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Temporal and Qualitative Dynamics of Conditioned Taste Aversion Processing: Combined Generalization Testing and Licking Microstructure Analysis

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The pattern of licking microstructure during various phases of a conditioned taste aversion (CTA) was evaluated. In Experiment 1, rats ingested lithium chloride (LiCl) for 3 trials and were then offered sodium chloride (NaCl) or sucrose on 3 trials. A CTA to LiCl developed and generalized to NaCl but not to sucrose. CTA intake suppression was characterized by reductions in burst size, average ingestion rate, and intraburst lick rate, and increases in brief pauses and burst counts. Compared with previous studies, LiCl licking shifted from a pattern initially matching that for normally accepted NaCl to one matching licking for normally avoided quinine hydrochloride by the end of the 1st acquisition trial. In Experiment 2, a novel paradigm was developed to show that rats expressed CTA generalization within 9 min of their first LiCl access. These results suggest that licking microstructure analysis can be used to assay changes in hedonic evaluation caused by treatments that produce aversive states.

Keywords: taste, licking, microstructure, aversion, learning

When intake of a novel tastant is followed by the injection of a toxin—for example, lithium chloride (LiCl)—animals form a conditioned taste aversion (CTA) and subsequently avoid that tastant (e.g., Garcia, Kimeldorf, & Koelling, 1955; see also Riley & Freeman, 2003). This model has been widely used to investigate behavioral and neural mechanisms of taste–visceral integration, learning, and memory and to identify therapeutic targets for clinical conditions such as cancer anorexia (Bernstein, 1985, 1999; Jacobsen et al., 1993; Rodriguez, Lopez, Symonds, & Hall, 2000; Welzl, D'Adamo, & Lipp, 2001).

The traditional measure of CTA is intake of the novel tastant before and after toxin exposure, relative either to control animals that do not receive toxin and/or to water intake in two-bottle preference tests. Although they are widely accepted, intake measurements per se cannot indicate how CTA-related neural or drug treatments affect hedonic evaluation of the tastant. For example, humans who develop a CTA after nausea not only minimize ingestion of the conditioned tastant but also report an acquired dislike for it (Pelchat & Rozin, 1982). In addition, intake measurements cannot distinguish whether CTA-blocking treatments selectively disrupt particular stages of the CTA learning sequence. A given manipulation may disrupt taste or visceral sensation, the ability to integrate taste and visceral information, and/or the ability to consolidate a CTA into or recall it from long-term memory (Spector, Breslin, & Grill, 1988). Disruption at any point in this sequence blocks CTA learning as assessed through intake measures.

To address some of these problems, Grill and colleagues (Berridge, Grill, & Norgren, 1981; Pelchat, Grill, Rozin, & Jacobs, 1983) used a videographic analysis of orofacial ingestion and rejection responses (taste reactivity) to brief intraoral sucrose infusions before and after CTA formation. They determined that CTA shifted the profile of sucrose taste reactivity from a pattern of uniformly ingestive acts to a mixture of ingestion and rejection orofacial movements comparable to quinine hydrochloride (QHCl). Spector et al. (1988) adapted this approach to dissociate the conditioned and unconditioned components of a CTA. They sampled the formation of a CTA through analysis of sucrose taste reactivity responses every 5 min after LiCl injection. They observed significantly more rejection and fewer ingestion responses 15–30 min after LiCl injection. However, no effect was observed if a 20-min delay was interposed between LiCl injection and the first sucrose infusion, which indicates that the changes in sucrose taste reactivity were due to associative conditioning rather than to a general effect of malaise. Eckel and Ossenkopp (1996) also failed to observe an unconditioned LiCl effect in a later replication

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of this study. Spector et al. (1988) speculated that the failure to observe unconditioned effects could have been related to the limited number of measures used (ingestion vs. rejection). Others have suggested that the intraoral taste reactivity test is a forced-choice and time-limited sampling procedure that does not accommodate appetitive behaviors (e.g., approaching or withdrawing from the drinking spout), through which treatment effects may also be expressed (Kent, Cross-Mellor, Kavaliers, & Ossenkopp, 2002; Wolgin & Wade, 1990; see also Seeley, Payne, & Woods, 1995).

In the present study we use licking microstructure analysis to provide a more detailed assay of CTA formation, generalization, and extinction. In this paradigm the temporal distribution of continuous licking from a spout is used to provide several measures of meal structure. Considerable research shows that some microstructural measures vary as a function of the orosensory or visceral feedback signals that control food intake. For example, under constant environmental conditions, the initial rate of ingestion and average size of bursts of licking tend to vary in a positive, monotonic fashion with increases in the concentration of a normally accepted tastant (e.g., sucrose), whereas meal duration and the number of bursts tend to decrease as a function of caloric feedback from the gut (e.g., Davis & Perez, 1993; Davis & Smith, 1992; Eisen, Davis, Rauhofer, & Smith, 2001; Spector, Klumpp, & Kaplan, 1998).

The continuous sampling and temporal precision (millisecond resolution) of microstructural analysis provides an ideal opportunity to evaluate the formation of a CTA. To measure CTA formation in real time, we allowed rats to drink 0.12 M LiCl, which represents a naturalistic model of CTA formation under conditions of toxin ingestion. We hypothesized that CTA formation would shift the pattern of microstructural responses from one characteristic of responses to palatable tastants to a pattern comparable to the profile of microstructural responses seen in response to aversive tastants, specifically QHCl (Berridge et al., 1981; Spector et al., 1988; Spector & St. John, 1998).

We also sought to build on the work of Spector et al. (1988) to distinguish associative from unconditioned effects in CTA processing. In the present study we use another measure of association—generalization. CTAs formed from LiCl ingestion generalize fully to sodium chloride (NaCl; Loy & Hall, 2002; Nachman, 1963b). Therefore, we combined the microstructural analysis with a taste generalization paradigm that exploits the gustatory similarities between LiCl and NaCl by offering rats NaCl on test trials subsequent to LiCl ingestion. Differences in the microstructure of the generalized response may help to identify associative aspects of the CTA. Further, a detailed analysis of licking throughout the entire meal may be sufficiently sensitive to measure possible unconditioned effects of LiCl on licking. Finally, in Experiment 2 we developed a rapid generalization test to further dissociate associative and unconditioned effects and to begin to explore a temporal threshold for CTA formation.

Experiment 1

Method

Subjects

Thirty-two experimentally naive, albino male Sprague–Dawley rats (Charles River, Wilmington, MA), weighing 208 ± 4 g (range = 163 to 239 g) at the start of the experiment, were tested. Rats were individually housed in Plexiglas cages (47 cm × 26 cm × 20.5 cm) in a temperature-controlled room on a 12-hr light–dark cycle (lights on at 0700). Rats had ad-lib access to water and food (Purina Lab Chow No. 5001, Purina Mills, St. Louis, Missouri) in the home cage prior to testing. Body weight was monitored throughout the experiment.

Apparatus

Behavioral testing was conducted via a lickometer (DiLog Instruments, Tallahassee, FL) in a separate test room. The lickometer was electronically interfaced to a computer and eight opaque plastic cages (47 cm × 26 cm × 20.5 cm). A 28 mm × 8 mm slot horizontally centered on the cage face 7 cm above the floor allowed rats to access a single drinking spout attached to an inverted 70-ml glass bottle. Tongue contacts with the spout completed a circuit that passed an imperceptible current (less than 50 μA) through the rat, which allowed the computer to record the time of each lick with 1-ms resolution. Files for each test session for each rat were saved for off-line analysis.

Procedure

Rats were maintained on a 23.75-hr schedule of water restriction throughout testing. Rats received 15-min fluid access in the test cages every day 2 hr after lights were turned on. Bottles were rinsed and filled prior to each session and were weighed to the nearest 0.1 g before and after the session to determine fluid intake. All rats were habituated to the test apparatus with 3 consecutive days of access to distilled water in the test cages. For each experiment, habituation was immediately followed by a 6-day acquisition phase and a 6-day test phase. For each phase, rats had access to a test tastant on Days 1, 3, and 5 (taste trials) and access to distilled water on Days 2, 4, and 6 (water repletion days). Rats were able to gain body weight in this paradigm. By the last day of testing, rats in groups that had received LiCl weighed significantly more (7% increase) than their pretest body weight, $t(15) = 2.57$, $p < .02$.

Experiment 1 consists of three separate experiments. Rats were randomly assigned to either a control group or a lithium group in each experiment. The lithium groups received 0.12 M LiCl on Days 1, 3, and 5 of the acquisition phase (three LiCl trials). In the test phase they were offered 0.12 M NaCl, 0.24 M NaCl, or 0.12 M sucrose on Days 1, 3, and 5 (see Table 1). The control groups received 0.12 M NaCl, 0.24 M NaCl, or 0.12 M sucrose concentrations during taste test days in both the acquisition and the test phases of the experiment; they never received LiCl. Sucrose was chosen as a normally preferred tastant that is qualitatively distinct from NaCl. Reduced sucrose intake would suggest a contribution of unconditioned effects (i.e., ongoing nausea) on behavior. No effect on sucrose intake would indicate that NaCl intake reduction after LiCl should be ascribed to associative effects (Lasiter, 1985).

Table 1
Experiment 1: Summary of conditions

Group	Acquisition phase Trials 1–3	Test phase Trials 1–3
1A (n = 8)	0.12 M NaCl	0.12 M NaCl
1B (n = 8)	0.12 M LiCl	0.12 M NaCl
2A (n = 4)	0.24 M NaCl	0.24 M NaCl
2B (n = 4)	0.12 M LiCl	0.24 M NaCl
3A (n = 4)	0.12 M sucrose	0.12 M sucrose
3B (n = 4)	0.12 M LiCl	0.12 M sucrose

Note. Each acquisition and test phase trial was followed by 1 day of 15-min access to distilled water. NaCl = sodium chloride; LiCl = lithium chloride.

Data Analysis

We divided *total session intake* (grams) by the specific gravity of the tastant to convert weight to volume (milliliters) and then divided it by the total number of licks in the session to yield the *lick volume* (microliters). We calculated *meal size* (milliliters) by multiplying the number of licks in the meal (first lick of the first burst to last lick of the last burst; Spector & St. John, 1998) by the average lick volume for that session. *Meal duration* (minutes) was defined as the session time of the last lick in the meal minus the session time of the first lick in the meal. *Average ingestion rate* (licks per second) was calculated as the number of licks in the meal divided by meal duration in seconds.

The temporal distribution of licking was analyzed via a variety of custom-made programs (Baird, Grill, & Kaplan, 1999; Kaplan, Baird, & Grill, 2001). A licking *burst* was defined as two or more consecutive licks with no interlick interval (ILI) equal to or exceeding 1 s. Thus, pauses greater than or equal to 1 s determined burst termination (Spector et al., 1998). We calculated *burst duration* (seconds) by subtracting the session time of the first lick in the burst from the time of the last lick in that burst. *Burst size* was calculated as the number of licks in each burst. To minimize artifact registrations due to nonlingual spout contacts, we defined meal onset as the first lick of the first burst containing at least three licks. *Latency* was defined as the time between placement of the rat into the test cage and the onset of the first burst of licking. *Initial lick rate* was the number of licks in the 1st min of the meal.

ILIs were analyzed in several ways. We determined the *average within-burst ILI* (milliseconds) by averaging all ILIs less than 1 s. Because more than 95% of all ILIs in a meal are less than 250 ms and are normally distributed below this cutoff (see Davis, 1996, for a discussion), we also determined the average of ILIs less than 250 ms (Spector & St. John, 1998). Because a second distribution of ILIs with a mode averaging twice (about 320 ms) the principal mode of the ILI distribution (about 160 ms) is also commonly observed (see Davis, 1996; Spector et al., 1998; Spector & St. John, 1998), we evaluated the distribution of ILIs from 250 to 499 ms. Finally, we analyzed ILIs ranging from 500 to 999 ms to complete analysis for the entire ILI distribution within bursts.

Pauses were defined as ILIs greater than or equal to 1 s. The *mean pause duration* (seconds) was determined as the meal duration minus the cumulative duration of bursts in the meal, divided by the number of meal pauses (number of bursts minus one). *Percentage of pause duration* for the meal was the cumulative time of all pauses (ILIs greater than or equal to 1 s) divided by the meal duration, multiplied by 100. The *pause ratio* was determined as the number of pauses divided by the number of ILIs in the meal (number of licks in the meal minus one). We also evaluated the frequency distribution of pauses by sorting them into bins of various durations.

To measure variations in ingestion pattern over the course of the meal, we analyzed the number of licks for each minute of each test session. In addition, bursts for each test meal were serially ordered and divided into thirds according to the method of Spector and St. John (1998). Average burst size, pause duration, and lick rate were then compared for each serial third.

Results and Discussion

CTA Acquisition

LiCl Trials 1–3. To assess CTA formation, we combined acquisition phase data (LiCl Trials 1–3) for the 16 rats exposed to LiCl across the three experiments (Groups 1B, 2B, and 3B) and assessed the data using a one-way repeated-measures analysis of variance (ANOVA). All reported significance values for post hoc tests were corrected for multiple comparisons (Bonferroni method).

LiCl ingestion was markedly affected by repeated LiCl exposure. Intake on LiCl Trials 2 and 3 was significantly lower than on Trial 1, $F(2, 30) = 44.93$, $p < .001$ (see Figure 1), indicating a potent CTA. Post hoc comparisons demonstrated that intake on LiCl Trials 2 and 3 did not differ from each other (all ps $> .12$). For all subsequent analyses of these data, when a main effect was observed, post hoc comparisons showed that differences were between responses on LiCl Trial 1 and responses on LiCl Trials 2 and 3, with no difference between Trials 2 and 3. Thus, only the main effects are reported below.

