects of LiCl

The proximal toxic effects of LiCl, although often invoked to explain its distal consequences, are not well defined. The daily intake of LiCl in this study should not have induced severe toxicosis. Intake of the 24 mM LiCl solution stabilized at about 25 ml / day, or 0.6 mmole Li/day, which is only about 5 % of the LD50 for Li in adult rats (12.6 mmole/kg) [33,48]. Daily intubation for 20 days of a comparable dose of Li in the form of lithium lactate resulted in only subtle changes in kidney and liver histopathology, although this foreshadowed more severe effects at 60 days [49]. The signs of mild Li intoxication, such as decreased locomotor activity, hypothermia, anorexia, pica, and nausea (in humans) are rapid and transient [7,8,25,50]: they are unlikely to be caused by direct gross disruption of cellular functioning. Rather, the acute effects may be secondary to neural and humoral physiological responses to elevated plasma Li, mediated by the chemosensitive neurons in the area postrema or endocrine responses of the hypothalamic-hypophyseal axis. Indeed, the acute effects of Li can be dissociated, such that area postrema lesions block LiCl-induced CTA [27] and central c-Fos expression [51], but do not block acute anorexic effects or oxytocin release [27], and do not block discrimination of LiCl from NaCl solutions in repeated 2 bottle tests [52].

At higher doses approaching its LD50, Li can cause renal neuropathy, central nervous system depression, seizures, and cardiac arrhythmias [53,54]. These pathologies are secondary to electrolyte imbalances related to Na and Li membrane transport, as well as specific interactions of Li with second messenger systems such as inositol, cAMP, or glycogen synthase kinase 3 beta – which at lower doses are candidate targets for the therapeutic mood stabilizing effects of Li [55].

5. Conclusions

The pattern of LiCl intake affects the self-administered dosing of the rats. The concentration of, e.g. intraneuronal Li, would be expected to equilibrate over weeks of LiCl consumption, however, and the concentration within distal compartments would be buffered from acute changes of plasma Li. Earlier studies have measured about 0.5-1 mM in plasma after 7 days on 0.8-1.25 g / L Li in drinking water [56,57]. Nonetheless, the minute-to-minute concentrations of plasma Li would be a superposition of repeated oral dosages. Such variation might well have therapeutic or toxicological consequences.

This study also demonstrated that rats quickly show an avoidance of LiCl solution, while on the same time scale rats show an avidity for an isoosmotic NaCl solution. This is likely due to a combination of gustatory cues, and post-ingestive effects, and a learned association between the two. The rapidity with which rats are able to respond differentially to LiCl vs. NaCl is remarkable, given the common gustatory properties and low level of self-administration in the first minutes of Li access.

Rats do show some accommodation to LiCl as the sole source of fluid, gradually increasing their intake with a lower volume per lick and spreading fluid intake across the day. However, their fluid and food intake remained well below baseline levels. Although chronic intake of LiCl at this concentration is likely not frankly toxic, the altered metabolic and hydrational state might impact neural and physiological measures in the rats independently of any putative therapeutic effects of Li.

CRediT authorship contribution statement

Denesa R. Lockwood: Conceptualization, Formal analysis, Funding acquisition, Investigation, Writing – original draft, Writing – review & editing. **Jennifer A. Cassell:** Formal analysis, Investigation. **James C. Smith:** Conceptualization, Formal analysis, Investigation, Methodology, Writing – original draft. **Thomas A. Houpt:** Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Supervision, Writing – original draft, Writing – review & editing.

Data availability

Data will be made available on request.

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