Although intake was suppressed by more than 85%, there was no overall difference in meal duration across test trials, which indicates that rats with a CTA expressed a sustained but very slow average rate of ingestion of 0.59 licks/s on Trials 2 and 3, almost four times slower than the rate observed on Trial 1 (2.10 licks/s), $F(2, 30) = 19.93$, $p < .001$.

An aversion associated with the taste of LiCl was clearly indicated by the potent decline in the number of licks in the 1st min,

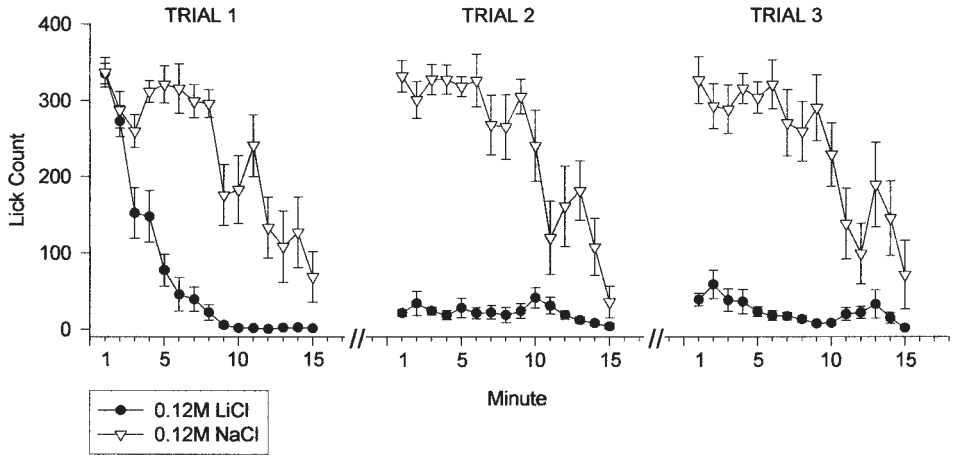

Figure 1. Mean (plus or minus standard error) lick rates (licks per minute) for rats ingesting 0.12 M lithium chloride (LiCl; $n = 8$) or 0.12 M sodium chloride (NaCl; $n = 8$).

$F(2, 30) = 480.71$, $p < .001$, on LiCl Trials 2 and 3 (see Figure 1). Although both intake and initial lick rate declined considerably, the number of bursts in LiCl meals was increased by 60% on Trials 2 and 3, $F(2, 30) = 3.64$, $p = .02$. The average size of bursts was reduced almost sevenfold, from a mean of 55.82 ± 10.62 licks/burst on Trial 1 to 8.27 ± 1.78 licks/burst and 7.92 ± 0.87 licks/burst on Trials 2 and 3, respectively, $F(2, 30) = 19.76$, $p < .001$. Further, the average lick volume was almost halved after LiCl exposure on Trial 1, from 6.07 ± 0.74 μL to 3.77 ± 0.45 μL on Trial 2 and 3.54 ± 0.45 μL on Trial 3, $F(2, 30) = 5.84$, $p = .01$.

Overall, on the second and third LiCl trials, the rats licked the spout in a more stop-and-go fashion: Bursts of licking were short but persisted throughout the session. A shortening of bursts with no change in meal duration might lead to the inference that pauses grew longer; however, the mean pause duration did not vary significantly across LiCl trials, $F(2, 30) = 0.98$, $p = .39$. Rather, rats expressed many more pauses, as indicated by the doubling of burst count (pause count = number of bursts minus one). On Trial 1 the average pause was twice as long as each burst; on Trials 2 and 3 pauses ($M = 22.17 \pm 4.04$ s) were more than 10 times longer than the average burst of licking ($M = 1.62 \pm 0.21$ s). Thus, the proportion of meal duration spent in pauses between bursts was increased from 67% on Trial 1 to a mean of 90% on Trials 2 and 3, $F(2, 30) = 27.32$, $p < .001$.

The formation of a CTA slowed the average rate of licking within bursts considerably, from 6.17 licks/s to 3.60 licks/s (see Figure 2A), as indicated by a significant increase in the average ILI (1–999 ms range) of licks within bursts on Trials 2 and 3, $F(2, 30) = 29.55$, $p < .001$. Because the vast majority of ILIs in meals ranged from 1 to 249 ms (93% of ILIs on Trial 1; see also *Method* section), we anticipated that the average duration of ILIs in this range would be greatly increased. However, there was no difference across LiCl trials, $F(2, 30) = 0.79$, $p = .46$. We therefore extended the analysis to the remaining ranges of ILIs within bursts and were further surprised to find little effect: The mean duration of ILIs ranging from 250 to 499 ms was not significantly changed, $F(2, 30) = 1.45$, $p = .25$, and the mean duration of ILIs ranging from 500 to 999 ms was significantly prolonged by only 39 ms, $F(2, 30) = 6.19$, $p < .006$ (see Figure 2A), a 6% increase insufficient to account for the near halving of lick rate within bursts. We therefore hypothesized that rats were expressing very

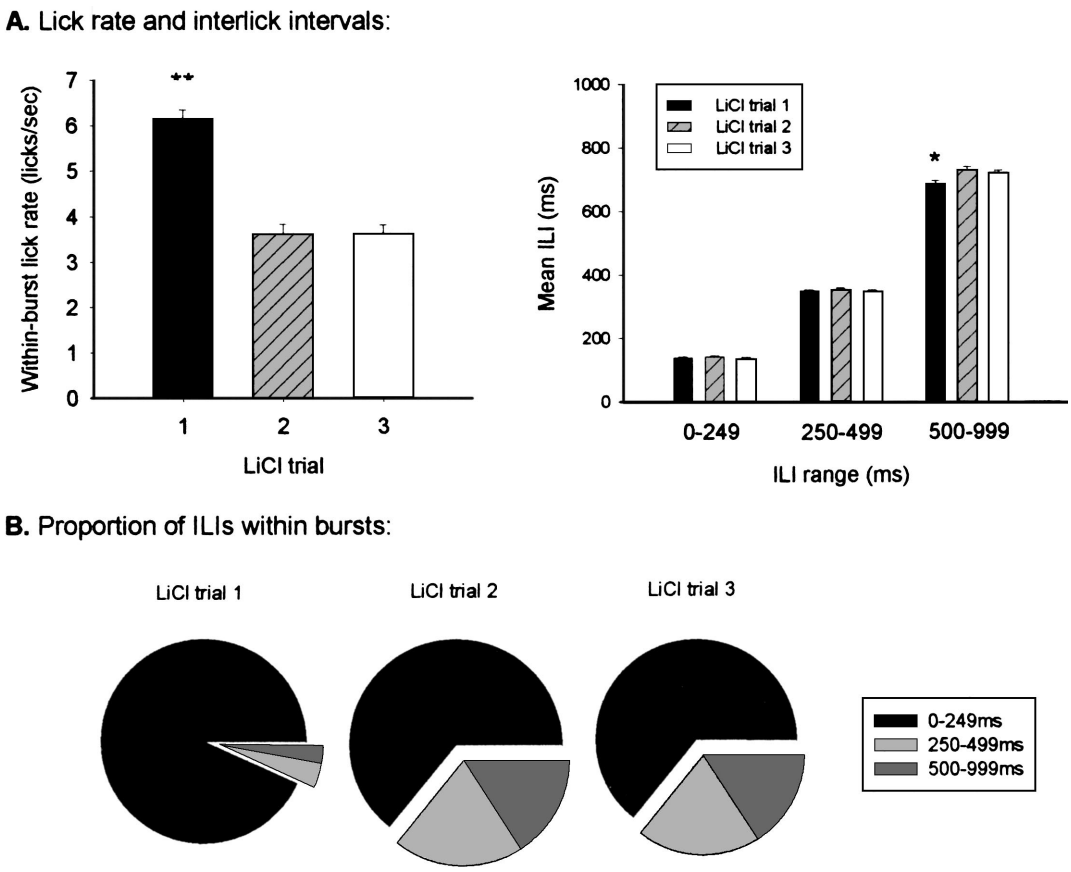

Figure 2. Comparison of within-burst licking measures for rats drinking 0.12 M lithium chloride (LiCl) across acquisition trials. Figure 2A: Mean (plus standard error) rate of licking within bursts was significantly (more than 40%) slower on LiCl Trials 2 and 3 (left panel). However, this rate reduction was not principally due to increases in the mean duration of interlick intervals (ILIs) for three distribution ranges within the burst (right panel). Figure 2B: The reduction of within-burst lick rate was largely due to a significant increase in the proportion of longer ILIs (at least 250 ms) within bursts. *$p < .006$. ** $p < .001$.

brief hesitations at the spout that would be reflected by a proportional increase in longer ILIs within bursts (see Figure 2B). Indeed, the proportion of ILIs within bursts ranging 0–249 ms dropped from a mean of 93% on Trial 1 to 64% on LiCl Trials 2 and 3, $F(2, 30) = 32.61$, $p < .001$. Accordingly, the proportion of ILIs in the 250–499 ms range increased from 3% on Trial 1 to a mean of 19% on Trials 2 and 3, $F(2, 30) = 29.67$, $p < .001$, and ILIs ranging 500–999 ms respectively increased from 3% to 17%, $F(2, 30) = 26.20$, $p < .001$ (see Figure 2B).

Comparison with QHCl licking microstructure. Although clear shifts in the microstructural responses to LiCl were evident in the transition from Trial 1 to Trials 2 and 3, it is possible that the effect magnitudes were reduced because of formation of the CTA during the first trial itself (see below). We therefore compared microstructural responses to LiCl on Trial 3 with those for water on the habituation day preceding the first LiCl trial, using paired *t* tests. This analysis also permitted comparison of the LiCl microstructure with the microstructure for bitter QHCl reported previously (Hsiao & Fan, 1993; Spector & St. John, 1998). In these studies, intact rats ingesting QHCl exhibited, relative to licking for water, decreases in intake, lick volume, burst size, and pause duration, whereas meal duration, burst count, and intraburst lick rate were increased (see Table 2). All of these trends, except for an effect on meal duration, were replicated for rats drinking LiCl (see Table 2). These results strongly suggest that the rats' hedonic evaluation of LiCl after CTA formation closely resembles that for bitter QHCl.

As noted, LiCl increased pause counts; however, this increase was not evenly distributed. Relative to water, the proportion of pauses ranging 1–2 s increased, $t(15) = -2.76$, $p < .02$, and those in the 5–10 s range decreased, $t(15) = -2.26$, $p = .04$. Comparisons of other pause ranges revealed no significant differences ($ps > .10$).

Dynamics of rapid CTA formation during LiCl Trial 1. Although rats exhibited clear differences between their responses to LiCl on Trial 1 and their responses on Trials 2 and 3, we hypothesized that a CTA was formed during LiCl Trial 1. We compared Acquisition Trial 1 meal progress measures (minute by minute and in meal thirds) for Group 1A (which received 0.12 M NaCl) and Group 1B (which received 0.12 M LiCl), using a mixed factors two-way ANOVA (Group × Meal Minute) and between-subjects *t* tests.

Initially, behavioral responses to LiCl and NaCl across both groups were indistinguishable. There was no statistically significant difference in the initial rate (1st min) of licking for either 0.12 M NaCl or 0.12 M LiCl on the first acquisition trial, $t(14) = 0.34$, $p = .74$ (see Figure 1). Further, comparison of meals divided serially into thirds by bursts revealed that, for both groups, the mean burst sizes, pause durations, and lick rates in the first third of the meal were almost identical (see Figure 3). However, as meals progressed, significant differences between the two groups began to emerge.

Nachman (1963a) showed that rats ingesting LiCl exhibited almost completely suppressed intake within 8 min of LiCl intake onset. We observed a virtually identical outcome. Relative to rats ingesting 0.12 M NaCl, the ingestion of 0.12 M LiCl was significantly slower by the 4th min (see Figure 1), as indicated by a significant interaction term, $F(14, 196) = 5.51$, $p < .001$, and post

Table 2
Comparison of LiCl Licking Microstructure With Reported QHCl Licking Microstructure

Measure	Baseline dH_20		LiCl Trial 3						
	M	*SE*	*M*	*SE*	*t*(15)	*p*	Effect size	0.2 mM QHCl vs. dH_20[a]	0.25 mM QHCl vs. dH_20[b]
Intake (ml)	13.70	0.67	1.22	0.25	−29.62	**.00**	↓ 91%	↓ 74%	↓
Lick count	2641.25	104.49	349.25	52.04	−21.66	**.00**	↓ 87%	↓ 56%	↓
Lick volume (μl)	5.21	0.20	3.88	0.44	−2.42	**.03**	↓ 26%	↓ 38%	↓
Meal duration (min)	12.78	0.43	11.35	1.00	−1.30	.21	↓ 11%	↑ 37%	
Ingestion rate (licks/s)	3.46	0.12	0.75	0.25	−12.01	**.00**	↓ 78%		
Initial lick rate	354.75	11.75	38.88	8.48	−29.62	**.00**	↓ 89%	↓ 52%	
Burst count	20.75	2.51	42.19	5.40	3.64	**.00**	↑ 103%	↑ 378%	↑
Mean burst size (licks)	161.40	23.40	7.92	0.86	6.72	**.00**	↓ 95%	↓ 87%	↓
Mean burst duration (s)	23.83	3.70	1.66	0.16	6.16	**.00**	↓ 93%		
Latency (s)	5.91	2.58	90.55	42.99	2.20	.06	↑ 1,400%		
Pause time (%)	49.72	1.89	89.44	1.28	22.61	**.00**	↑ 79%		
Mean pause duration (s)	25.48	3.80	18.63	2.49	1.52	.15	↓ 27%	↓ 52%	
Pause ratio	0.74	0.09	13.59	1.18	10.83	**.00**	↑ 1,686		↑
ILI range groups									
0–999 ms	146.40	2.64	275.59	14.26	8.17	**.00**	↑ 88%		
0–249 ms	142.76	2.58	136.97	2.13	−1.85	.08	↓ 4.1%	↓ 3.3%	No significant change[c]
250–499 ms	335.37	4.76	347.09	3.22	1.96	.08	↑ 3.5%		
500–999 ms	698.62	12.57	720.44	6.87	1.42	.17	↑ 3.1%		
Range proportions (% ILIs in burst)									
0–249 ms	98.91	0.65	63.97	3.63	−9.61	**.00**	↓ 35%		
250–499 ms	0.66	0.11	19.11	2.17	8.54	**.00**	↑ 2,790%		
500–999 ms	0.43	0.06	16.92	1.97	8.31	**.00**	↑ 3,862%		

Note. Bold font indicates that *p* met the criterion for statistical significance, which was set at $p \leq .05$. LiCl = lithium chloride; QHCl = quinine hydrochloride; dH_2O = distilled water; ILI = interlick interval.
[a] From Spector and St. John (1998). [b] From Hsiao and Fan (1993). [c] ILIs < 230 ms were evaluated.

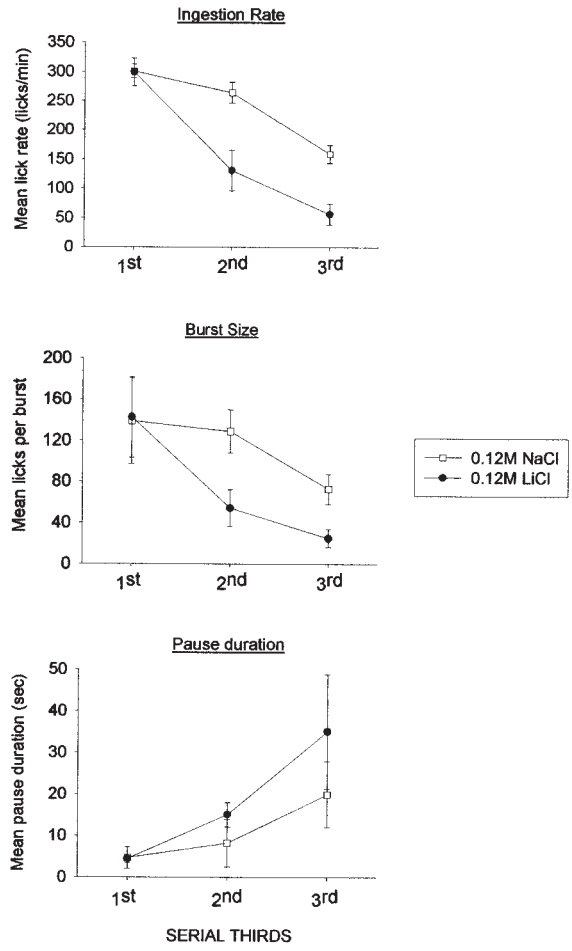

Figure 3. Bursts and pauses for each rat were serially ordered for the first trial for 0.12 M lithium chloride (LiCl) or 0.12 M sodium chloride (NaCl) ingestion in Groups 1A and 1B and broken down into roughly equal one-third segments. Mean (plus or minus standard error) ingestion rate (licks per minute), mean burst size (licks per burst), and mean pause duration (seconds) associated with each segment are presented. Burst size and ingestion rate measures were virtually identical in the first third of the meal: size, $t(14) = 0.06$, $p = .95$; rate, $t(14) = 0.49$, $p = .64$. As meals progressed, burst size for LiCl was significantly reduced for the remaining two thirds: middle third, $t(14) = 2.70$, $p < .02$; last third, $t(14) = 2.82$, $p < .02$. Ingestion rate was significantly reduced for LiCl in the middle third, $t(14) = 4.83$, $p = .001$, but not the last third ($p = .09$). No significant differences were observed for mean pause duration ($ps > .21$).

hoc comparisons ($ps < .02$). In addition, as LiCl meals progressed, rats expressed significantly smaller bursts in the second and last thirds of the meal relative to rats ingesting NaCl (see the legend of Figure 3 for statistics).

CTA Generalization

To assess generalization to NaCl, we evaluated two planned comparisons for each behavioral measure. The first was a paired-samples *t* test to evaluate carryover from LiCl Trial 3 to NaCl/sucrose Trial 1, within each of the LiCl-exposed groups (Groups 1B, 2B, and 3B). A second independent-samples *t* test was used to compare responses of the LiCl groups (1B, 2B, and 3B) on NaCl Trial 1 (as above) with responses on the matching NaCl/sucrose test day (Test Trial 1) of the control groups (1A, 2A, and 3A), which received only NaCl or sucrose in the three preceding trials of the acquisition phase. Means (plus or minus standard error) and statistics for these comparisons are reported in Tables 3, 4, and 5.

Overall, LiCl aversion strongly generalized to 0.12 M and 0.24 M NaCl (see Tables 3 and 4) but not to sucrose (see Table 5). NaCl intakes after LiCl exposure were markedly lower (75% for 0.12 M NaCl and 81% for 0.24 M NaCl) than the respective control group NaCl intakes (see Figures 4 and 5 and Tables 3 and 4). Initial lick rates were comparably slow between LiCl Trial 3 and NaCl Trial 1 at both NaCl concentrations and significantly slower relative to NaCl control groups (see Tables 3 and 4, Figures 4 and 5). The suppressed initial rate of licking for NaCl was not likely due to neophobia, because the initial lick rate for 0.12 M NaCl in the control group (1A) between Acquisition Trial 1 and Test Trial 1 was not significantly different, $t(7) = -0.61$, $p = .56$ (see also Figure 4). Thus, initial rate measures suggest a full generalization of LiCl CTA to NaCl.

The pattern of licking for NaCl after LiCl exposure resembled that for LiCl. Figure 5 shows that most measures of NaCl licking after LiCl exposure resembled the profile of licking responses for LiCl much more than they resembled the profile of licking for the same NaCl concentration by rats never exposed to LiCl. Relative to rats with no LiCl experience (Groups 1A and 2A), the licking pattern for LiCl and conditioned NaCl was stop-and-go: Bursts were smaller but more numerous, rats spent more session time away from the spout, and licking within bursts was slower (see Tables 3 and 4). Mean burst sizes and burst durations were much smaller for NaCl after LiCl than for the NaCl control groups (see Tables 3 and 4, Figure 5), and the proportion of meal duration expressed as pauses was also increased (see Tables 3 and 4, Figure 5).

The slower rate of licking within bursts observed during LiCl drinking also generalized to NaCl (Tables 3 and 4). This slowing effect was mostly due to an increase in the proportion of ILIs greater than 249 ms within bursts, and it significantly generalized to NaCl (see Tables 3 and 4). Although there was no effect of LiCl exposure on the average duration of pauses in the meal, there was a significant increase in the relative proportion of pauses falling in the 1–2 s range for both NaCl concentrations: 0.12 M NaCl, $t(14) = 3.07$, $p < .01$; 0.24 M NaCl, $t(6) = 2.58$, $p = .04$. The frequency of pauses for NaCl after LiCl in this range was doubled in comparison with NaCl controls (see Figure 6, only 0.12 M NaCl shown). Overall, CTA had the effect of selectively increasing the incidence of ILIs ranging from 250 ms to 2 s.

Finally, the average lick volume was roughly halved by LiCl exposure (see *CTA Acquisition* section). This reduction also generalized to both NaCl concentrations (see Tables 3 and 4, Figures 4 and 5) and also to sucrose (see Table 5).

CTA Extinction

An independent samples *t* test was used to assess whether the generalization of CTA to NaCl Trial 1 was fully extinguished by the third NaCl trial for whole-meal and microstructural measures. The minute-to-minute lick rate was also compared across test groups (Groups A vs. B) for each test period trial via a two-way

Table 3
Generalization of 0.12 M LiCl CTA to 0.12 M NaCl

Measure	(A) Group 1B: LiCl Trial 3 (CTA)		(B) Group 1B: NaCl Test Trial 1 (generalization)		(C) Group 1A: NaCl Test Trial 1 (control)		A vs. B		B vs. C	
	M	*SE*	*M*	*SE*	*M*	*SE*	*t*(7)	*p*	*t*(14)	*p*
Intake (ml)	0.97	0.23	4.78	1.06	24.96	0.87	−3.84	**.01**	−14.72	**.00**
Lick count	361.38	156.50	1,160.75	229.38	3,796.00	108.65	−3.95	**.01**	−10.38	**.00**
Lick volume (μl)	3.11	0.39	4.07	0.25	6.61	0.80	−2.15	**.05**	−6.68	**.00**
Meal duration (min)	12.12	1.10	14.51	0.20	14.32	0.36	−2.03	.08	0.47	.64
Ingestion rate (licks/s)	0.46	0.11	1.33	0.27	4.42	0.09	−3.55	**.01**	−10.40	**.00**
Initial lick rate	32.63	9.04	104.50	46.26	330.88	33.30	−1.76	.12	−3.97	**.00**
Burst count	37.75	8.82	57.87	12.18	35.37	3.17	−2.08	.07	1.79	.10
Mean burst size (licks)	8.37	1.64	24.43	7.59	113.11	10.13	−2.12	.07	−7.01	**.00**
Mean burst duration (s)	1.61	0.31	4.07	1.14	16.39	1.33	−2.19	.06	−7.03	**.00**
Latency (s)	121.19	85.25	5.79	3.43	1.98	1.04	1.35	.22	1.06	.31
Pause time (%)	90.98	2.27	77.50	4.05	35.59	1.96	3.72	**.01**	9.32	**.00**
Mean pause duration (s)	26.45	7.17	19.88	7.57	9.48	1.10	0.69	.51	1.36	.19
Pause ratio	0.15	0.03	0.07	0.02	0.01	0.00	2.78	**.03**	3.82	**.00**
ILI range groups										
0–999 ms	256.29	20.27	207.29	17.09	146.99	3.16	1.82	.11	3.47	**.00**
0–249 ms	134.70	3.45	137.99	1.89	142.71	2.38	−0.91	.39	−1.56	.14
250–499 ms	341.43	4.83	355.09	2.79	339.58	8.60	−1.85	.11	1.72	.11
500–999 ms	712.03	11.78	725.33	11.60	679.28	13.44	−0.84	.43	2.59	**.02**
Range proportions (% ILIs in burst)										
0–249 ms	68.15	5.36	82.30	4.34	98.64	0.36	−1.98	.09	−3.75	**.00**
250–499 ms	16.96	3.15	9.46	2.39	0.91	0.25	1.70	.13	3.56	**.00**
500–999 ms	14.89	3.04	8.24	2.00	0.45	0.37	1.93	.10	3.89	**.00**

Note. Bold font indicates that *p* met the criterion for statistical significance, which was set at $p \leq .05$. LiCl = lithium chloride; CTA = conditioned taste aversion; NaCl = sodium chloride; ILI = interlick interval.

mixed factors ANOVA (Minute × Group). Only the results for 0.12 M NaCl are presented. Results for rats tested with 0.24 M NaCl were comparable, except that some measures fully extinguished with only two extinction trials (e.g., see Figure 4).

On the first NaCl trial after LiCl, intake, burst size, burst duration, lick volume, intake, intraburst lick rate, initial lick rate, and various pause measures were significantly different from controls (see Table 3; see also Figures 4 and 5). By the third NaCl trial, almost all of these differences were abolished; only intake and lick volume remained significantly suppressed (see Table 6, Figures 4 and 5). However, even these measures had recovered appreciably (62% and 49%, respectively) relative to their levels on NaCl Test Trial 1.

Rapid extinction was also apparent in the rate of licking in each NaCl trial in the test phase. On Test Trial 1, both main effects were significant—group, $F(1, 14) = 101.75$, $p < .0001$; minute, $F(14, 196) = 3.87$, $p < .01$—as was the interaction term, $F(14, 196) = 3.45$, $p = .02$. Thus, NaCl ingestion after LiCl exposure was significantly slower than that for the control group, although the ingestion rates tended to converge at the end of the test (see Figure 7). On the second extinction trial, the initial rates of ingestion were faster than those in the preceding trial, resulting in more parallel although separate ingestion rate curves between both groups. Hence, the interaction term was no longer significant, $F(14, 196) = 0.86$, $p = .51$, although both of the main effects were significant: minute, $F(14, 196) = 5.61$, $p < .001$; group, $F(1, 14) = 23.40$, $p < .001$. By the third extinction trial, ingestion rate curves between the two groups were mostly overlapping, which suggests that the CTA was extinguished (see Figure 7). This is supported by the lack of a significant main effect of group on Trial 3, $F(1, 14) = 3.51$, $p = .082$, and the lack of an interaction term, $F(14, 196) = 1.58$, $p = .09$, although a main effect of minute, $F(14, 196) = 11.16$, $p < .001$, indicated a gradual decline in ingestion rates.

Experiment 2

In Experiment 1, rats ingesting LiCl exhibited a rapid decline in ingestion rate that significantly departed from ingestion of an equimolar concentration of NaCl by the 4th min of the test. A CTA was definitely established at some time prior to LiCl Trial 2, because lick rate was markedly suppressed at the very beginning (1st min) of the second LiCl trial. We hypothesized that the CTA was formed within the first 8 min of the first LiCl trial, the time at which LiCl licking was suppressed to an almost negligible rate (22.00 ± 10.24 licks/min). However, it is also possible that intake suppression at this time was due to malaise rather than associative factors and that the taste–malaise association was made at a later time.

To evaluate this hypothesis, we used an MS-160 Davis Rig (DiLog Instruments, Tallahassee, FL) to rapidly switch tastants offered to rats during a single test trial. In the key experiments, rats were offered 0.12 M LiCl for 8 min and then offered a second tastant for another 8 min. If a CTA to the taste of LiCl is formed during the first 8 min of LiCl ingestion, the ingestion rate suppression exhibited during LiCl exposure should remain suppressed when rats are offered either 0.12 M LiCl or NaCl in the second half of the trial but not when they are offered water or 0.12 M sucrose.

Table 4
Generalization of 0.12 M LiCl CTA to 0.24 M NaCl Intake

Measure	(A) Group 2B: LiCl Trial 3 (CTA)		(B) Group 2B: NaCl Test T1 (generalization)		(C) Group 2A: NaCl Test T1 (control)		A vs. B		B vs. C	
	M	*SE*	*M*	*SE*	*M*	*SE*	*t*(3)	*p*	*t*(6)	*p*
Intake (ml)	1.44	0.39	3.39	1.01	13.41	0.72	−2.07	.13	−8.07	**.00**
Lick count	370.50	83.40	990.25	264.74	2,404.00	129.54	−2.26	.11	−4.80	**.00**
Lick volume (μl)	3.69	0.71	3.38	0.18	5.60	0.31	0.55	.62	−6.17	**.00**
Meal duration (min)	13.04	1.26	14.68	0.09	13.71	0.72	−1.29	.29	1.33	.23
Ingestion rate (licks/s)	0.50	0.12	1.13	0.30	2.95	0.24	−2.39	.10	−4.72	**.00**
Initial lick rate	32.25	10.59	38.00	12.94	246.75	80.50	0.50	.65	−2.56	**.04**
Burst count	54.25	9.76	54.25	9.68	26.75	4.13	0.00	1.00	2.61	**.04**
Mean burst size (licks)	6.78	1.09	21.82	8.56	97.60	16.98	−1.85	.16	−3.99	**.01**
Mean burst duration (s)	1.51	0.18	3.74	1.09	14.33	2.34	−2.22	.11	−4.11	**.01**
Latency (s)	0.98	0.57	0.12	0.05	2.76	1.79	1.45	.24	−1.47	.19
Pause time (%)	89.13	2.37	79.84	3.15	56.59	2.90	3.74	**.03**	5.43	**.00**
Mean pause duration (s)	15.07	4.33	14.18	1.73	21.06	6.14	0.23	.83	−1.08	.32
Pause ratio	0.16	0.02	0.07	0.03	0.01	0.00	3.59	**.04**	2.27	.06
ILI range groups										
0–999 ms	297.23	31.48	221.43	34.63	149.89	5.19	1.62	.20	2.04	.09
0–249 ms	139.90	3.72	134.02	3.91	141.82	3.59	1.84	.16	−1.47	.19
250–499 ms	349.65	4.42	361.89	3.75	345.48	10.50	−2.43	.09	1.47	.19
500–999 ms	722.88	11.90	723.60	6.83	686.63	16.18	−0.05	.96	2.11	.08
Range proportions (% ILIs in burst)										
0–249 ms	58.50	6.74	78.50	7.67	97.79	0.37	−2.25	.11	−2.50	**.05**
250–499 ms	22.23	2.64	10.59	3.52	1.20	0.10	5.66	**.01**	2.66	**.04**
500–999 ms	19.26	4.65	10.91	4.32	1.01	0.31	1.20	.32	2.28	.06

Note. Bold font indicates that *p* met the criterion for statistical significance, which was set at $p \leq .05$. LiCl = lithium chloride; CTA = conditioned taste aversion; NaCl = sodium chloride; ILI = interlick interval.

However, if the behavioral suppression at the 8th min is instead or also due to unconditioned malaise, intake should remain suppressed for all four stimuli offered subsequent to LiCl ingestion.

Method

Subjects

Thirty-eight naive, male albino Sprague–Dawley rats (Charles River, Wilmington, MA) weighing 300 ± 9 g (range = 223 to 411 g) were subjects of the experiment. Rats were housed individually in cages similar to those used in Experiment 1 in a room where temperature and lighting (12-hr light–dark cycle) were automatically controlled. All manipulations were performed during the lights-on portion of the cycle. Food (Harlan Teklab 8604, Madison, WI) and tap water were available ad libitum except where noted.

Apparatus

Rats were tested daily in an automated lickometer referred to as the Davis Rig (Davis MS-160, DiLog Instruments, Tallahassee, FL). Unlike the single-bottle lickometer in Experiment 1, the Davis Rig allows the presentation of up to 16 different taste stimuli within a single behavioral session, with a minimum interstimulus interval of 7.5 s (Rhinehart-Doty, Schumm, Smith, & Smith, 1994; G. P. Smith, 2001). Rats are placed in a plastic rectangular cage (30 cm × 14.5 cm × 18 cm) with a wire mesh floor and have access to sipper tubes (when a computer-operated shutter is lifted) via an oval opening centered in the front wall of the test chamber. Licks on the spout are recorded by microcomputer via a circuit similar to that of the lickometer used in Experiment 1.

Procedure

Following overnight water restriction, rats were habituated to the Davis Rig over four daily sessions and were tested on the 5th day. On Sessions 1 and 2, rats were offered a single 15-min trial of distilled water (the clock began with the first lick). On Sessions 3 and 4, rats were given two 8-min trials of distilled water with two different stimulus bottles housed on a motorized track outside the test chamber. Each 8-min trial began with the rat's first lick. At the end of the first trial (T1), the shutter closed for 7.5 s while the next sipper tube was positioned. If the rat did not initiate the second trial (T2) within 5 min, the session ended. Stimulus bottles were weighed to the nearest 0.01 g before and after the session to monitor total intake.

Conditions were identical on the test day, except for the taste stimuli used during T1 and T2. In brief, four groups received 0.12 M LiCl at T1 and then received LiCl, NaCl, sucrose, or water at T2 (see Table 7). Two control groups were also tested; one received NaCl (T1) and water (T2), and the other received NaCl on both T1 and T2. These conditions allowed assessment of behavior toward the conditioned stimulus (CS), a qualitatively similar stimulus, a dissimilar stimulus, and water.

Data Analysis

To address specific hypotheses, we performed a number of planned contrasts. For each test group, we used repeated-measures ANOVA to compare differences across the two halves of the test session (T1 vs. T2). To ensure stable LiCl responses across groups, we used a one-way between-subjects ANOVA to compare T1 measures for groups that were first exposed to LiCl. These four groups were then pooled for a *t* test comparison against the T1 measures for the NaCl–NaCl group to provide a LiCl–NaCl comparison. To evaluate the relative effects of T1 LiCl

Table 5
Comparison of 0.12 M LiCl With 0.12 M Sucrose

Measure	(A) Group 3B: LiCl Trial 3 (CTA)		(B) Group 3B: Sucrose Test Trial 1 (generalization)		(C) Group 3A: Sucrose Test Trial 1 (control)		A vs. B		B vs. C	
	M	*SE*	*M*	*SE*	*M*	*SE*	*t*(3)	*p*	*t*(6)	*p*
Intake (ml)	1.00	0.30	17.90	1.71	25.43	3.21	−10.42	.00	−2.07	**.08**
Lick count	330.00	71.26	3,843.25	275.31	4,105.75	358.77	−12.94	**.00**	−0.58	.58
Lick volume (μl)	2.79	0.37	4.64	0.16	6.15	0.35	−7.52	**.01**	−3.96	**.01**
Meal duration (min)	12.18	0.79	13.84	0.59	14.67	0.30	−2.07	.13	−1.26	.26
Ingestion rate (licks/s)	0.46	0.11	4.65	0.34	4.65	0.35	−12.61	**.00**	−0.02	.99
Initial lick rate	42.50	14.62	401.25	6.09	355.00	13.80	−19.22	**.00**	3.07	**.02**
Burst count	43.75	9.03	26.00	4.88	20.00	3.76	1.79	.17	0.97	.37
Mean burst size (licks)	7.61	0.99	162.51	27.15	219.46	25.74	−5.55	**.01**	−1.52	.18
Mean burst duration (s)	1.73	0.21	23.78	3.79	32.35	3.45	−5.53	**.01**	−1.67	.15
Latency (s)	88.72	34.21	0.67	0.19	1.13	0.78	2.58	.08	−0.58	.58
Pause time (%)	89.62	2.32	31.79	4.32	31.04	5.53	12.77	**.00**	0.11	.92
Mean pause duration (s)	17.80	3.76	10.95	1.18	17.73	5.84	2.15	.12	−1.13	.30
Pause ratio	0.003	0.00	0.005	0.00	0.005	0.00	−7.25	**.01**	0.19	.86
ILI range groups										
0–999 ms	294.51	23.54	148.42	1.96	148.85	3.17	6.03	**.01**	−0.12	.91
0–249 ms	137.97	3.38	141.73	1.69	146.75	3.16	−1.01	.39	−1.40	.21
250–499 ms	355.97	5.46	358.51	14.42	341.78	4.38	−0.26	.81	1.11	.31
500–999 ms	736.31	8.56	734.16	34.57	707.21	16.10	0.08	.94	0.71	.56
Range proportions (% ILIs in burst)										
0–249 ms	60.88	7.69	98.22	0.82	99.47	0.08	−4.86	**.02**	−1.50	.18
250–499 ms	20.13	5.96	1.01	0.38	0.23	0.08	3.25	**.05**	2.02	.09
500–999 ms	18.99	1.93	0.77	0.46	0.29	0.03	8.57	**.01**	1.04	.34

Note. Bold font indicates that *p* met the criterion for statistical significance, which was set at $p \leq .05$. LiCl = lithium chloride; CTA = conditioned taste aversion; ILI = interlick interval.

exposure on subsequent T2 tastant responses, we used one-way ANOVA with post hoc comparisons to compare T2 responses across groups. For analysis of lick rates by minute, we used two-way mixed factors (Minute × Group) ANOVA with post hoc comparisons.

Results and Discussion

Four rats, 1 in each LiCl test group, failed to sample the tastant offered in the second half of the test session (T2). Data for these rats were removed from analysis, although the failure of these rats to further sample is discussed.

Intake

Results indicate that the rapid reduction of LiCl intake is due in part to avoidance based on a taste association rather than an unconditioned effect of LiCl per se. LiCl intake did not differ in the first half of the test session (T1) for the four groups exposed to LiCl in T1, $F(3, 20) = 2.10$, $p = .13$ (see Figure 8). In the second half of the test session (T2), the rats receiving either LiCl or NaCl after LiCl exhibited significantly less intake compared with their T1 LiCl intake: LiCl, $F(1, 6) = 148.71$, $p < .001$; NaCl, $F(1, 6) = 38.45$, $p < .001$. For rats that received either sucrose or water after LiCl exposure, intake was not significantly less than it was for LiCl in T1—sucrose, $F(1, 2) = 9.17$, $p = .09$; water, $F(1, 6) = 2.25$, $p = .18$—despite the fact that rats were undoubtedly less dehydrated and were also likely experiencing malaise. However, a direct comparison of T2 water intakes after T1 LiCl (Group LiCl–water) and after T1 NaCl exposure (Group NaCl–water) indicates that water intake was significantly lower in the group exposed to LiCl, $F(1, 9) = 10.37$, $p < .01$, although it was also significantly greater (238%) than T2 NaCl intake after LiCl exposure, $F(1, 12) = 6.93$, $p < .02$. The pattern of generalization results observed in Experiment 1 was replicated in that a comparison of T2 NaCl and T2 LiCl intakes after LiCl was not significantly different, $F(1, 12) = 0.38$, $p = .55$, and NaCl intake was significantly lower compared with T2 NaCl in the control group (see Table 8 and Figure 8). Thus, T2 behavior was affected by acute malaise, but it is important to note that there is also evidence that rats can express associative taste aversion learning within 8 min of exposure.

Time Course of Ingestion

Ingestion rates for LiCl in T1 replicated those observed in Experiment 1. Rats initially drank LiCl avidly at a rate that did not vary across the four groups exposed to it, $F(3, 20) = 0.08$, $p = .97$ (range = 342–362 licks/min), and was not different from the initial lick rate for T1 NaCl, $F(1, 27) = 1.03$, $p = .32$. As the meal progressed, rats ingesting LiCl exhibited a rapid decline in lick rate, and ingestion almost ceased by the 8th min (see Figure 9). The shape of the LiCl ingestion rate curves did not vary substantially across these four groups, as indicated by the lack of a significant main group effect, $F(3, 20) = 1.49$, $p = .25$, and the fact that there was no significant Group × Minute interaction, $F(21, 140) = 0.72$, $p = .81$. By contrast, rats in the NaCl–NaCl group ingested NaCl at a sustained and robust pace throughout the entire 8-min T1 test (see Figure 9B), and this curve departed

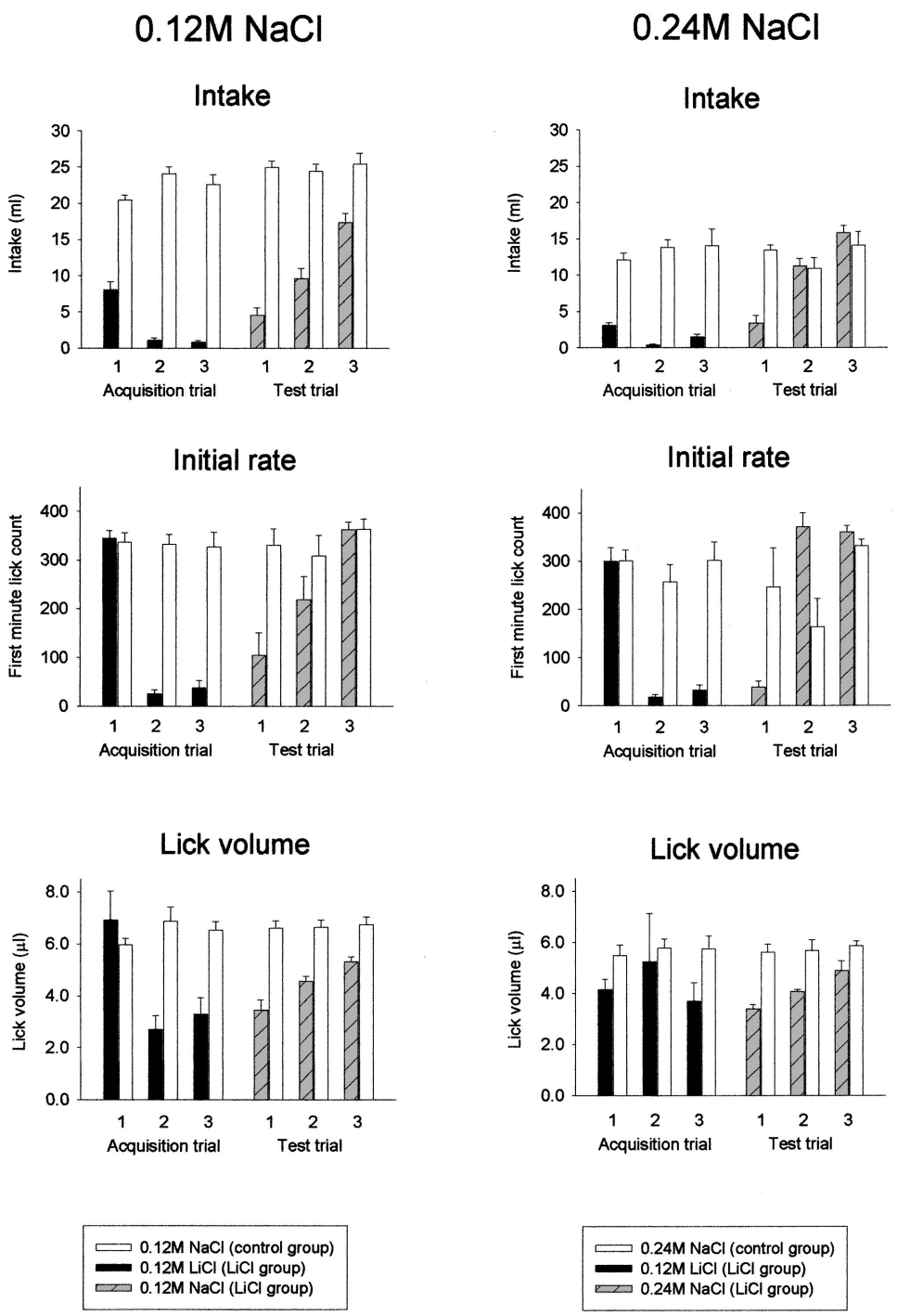

Figure 4. Mean (plus standard error) intake (milliliters), initial lick rate (licks per minute), and lick volume (microliters) across acquisition and test trials for four experimental groups in Experiment 1. Left panels: Data for Groups 1A and 1B. Control rats (Group 1A) drank 0.12 M sodium chloride (NaCl) on all test trials. Group 1B drank 0.12 M lithium chloride (LiCl) on three acquisition trials and 0.12 M NaCl over three subsequent extinction trials. Right panels: The same measures are plotted for Groups 2A and 2B, for which 0.24 M NaCl was substituted for 0.12 M NaCl. Thus, Group 2B received 0.12 M LiCl for three acquisition trials, followed by 0.24 M NaCl across three test trials, and Group 2A received 0.24 M NaCl across both acquisition and test trials.

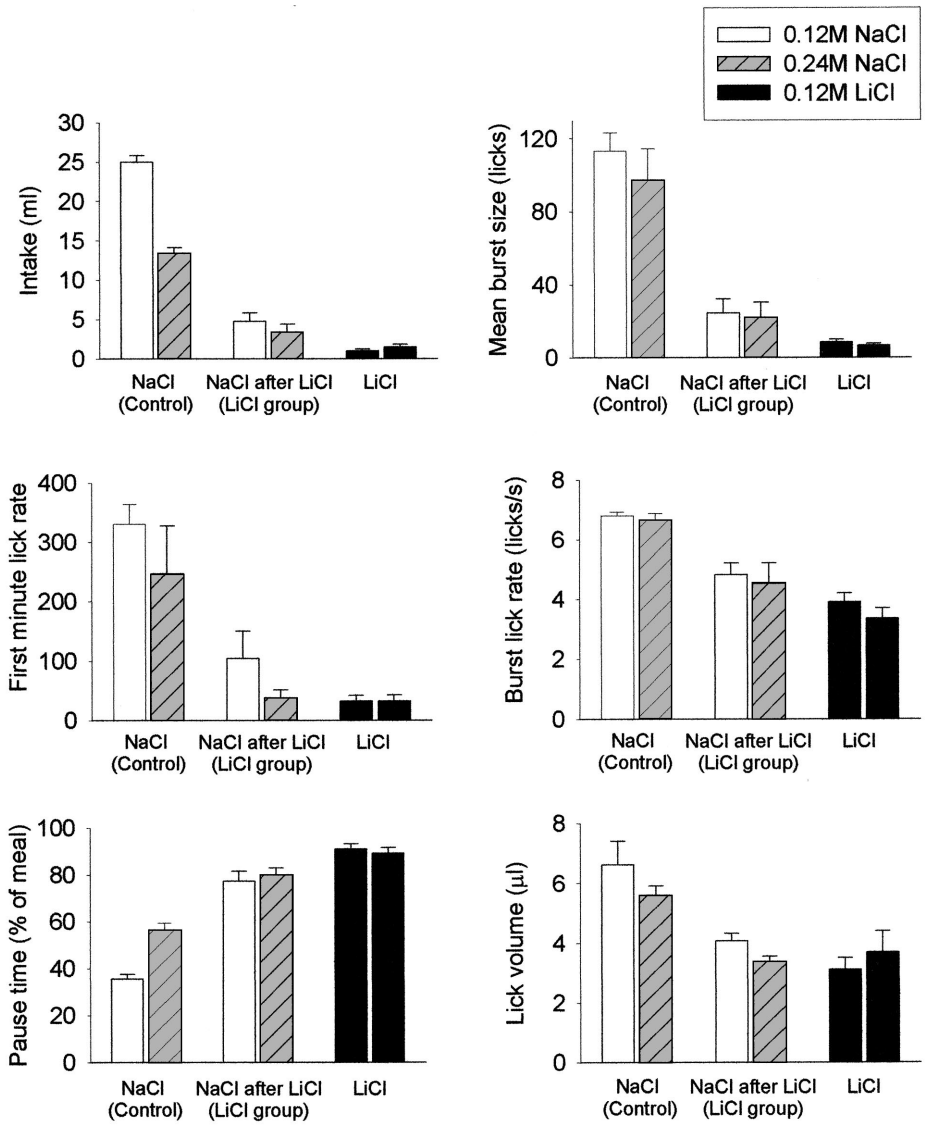

Figure 5. Mean (plus standard error) of six dependent variables for four experimental groups in Experiment 1 depict generalization of lithium chloride (LiCl) conditioned taste aversion to sodium chloride (NaCl). Measures include intake (milliliters), mean burst size (licks per burst), initial lick rate (licks per minute), within-burst lick rate (licks per second), proportion of pause time in the meal (percentage), and lick volume (microliters). The first pair of bars depicts measures on Test Trial 1 for Control Groups 1A (0.12 M NaCl) and 2A (0.24 M NaCl). The middle pair of bars indicates licking measures for matched NaCl concentrations in Groups 1B (0.12 M NaCl) and 2B (0.24 M NaCl) on the first generalization test after three LiCl exposures. The final pair indicates values for licking 0.12 M LiCl on the third acquisition trial in Groups 1B and 2B.

significantly from the LiCl ingestion rate curve (Group LiCl–NaCl) by the 7th min: interaction, $F(7, 77) = 5.90$, $p < .001$.

In T2, the ingestion rate curves, which had been roughly uniform when the rats were ingesting LiCl in T1, now diverged significantly depending on what tastant was offered. It should be noted that 1 rat in each treatment group failed to sample the T2 tastant. Of rats that did sample the T2 tastant, there was no systematic or statistical difference in latency either across groups, $F(4, 27) = 2.35$, $p = .09$, or relative to water habituation trials (mean latency = 23.9 s), $F(1, 20) = 3.55$, $p > .07$.

As shown in Figure 9A, rats that sampled NaCl or LiCl in T2 exhibited an approximate halving of ingestion rate in the 1st min relative to the 1st min of licking for either T1 LiCl (52% suppression), $F(1, 6) = 6.12$, $p < .05$, or T1 NaCl (49% suppression), $F(1, 6) = 6.52$, $p < .03$. The T2 NaCl initial rate after T1 LiCl exposure was also 39% lower relative to T2 NaCl control (NaCl–NaCl group) values, $F(1, 11) = 4.85$, $p = .05$ (see Figure 9B). In the remaining minutes of the test session, ingestion rate for both LiCl and NaCl rapidly declined, in almost parallel fashion, to negligible values within 6 min for rats exposed to T1 LiCl (see Figure 9A).

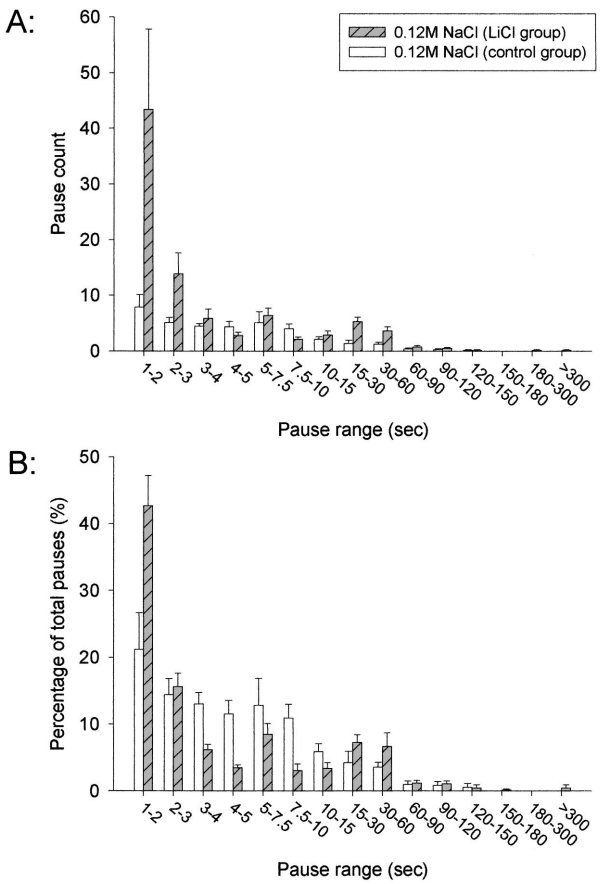

Figure 6. A: Frequency distribution of pauses for 0.12 M sodium chloride (NaCl) on Test Trial 1 by rats in the control Group 1A and the lithium chloride (LiCl)-exposed Group 1B. Mean (plus standard error) pause counts in each range are plotted. Range labels have been simplified for clarity of presentation. Range categories include pauses equal to or greater than the lower value through to pauses up to 1 ms less than the upper value indicated. For example, the 15–30 s category includes pauses of 15,000 ms through 29,999 ms. B: Same data as in Panel A, except that pause counts in each range are plotted as a proportion of all pauses in the meal.

At no time point were the ingestion rates significantly different for either T2 NaCl or T2 LiCl after T1 LiCl, as there was no significant between-groups difference in lick rate, $F(1, 12) = 0.88$, $p = .37$, and no significant Group × Minute interaction, $F(4, 48) = 0.29$, $p = .88$. There was a significant main effect of minute, $F(4, 48) = 15.19$, $p = .002$, indicating a general decline in lick rate. A comparison of T2 NaCl lick rates after either T1 LiCl or T1 NaCl also revealed that licking for NaCl after LiCl was markedly suppressed relative to control rats' licking of NaCl after T1 NaCl (see Figure 9B), as there was a significant main effect of group, $F(1, 11) = 53.03$, $p < .001$. There was no significant interaction, $F(7, 77) = 0.75$, $p = .64$, although there was a main effect of minute, $F(7, 77) = 6.11$, $p < .001$, indicating a declining lick rate for both groups (see Figure 9B).

In contrast, Figure 9C shows that rats offered water or sucrose after LiCl exhibited a rapid reinvigoration of ingestion rate. The 1st-min lick rate for sucrose more than doubled that for T2 LiCl, $F(1, 9) = 7.46$, $p = .03$, or NaCl, $F(1, 9) = 5.51$, $p = .05$. Water licking was initially unaffected by prior T1 LiCl ingestion: The 1st-min lick rate for T2 water licking in the LiCl–water group was almost identical to that for the NaCl–water control group (see Table 9). The initial lick rate for T2 water more than doubled that for T2 LiCl, $F(1, 13) = 6.03$, $p = .03$, and was 72% greater than the T2 NaCl initial lick rate, $F(1, 13) = 3.73$, $p = .07$.

Throughout the remainder of T2, both sucrose and water lick rates gradually declined: minute, $F(7, 56) = 4.82$, $p < .001$. The rate of licking for water overall was slower than for sucrose, as shown by a significant main effect of group, $F(1, 8) = 42.28$, $p < .001$, although the interaction term was not significant, $F(14, 56) = 2.22$, $p = .08$. The lick rate for T2 water after T1 LiCl was also significantly slower than for T2 water after T1 NaCl, $F(1, 9) = 91.28$, $p < .001$, but it was also significantly greater than for T2 NaCl after T1 LiCl, $F(1, 12) = 26.76$, $p < .001$. Thus, water ingestion after LiCl was partially suppressed, but significantly less so than licking for NaCl after T1 LiCl.

Microstructure

In Experiment 1, LiCl exposure resulted in reduced lick volume and burst size and duration, increased burst count, and increases in ILI and pause measures, such that the rate of ingestion within bursts (and for the overall meal) was slower. In this section we evaluate whether such changes occur within a single test session by comparing T1 and T2 microstructural measures.

Burst structure. The number of bursts of LiCl licking in T1 did not differ across the four LiCl-exposed groups (range = 13.33 ± 2.60 to 18.57 ± 2.06), $F(1, 6) = 0.54$, $p = .66$, and, in replication of Experiment 1, roughly doubled the number of bursts of T1 NaCl licking by the NaCl–NaCl group, $F(1, 27) = 5.08$, $p < .03$. Further, the average burst size for T1 LiCl was roughly one third of that for T1 NaCl, $F(1, 6) = 29.53$, $p < .001$. When LiCl-exposed rats were offered LiCl or NaCl in T2, both the number of bursts—LiCl–LiCl, $F(1, 6) = 51.98$, $p < .001$; LiCl–NaCl, $F(1, 6) = 15.29$, $p < .01$—and the mean burst sizes—LiCl–LiCl group, $F(1, 6) = 21.92$, $p = .003$; LiCl–NaCl group, $F(1, 7) = 7.20$, $p < .04$—were dramatically reduced relative to T1. Compared with controls (NaCl–NaCl group), burst size for T2 NaCl ingestion was reduced more than threefold as a result of LiCl preexposure (see Table 8).

In groups LiCl–sucrose and LiCl–water, for which T2 intake was less affected by T1 LiCl drinking, the number of bursts and average burst sizes were not significantly different from those for T1 LiCl: LiCl–sucrose, $F(1, 2) = 2.17$, $p = .28$; LiCl–water, $F(1, 6) = 1.14$, $p = .33$. However, the mean burst size for water intake in T2 was more than halved relative to the NaCl-water control group (see Table 9). The number of bursts was not significantly different in this T2 water–water group comparison (see Table 9).

ILIs. In Experiment 1 we observed that CTA slowed licking within bursts (see Figure 2). This effect was carried by a proportional increase in longer ILIs (250–999 ms) within bursts. The results of Experiment 2 convey a consistent trend toward replication of this effect, although effects were less robust.

Overall, licking within bursts was slowed from an average of 6.29 ± 0.19 licks/s for T1 LiCl to 4.76 ± 0.56 licks/s for T2 LiCl, $F(1, 6) = 3.37$, $p = .11$, and 5.05 ± 0.55 licks/s for T2 NaCl in Group LiCl–NaCl, $F(1, 6) = 2.60$, $p = .16$ (see also Table 8). By comparison, licking within bursts was slightly faster for sucrose

Table 6
NaCl CTA Extinction Trial 3 Compared With NaCl Controls

Measure	Group 1A: NaCl Test Trial 3 (control group)		Group 1B: NaCl Test Trial 3 (CTA group)			
	M	*SE*	*M*	*SE*	*t*(7)	*p*
Intake (ml)	25.42	1.47	17.63	1.21	−4.08	**.00**
Lick count	3,786.88	191.27	3,263.75	203.47	−1.87	.08
Lick volume (μl)	6.73	0.29	5.40	0.19	−3.70	**.00**
Meal duration (min)	13.93	0.56	13.85	0.37	0.12	.90
Ingestion rate (licks/s)	4.56	0.23	3.96	0.30	−1.59	.13
Initial lick rate	363.13	21.68	362.75	15.61	0.01	.99
Burst count	27.62	4.94	27.38	6.11	0.03	.98
Mean burst size (licks)	169.88	27.13	170.29	36.08	0.01	.99
Mean burst duration (s)	24.78	3.86	25.08	5.16	0.05	.96
Latency (s)	3.03	1.71	1.28	0.84	−0.92	.37
Pause time (%)	33.09	3.50	41.40	4.07	1.55	.14
Mean pause duration (s)	16.62	4.93	17.76	3.28	0.19	.85
Pause ratio	0.01	0.00	0.01	0.00	0.73	.48
ILI range groups						
0–999 ms	147.78	1.96	150.13	3.20	0.63	.54
0–249 ms	142.82	2.46	141.03	2.54	0.51	.62
250–499 ms	343.19	8.27	346.08	6.23	0.28	.79
500–999 ms	674.85	29.06	702.17	11.60	0.84	.41
Range proportions (% ILIs in burst)						
0–249 ms	98.39	0.51	97.24	0.56	−1.52	.15
250–499 ms	1.07	0.37	1.81	0.28	1.57	.14
500–999 ms	0.53	0.15	0.95	0.29	1.25	.23

Note. Bold font indicates that *p* met the criterion for statistical significance, which was set at $p \leq .05$. NaCl = sodium chloride; CTA = conditioned taste aversion; ILI = interlick interval.

after T1 LiCl (6.53 ± 0.32 lick/s), $F(1, 6) = 17.69$, $p = .05$, and T2 lick rates for water were not different from the rates for the NaCl–water group (see Table 9). The mean durations of ILIs in the 0–249 ms range were not significantly different in the T2 test between the LiCl–NaCl and LiCl–LiCl groups, $F(1, 13) = 0.02$, $p = .90$; between the LiCl–water and NaCl–water groups (see Table 9); or between the LiCl–NaCl and NaCl–NaCl groups (see Table 8). The average of ILIs in the 250–499 ms range was somewhat (about 10%) shortened for T2 water after T1 LiCl (see Table 9), but no other effects were observed (*p*s > .10; see Table

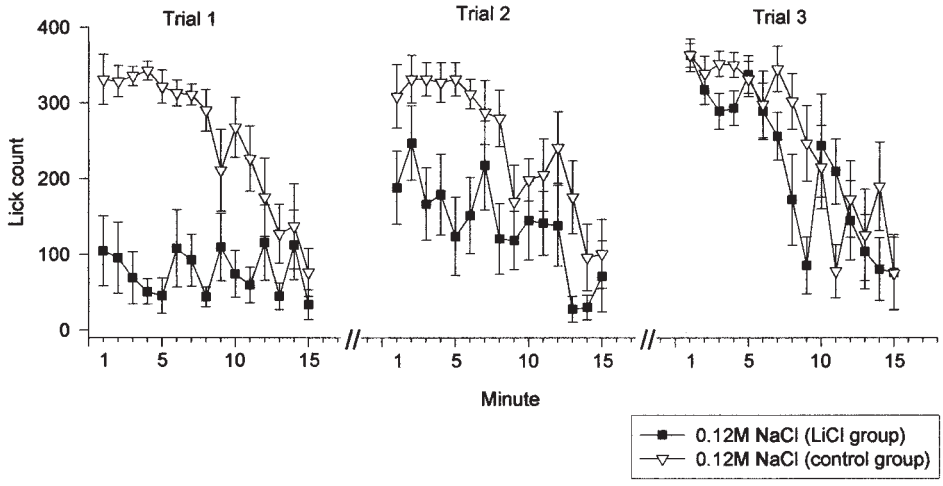

Figure 7. Mean (plus or minus standard error) lick rates (licks per minute) for rats drinking 0.12 M sodium chloride (NaCl) across three extinction trials. Lick rates for rats in the control group (Group 1A; $n = 8$) were consistent across all three trials. For rats in the lithium chloride (LiCl) group (Group 1B; $n = 8$), prior exposure to LiCl markedly suppressed NaCl drinking throughout the first test trial. Gradual extinction was apparent through successive increases in the rates of licking over the first half of the meal on Trials 2 and 3.

Table 7
Experiment 2: Summary of Conditions

Group	T1 tastant (first 8 min)	T2 tastant (second 8 min)
LiCl–LiCl (n = 8)	0.12 M LiCl	0.12 M LiCl
LiCl–NaCl (n = 8)	0.12 M LiCl	0.12 M NaCl
NaCl–NaCl (n = 6)	0.12 M NaCl	0.12 M NaCl
LiCl–sucrose (n = 4)	0.12 M LiCl	0.12 M sucrose
LiCl–water (n = 8)	0.12 M LiCl	Water
NaCl–water (n = 4)	0.12 M NaCl	Water

Note. T = trial; LiCl = lithium chloride; NaCl = sodium chloride.

8). No significant differences were observed for ILIs in the 500–999 ms range ($ps > .17$; see Tables 8 and 9).

The proportion of within-burst ILIs in the 0–249 ms range was reduced from a mean of 97.25% ± 0.71% for T1 LiCl to 81.35% ± 7.47% for T2 LiCl, $F(1, 6) = 5.54$, $p = .06$, and to 83.95% ± 7.04% for T2 NaCl in the LiCl–NaCl group, $F(1, 6) = 3.97$, $p = .09$ (see Table 8). The proportions of these ILIs were not appreciably reduced for T2 sucrose (96.72% ± 0.19%) or T2 water (94.72% ± 1.50%) after T1 LiCl. The proportional loss of T2 ILIs 0–249 ms for the LiCl–NaCl and LiCl–LiCl groups was offset by increases in T2 ILIs 250–499 ms (LiCl–LiCl: 10.52% ± 5.32%; LiCl–NaCl: 9.92% ± 4.30%) and T2 ILIs 500–999 ms (LiCl–LiCl: 8.13% ± 3.47%; LiCl–NaCl: 6.13% ± 2.82%).

Lick volume. Lick volume fluctuated little between T1 and T2 tests (no more than 12%), and no comparisons were significantly different ($ps > .13$), except for water intake after T1 LiCl, which was reduced 12% relative to T1 LiCl, $F(3, 23) = 6.95$, $p < .04$. Experiment 3 was developed to explore the nature of this discrepancy with Experiment 1.

Experiment 3

Method

In Experiment 1, lick volume was reduced by more than 40% on LiCl Trials 2 and 3 and was more resistant to extinction than most other measures (see Figure 4). In Experiment 2, lick volumes fluctuated little throughout the single test trial. Rats can vary lick topography rapidly on the basis of taste. For example, rats exposed to QHCl in a single trial expressed a halved lick volume relative to water licking (Spector & St. John, 1998). Therefore, the delayed expression of lick volume changes may indicate a delayed associative effect of CTA conditioning. One caveat to this interpretation is that for Experiment 2 we used a different apparatus, in which the spout was recessed about 5 mm more than in the lickometer used in Experiment 1. It is plausible that rats in Experiment 2 had less freedom to control lick volume because the longer tongue protrusion requirements diminished their ability to extrude larger drops from the spout (e.g., Weijnen, 1998). To test this hypothesis, we used the same procedures as those for the LiCl–LiCl group in Experiment 2 to expose 4 naive rats (278 ± 4 g) to 0.12 M LiCl, with the modification that rats were tested for 2 consecutive LiCl days.

Results and Discussion

Consistent with Experiment 2, 1 rat failed to sample LiCl in the T2 phase of the 1st LiCl test day. Also, 2 rats failed to sample LiCl in the T1 and/or the T2 session of the 2nd LiCl test day. Although a strong aversion was formed, all rats made at least 88 licks when they did sample LiCl, permitting analysis of lick volume. As there was no obvious difference (data not shown) in the T1 to T2 lick volume measures (consistent with Experiment 2), we collapsed lick volume data for each rat across T1 and T2 periods to yield an average lick volume score for each test day.

Results for the two daily LiCl trials were compared with the water trial preceding the 1st LiCl test day via a one-way repeated-measures ANOVA with post hoc comparisons. For the water habituation test, mean lick volume was 4.47 ± 0.10 μL. Mean lick volume declined little on the 1st LiCl test day; it was 3.87 ± 0.34 μL. However, mean lick volume was more than halved on the 2nd LiCl test day, to 2.19 ± 0.21 μL. This reduction was significantly different from the water trial, $F(2, 4) = 2.64$, $p = .001$ (comparison $p = .02$). We conclude that lick volume reduction is a delayed associative outcome of CTA learning.

General Discussion

CTA Microstructure

We used lick microstructure analysis to characterize the formation of a CTA in rats ingesting LiCl. This method approximates toxin exposure under feral conditions and allows for real time analysis of the formation of the aversion. In Experiment 1 rats showed a rapid decline in lick rate for LiCl on the first trial. By the second and third LiCl trials, profound shifts in the meal pattern were evident: The initial lick rate was reduced tenfold, bursts were dramatically reduced in size but increased in number, and the rate of ingestion both within and across bursts was slowed markedly. By all measures, it appears that CTA-treated rats were thirsty but also were hesitant to remain at the spout.

The pattern of microstructural changes we observed after CTA formation has not been reported for other treatments that suppress

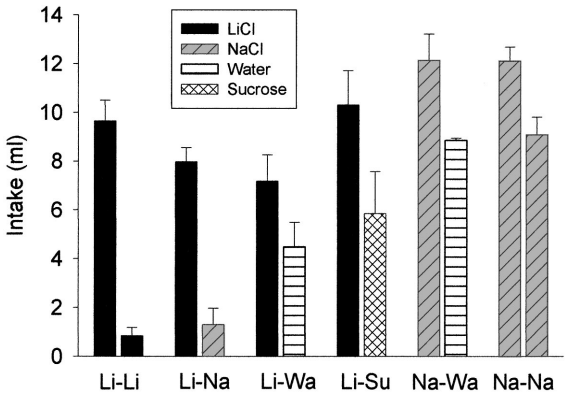

Figure 8. Mean (plus standard error) intake values for tastants offered in the first 8 min (first bar in each pair) and second 8 min (second bar in each pair) of the test session, for all test groups in Experiment 2. Tastants were 0.12 M lithium chloride (LiCl), 0.12 M sodium chloride (NaCl), 0.12 M sucrose, and distilled water. Labels on the abscissa indicate the experimental test groups (see Table 7). Li–Li = LiCl offered in both parts of the test session; Li–Na = LiCl offered first, then NaCl; Li–Wa = LiCl offered first, then water; Li–Su = LiCl offered first, then sucrose; Na–Wa = NaCl offered first, then water; Na–Na = NaCl offered in both parts of the test session.

Table 8
NaCl Responses After LiCl or NaCl Ingestion

Measure	T2 NaCl after T1 NaCl		T2 NaCl after T1 LiCl			
	M	*SE*	*M*	*SE*	*t*(13)	*p*
Intake (ml)	9.08	0.73	1.49	0.64	−7.88	**.00**
Lick count	1,635.63	246.91	343.43	158.02	−4.26	**.00**
Lick volume (μl)	4.78	0.24	4.69	0.33	−0.22	.83
Drinking duration (min)	7.79	0.10	3.36	0.98	−4.12	**.00**
Ingestion rate (licks/s)	4.07	0.29	2.31	0.86	−1.82	.10
Initial lick rate	331.67	22.60	184.85	58.25	−2.20	**.05**
Burst count	16.33	2.64	8.86	2.73	−1.95	.08
Mean burst size (licks)	137.07	25.70	45.62	19.97	−2.85	**.02**
Mean burst duration (s)	21.64	4.00	7.40	3.11	−2.85	**.02**
Latency (s)	0.14	0.00	40.03	12.26	5.78	**.04**
Pause time (%)	35.47	3.60	62.26	13.34	1.80	.10
Mean pause duration (s)	13.62	3.80	39.31	14.70	1.57	.15
Pause ratio	0.01	0.00	0.06	0.02	2.46	**.03**
ILI range groups						
0–999 ms	158.59	4.86	198.71	21.82	1.66	.13
0–249 ms	153.93	4.65	145.05	3.35	−1.58	.14
250–499 ms	346.87	3.40	350.98	7.62	0.47	.65
500–999 ms	680.78	24.59	607.30	106.22	−0.62	.55
Range proportions (% ILIs in burst)						
0–249 ms	98.51	0.02	83.95	0.07	−1.90	.08
250–499 ms	0.97	0.14	9.93	4.30	1.91	.08
500–999 ms	0.52	0.12	6.13	2.82	1.83	.10

Note. Bold font indicates that *p* met the criterion for statistical significance, which was set at $p \leq .05$. NaCl = sodium chloride; LiCl = lithium chloride; T = trial; ILI = interlick interval.

feeding by state or pharmacological manipulation. Caloric gastric preloads reduced the number and size of bursts in the meal and, like hepatic-portal glucose infusions, also suppressed the average ingestion rate (Baird et al., 1999; Eisen et al., 2001). Satiety-related drug treatments, including cholecystokinin, d-fenfluramine, and Melanotan II infusions, were shown to suppress meal duration, number of bursts,[1] and average ingestion rate (Davis, Smith, & Kung, 1995; Kaplan, Donahey, Baird, Simansky, & Grill, 1997; Williams, Grill, Weiss, Baird, & Kaplan, 2002). We also observed a reduction in mean burst size and average ingestion rate; however, CTA-induced intake reduction was accompanied by reduced lick volume and within-burst lick rate and by a paradoxical increase in the number of bursts. These latter effects were not reported in the studies noted above.

Our results do compare favorably with the prevailing scattered evidence of CTA licking responses in the literature. Kent et al. (2002) evaluated 0.3 M sucrose licking on 3 CTA acquisition trials. Rats progressively reduced intake and burst size but did not increase the number of bursts, as we observed. This discrepancy may relate to CTA strength: Rats in our study ingested a relatively larger dose of LiCl (3.50 mEq/kg vs. 0.75 mEq/kg) and reduced intake to a much greater degree (92% vs. 21% suppression). Davis and Perez evaluated licking responses to saccharin during and after LiCl exposure (reported in Davis, 1998). They observed a decline in both intake and initial lick rate during LiCl training, which gradually reversed over 10 extinction trials. Additional microstructure measures were not reported. Our results are consistent, except that extinction occurred more rapidly, within 3 trials. This was likely due to our water restriction paradigm, in which rats were only allowed access to fluids during the 15-min test sessions. Finally, Aja and colleagues (Aja, Robinson, Mills, Ladenheim, & Moran, 2002; Aja, Schwartz, Kuhar, & Moran, 2001) observed that intracerebroventricular (ICV) infusions of cocaine- and amphetamine-related transcript (CART) suppressed intake but also produced a CTA and tremors. They found a slower rate of licking within bursts and a slowed rate of ingestion overall, as we also observed.

During CTA, rats ingested at an almost 50% slower rate within bursts because of an increase in the proportion of longer ILIs (250–999 ms) relative to shorter ILIs (less than 250 ms). The lack of influence on ILIs less than 250 ms suggests that LiCl exposure did not significantly disrupt the rhythmic timing functions of the central pattern generator for licking in the reticular formation (Travers, Dinardo, & Karimnamazi, 1997) but rather affected processes that engage and disengage bursts of licking.

In contrast to the increased average ILI duration within bursts, we observed no overall effect on average pause duration. Rather, the average size and duration of bursts were markedly reduced, and the pause count was increased, such that the proportion of meal time expressed in pauses significantly increased. Combined with a slower rate of licking within bursts and no change in meal duration, rats ingested at an average lick rate that was 60% to 90% slower under CTA conditions.

Although there was no overall effect on mean pause duration, CTA treatment produced an increase in brief pauses (1–2 s) in the meal (see Figure 6). Considering the parallel increase in long ILIs within bursts, we conclude that CTA treatment specifically in-

[1] Not reported for d-fenfluramine.

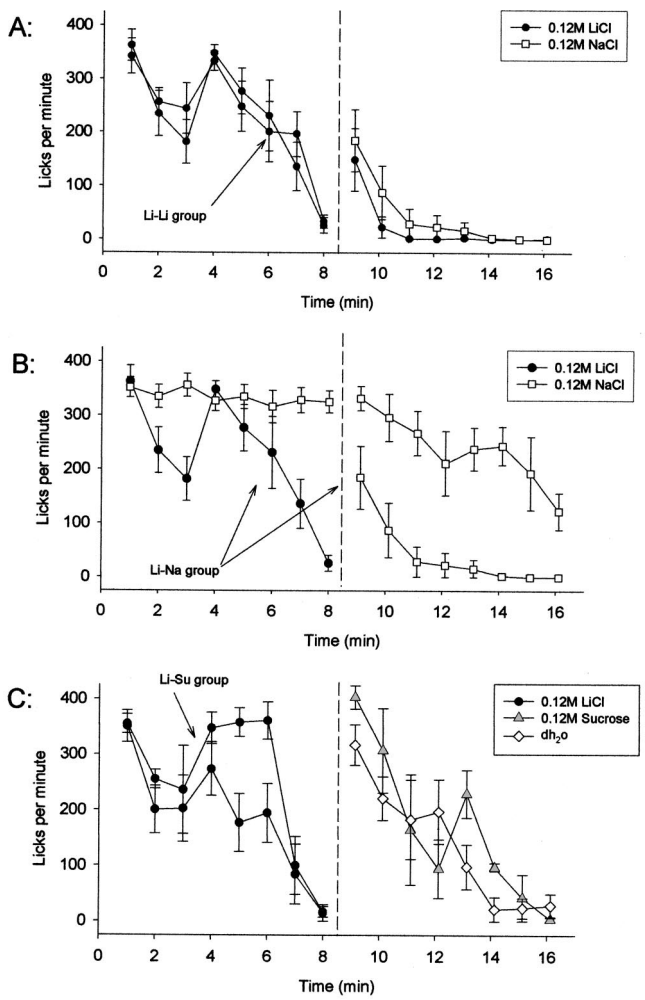

Figure 9. Mean (plus or minus standard error) lick rates (licks per minute) for rats drinking 0.12 M lithium chloride (LiCl), 0.12 M sodium chloride (NaCl), 0.12 M sucrose, or distilled water (dh_2o) across the first (T1) and second (T2) 8-min test session halves. Panel A: Comparison of LiCl–LiCl (Li–Li) and LiCl–NaCl (Li–Na) groups. Lick rates for both LiCl and NaCl in T2 were suppressed by LiCl ingestion in T1. T2 lick rates were overlapping and not statistically different, which suggests the generalization of suppression to NaCl. This inference is supported by Panel B, which directly compares Li–Na and Na–Na groups. Panel C: Rats in the LiCl–sucrose (Li-Su) and LiCl–water groups expressed renewed lick rates for sucrose or water after LiCl drinking had been suppressed to minimal values by the 8th min of T1, which indicates that suppression of T2 NaCl and LiCl lick rates was not primarily due to malaise.

creases the incidence of ILIs in the range of 250 ms to 2 s. This distributional shift appears to reflect a disruption of the Poisson process, by which behaviors that break continuation of the ongoing stereotyped lick cycle are selected (Davis, 1996); in this case, the system was biased by CTA treatment toward a much more frequent selection of behaviors that produce brief pauses. The increase in intervals ranging from 250 ms to 2 s could be due to increased expression of orofacial rejection responses that develop with CTA training (e.g., Berridge et al., 1981; Cross-Mellor, Clarke, & Ossenkopp, 2004; Pelchat et al., 1983; Spector et al., 1988). Pelchat et al. (1983) encoded orofacial responses to CTA-paired sucrose in rats licking from a spout and observed a range of rejection behaviors, including gapes, chin rubs, forelimb flails, face washing, and head shakes. Each of these rejection behaviors can be expressed within 2 s in rats (John-Paul Baird, unpublished observations) but also requires more time to express than a lick cycle (about 160 ms). For example, a gape cycle (the briefest rejection behavior) is typically 250 ms in duration (Chen & Travers, 2003; Dinardo & Travers, 1994). Davis (1996) suggested that ILIs in the 250–499 ms range represent lateral tongue protrusions (ingestive taste reactivity) or missed lick contacts, as the mean of ILIs in this range usually doubles the local lick cycle duration. However, CTA treatments reduce the incidence of tongue protrusions (Berridge et al., 1981; Eckel & Ossenkopp, 1996; Spector et al., 1988). Although CTA may enhance missed licks, the increase in ILIs 500 ms–2 s suggests that other nonlicking behaviors were engendered by the CTA treatment. Nevertheless, definitive proof requires a coordinated licking/electromyography and videographic analysis.

The emergence of more intervals in the 250 ms–2 s range, coupled with a severe reduction in burst size and no change in the mean pause duration, suggests that CTA treatment enhances processes that terminate rather than initiate bursts. Spector and St. John (1998) reached the same conclusion for the effects of QHCl on licking, observing that intact rats ingesting QHCl exhibited smaller bursts and a reduced initial rate of ingestion but no change in the mean pause duration relative to water ingestion. The present CTA results are comparable (see Table 2), and the failure to observe changes in mean pause duration or longer pauses may be consistent with a lack of growth of postingestive feedback inhibition during the meal because of minimal fluid intake under CTA conditions (see Spector et al., 1998).

It is unclear whether CTA treatment also affects burst initiation. The increase in burst count in the CTA conditions of Experiment 1 might have been due to thirst and/or to CTA. We believe that thirst moderates this effect because burst count was decreased for T2 LiCl and T2 NaCl drinking after T1 LiCl exposure (thus, hydration) in Experiment 2.

CTA Produces Shifts in Hedonic Evaluation

On first exposure to LiCl in Experiment 1, the rate of drinking, mean burst size, and mean pause duration in the first third of the meal were indistinguishable from those of control rats drinking NaCl. These data support the hypothesis that LiCl shares a gustatory similarity with NaCl (Nachman, 1962, 1963a, 1963b; see also Kiefer, 1978; Ossenkopp, Ladowsky, & Eckel, 1997; Strom, Lingenfelter, & Brody, 1970; Trifunovic & Reilly, 2002). In the remainder of the first LiCl trial, measures for LiCl licking markedly departed from those for NaCl in control rats. LiCl drinking shifted to a pattern of slowed ingestion characterized by bursts that were severely truncated (see Figure 3). This emergent pattern at the end of the first LiCl trial later typified ingestion throughout subsequent LiCl trials. The pattern of responses on LiCl Trial 3 versus water drinking was almost identical to that observed in intact rats drinking QHCl versus water (see Table 2). It is worth noting that in Spector and St. John (1998) an analysis of meals by thirds revealed an immediate difference in the first meal third between QHCl and water for burst size and drinking rate. This

Table 9
Comparison of Water Drinking Responses After 0.12 M LiCl or 0.12 M NaCl Ingestion

Measure	T2 dH_2O after T1 NaCl		T2 dH_2O after T1 LiCl			
	M	*SE*	*M*	*SE*	*F*(1, 10)	*p*
Intake (ml)	8.86	0.09	4.48	1.00	10.37	**.01**
Lick count	1,920.50	87.06	1,090.29	228.09	6.94	**.03**
Lick volume (μl)	4.64	0.22	4.23	0.28	0.96	.39
Drinking duration (min)	7.63	0.37	4.73	0.90	5.39	**.05**
Ingestion rate (licks/s)	4.25	0.38	3.73	0.42	0.69	.43
Initial lick rate	313.50	30.21	317.43	36.23	0.01	.94
Burst count	11.75	2.25	14.43	3.287	0.32	.59
Mean burst size (licks)	184.16	14.07	80.67	37.60	9.71	**.01**
Mean burst duration (s)	29.39	5.90	12.36	1.96	11.48	**.01**
Latency (s)	9.48	3.85	63.42	16.35	5.84	**.04**
Pause time (%)	32.19	5.53	42.58	5.86	1.37	.27
Mean pause duration (s)	14.92	3.57	14.11	5.73	0.10	.92
Pause ratio	0.01	0.00	0.01	0.00	5.51	**.04**
ILI range groups						
0–999 ms	160.97	2.45	157.77	3.60	0.39	.78
0–249 ms	154.22	3.04	143.31	3.40	4.58	.06
250–499 ms	360.87	10.57	322.99	9.17	6.75	**.03**
500–999 ms	717.14	30.26	721.49	21.76	0.01	.91
Range proportions (% ILIs in burst)						
0–249 ms	97.94	0.33	94.72	1.50	2.47	.15
250–499 ms	1.37	0.28	4.01	1.46	1.76	.22
500–999 ms	0.69	0.20	1.27	0.22	3.16	.11

Note. Bold font indicates that *p* met the criterion for statistical significance, which was set at $p \leq .05$. LiCl = lithium chloride; NaCl = sodium chloride; T = trial; dH_2O = distilled water; ILI = interlick interval.

pattern of burst responses in our experiment did not emerge immediately but rather developed gradually over the course of the first LiCl drinking trial in Experiment 1. This outcome supports the interpretation that the hedonic evaluation of LiCl was dynamically shifted from a preferred profile comparable to NaCl to one that paralleled bitter QHCl by the end of the first acquisition trial. Further, the results of Experiment 2 indicate that this change was based on a palatability shift rather than on the development of acute malaise.

In Experiment 1, the LiCl CTA generalized to both concentrations of NaCl but not to sucrose. NaCl licking on the 1st test day after LiCl more closely resembled licking for LiCl than licking for equimolar NaCl by the control group (see Figure 5 and Tables 3 and 4). Furthermore, the microstructural pattern of the generalization responses was not comparable to treatments that suppress intake through satiety (discussed above). These generalization results also support the hypothesis that LiCl exposure shifted the hedonic evaluation of LiCl (and, subsequently, NaCl) from an appetitive to an aversive appraisal (Berridge et al., 1981; Cross-Mellor et al., 2004; Spector et al., 1988).

Dissociation of Associative and Unconditioned Effects of LiCl Exposure

The unconditioned effects of LiCl on drinking behavior are not easily inferred. In Experiment 1, the comparatively parallel patterns of licking for NaCl after LiCl and licking for LiCl itself on Acquisition Trial 3 might be taken to indicate that licking responses during LiCl exposure were mostly associative and not an unconditioned effect of malaise generated by LiCl per se. However, responses during the first NaCl trial could include the beginnings of extinction processes. Furthermore, intake on LiCl Trial 3 was small, corresponding to a dose (0.38 mEq/kg) that likely produces only a moderate malaise and CTA (Nachman & Ashe, 1973),[2] and is consistent with the suggestion that rats learn to regulate LiCl drinking at a rate that minimizes toxicosis (Cross-Mellor et al., 2004). Analysis of the first LiCl acquisition trial to reveal the unconditioned effects of LiCl is also indeterminate. Although rats consumed much more LiCl on this trial, Experiment 2 shows that rats rapidly formed a taste–visceral association during this trial. One possible unconditioned effect of LiCl is that it reduces the probability of spout engagement in thirsty rats. One rat from each LiCl group in Experiment 2 and 1 rat in Experiment 3 (first LiCl trial) refused to sample the tastant offered in the T2 test period. Rats could have retreated from the spout because of nausea (di Lorenzo, 1988; Nachman, 1963a) or because they expected the T2 tastant to also be LiCl and simply avoided it. In opposition to the former possibility, the mean T1 LiCl consumption of the retreating rats (5.92 $\pm$ 0.93 mL) was 33% less than that (8.78 $\pm$ 0.98 mL) for rats that continued to sample during T2.

A comparison of licking for water offered immediately after LiCl or NaCl intake in Experiment 2 more directly indicates unconditioned LiCl effects. Intake for water after T1 LiCl exposure was reduced almost 50% in comparison with T2 water drinking after T1 NaCl. This suppression cannot be attributed to differ-

[2] Nachman and Ashe (1973) showed that injection of 0.3 mEq/kg LiCl caused a roughly 50% reduction in intake of sucrose, the conditioned stimulus.

ences in T1 hydration because rats consumed less LiCl (thus, less water) compared with rats that drank NaCl. In addition, burst size was halved, the duration of water drinking was reduced 38%, and latency was significantly increased after LiCl (see Table 9). Nevertheless, it is clear that a large dose of LiCl (mean ingested = 147 ± 16 mg/kg) was insufficient to completely abolish ingestion in the majority (23 of 28) of thirsty rats tested; rats capably ingested appreciable volumes of water or sucrose after T1 LiCl.

It is interesting to note that Eckel and Ossenkopp (1996), Houpt and Berlin (1999), and Spector et al. (1988) failed to observe unconditioned effects of LiCl on intake or taste reactivity responses when rats were first tested 15 or 20 min after LiCl injection. Consistent with this finding, we observed no shifts in the ILI distribution during T2 water licking after T1 LiCl in Experiment 2, as we did for LiCl and NaCl CTA conditions in both experiments. Our results support prior suggestions that the emergence of orofacial rejection responses during CTA formation is an outcome of associative taste–visceral processing (see Eckel & Ossenkopp, 1996; Spector et al., 1988). The failure to observe a decline in intraoral intake 15 min after LiCl injection may relate to differences in route of LiCl delivery (intraperitoneal vs. oral), LiCl dose, or intake sampling method.

Temporal Dynamics of CTA Formation

The rapid generalization test design of Experiment 2 permitted us to begin to explore a temporal threshold for CTA formation. Most studies of CTA learning assess intake of the CS on a test day subsequent to acquisition training, allowing 24 hr or more for the taste memory trace to be associated with visceral malaise. This delay conceivably allows the malaise to run its course, which could strengthen the association. In Experiment 2 we shortened the test interval to assess whether the taste association could be formed within 8 min. Licking for LiCl was rapidly suppressed, and a CTA was clearly formed during this time, because the suppression generalized to NaCl in a manner that overlapped the response to T2 LiCl. This suppression was not principally due to malaise, because rats offered sucrose or water immediately after LiCl exhibited renewed and sustained licking. We conclude that CTA formation does not require significant behavioral expression of malaise (as rats actively ingested LiCl through most of T1) or more than 8 min to viscerally process LiCl.

Previous studies have revealed that CTA learning occurs rapidly. Spector et al. (1988) first observed the emergence of oral rejection responses within 15 min of intraperitoneal LiCl injection. Eckel and Ossenkopp (1996) observed increased aversive responses 10 min after LiCl injection in intact rats. Houpt and Berlin (1999) showed that rats decreased intraoral sucrose intake 15 min after contingent pairing of intraoral sucrose and intraperitoneal LiCl. The present study reduces the time to observe CTA formation to less than 9 min. It is interesting to note that changes in taste reactivity were not observed 5 min after LiCl injection, when we observed a significant decline in LiCl ingestion rate (see Figure 1), and were inconsistently observed 10 min after injection, when we observed clear CTA generalization to NaCl. These discrepancies could be due to differences in the route of LiCl delivery (intraperitoneal vs. oral), dosage (3.5 mEq/kg vs. 3 mEq/kg), sampling procedure (discrete vs. continuous), or volume of CS sampled. Although CTA can be formed after only 0.1 mL CS tastant sampling, the strength of association increases with CS volumes through 2.5 mL (Barker, 1976; Peck & Ader, 1974; T. Yamamoto, Shimura, Sako, Yasoshima, & Sakai, 1994).

Previous studies (Baird, Travers, & Travers, 2001) have shown that taste and gastric distension responses are integrated within the parabrachial nucleus (PBN), a brainstem nucleus necessary for CTA formation (Spector, 1995), within 90 s, although cortical or vagal responses to intraperitoneal LiCl appear after 4–5 min (Niijima & Yamamoto, 1994; Yamamoto & Yuyama, 1987). Several studies (Chang & Scott, 1984; McCaughey, Giza, Nolan, & Scott, 1997; Shimura, Tanaka, & Yamamoto, 1997; Shimura, Tokita, & Yamamoto, 2002; Tamura & Norgren, 1997) have shown significant differences in neural gustatory coding in the PBN or nucleus of the solitary tract between groups of rats with or without prior CTA training, although evidence of single-neuron shifts is limited to one study of insular cortex recordings (Yasoshima & Yamamoto, 1998). The rapid formation of CTA evidenced here suggests that direct, real-time neural evidence of CTA formation can be obtained at other nuclei implicated in CTA (e.g., the PBN).

Extinction

Although CTA profoundly changed several microstructural measures, rats exhibited rapid extinction such that all measures except intake and lick volume were comparable to control conditions by the third extinction trial for 0.12 M NaCl. Extinction rates reported in the literature vary considerably as a result of the extent of food or water deprivation, time of testing, and method of CS delivery (e.g., Abelson, Pierrel-Sorrentino, & Blough, 1977; Fouquet, Oberling, & Sandner, 2001; Nolan et al., 1997; Ternes, 1976; J. Yamamoto, Fresquet, & Sandner, 2002). In Experiment 1 rats had only 15 min fluid access every 24 hr. This likely resulted in more frequent CS sampling (increased burst count) for purposes of hydration, resulting in a rapid extinction within three trials. This outcome supports observations that CTA learning obeys Pavlovian extinction principles provided that adequate CS sampling occurs under extinction conditions (e.g., Wolgin & Wade, 1990).

Extinction occurred rapidly but in a graded fashion across trials (see Figure 7). It is interesting to note that although most microstructural measures had recovered by the third trial, two lagging indicators of this recovery were lick volume and intake (see Table 6). This outcome suggests the provocative notion that intake per se may be a less sensitive measure of aversion and extinction than licking microstructure and taste reactivity measures. For example, ICV CART infusions produced rightward shifts in the ILI distribution, indicating a slower lick rate within bursts (as observed here under CTA), at a dose of CART (0.5 μg) that was subthreshold to the dose necessary to suppress intake (1 μg) and cause a CTA (Aja et al., 2001, 2002). However, further work is needed to explicitly compare licking microstructure or taste reactivity measures at doses of an emetic agent that straddle the intake threshold for CTA formation.

Limitations

The microstructural changes induced by CTA in this study may not be pertinent to other forms of CTA. For example, ICV CART infusions produced a CTA, tremors, and slowing of ingestion rate

and intraburst lick rate but had no effect on burst size or number as observed with CTAs in this study. Researchers should test additional forms of CTA (e.g., different emetics and different tastants) with a microstructural analysis to assess the general validity of the present outcomes. Several studies have reliably characterized the effects of other intake-modifying treatments on licking microstructure for normally preferred tastants. We believe that the present study provides normative data that contribute to a growing literature on licking microstructure for aversive tastants. One may argue that without significant motivation (e.g., thirst) animals would not ingest aversive tastants. However, many natural foods (e.g., fruits) contain mixtures of both palatable and aversive taste stimuli. Furthermore, a better understanding of the character of licking responses to a variety of gustatory stimuli, including aversive stimuli, could help to reveal the functional underpinnings of novel treatments that affect intake (see Grill, Spector, Schwartz, Kaplan, & Flynn, 1987, for discussion). Nevertheless, it should be noted that rats express considerable behavioral flexibility when intake access is challenged. We recently showed that explicit manipulation of lick volume produced marked shifts in the licking pattern with no overall effect on the intake outcome (Kaplan et al., 1997, 2001; Williams et al., 2002). Although licking parameters are not strictly fixed to sensory or state variables, microstructural analysis appears to have exploratory value to the extent that effects can be reliably observed and replicated under constant conditions. For example, in this study we observed a reliable within- and across-experiments replication of the pattern of behavioral microstructure for LiCl licking in seven separate groups of rats.

Perspectives

Taste reactivity analyses show that CTA treatments produce conditioned sucrose responses that resemble those for QHCl in untreated rats (Berridge et al., 1981; Cross-Mellor et al., 2004; Eckel & Ossenkopp, 1996; Pelchat et al., 1983). The pattern of licking microstructure also shifted to resemble QHCl licking during and after CTA treatment (see Table 2). The congruence of the outcomes of these two methods strongly supports the theory that CTA treatments produce an acquired dislike for once-preferred tastants in rats and humans alike (Pelchat & Rozin, 1982).

As a measure of hedonic evaluation, each method offers different strengths. Taste reactivity analysis provides categorical data and can use intraoral infusions to ensure uniform CS sampling. Licking microstructure analysis provides a wider array of response measurements and permits a less cumbersome analysis of behavior continuously throughout the test session, with millisecond resolution. This technique may therefore provide an effective and efficient means to evaluate both the quantitative (intake) and the qualitative (hedonic evaluation) effects of novel treatments and help to distinguish whether intake effects are due to catatonia, malaise, satiety, or other factors.

In Experiment 2 we developed a rapid CTA generalization test. We believe that this paradigm could be used to build on the efforts of Spector et al. (1988) to distinguish the differential effects of particular treatments that block CTA processing, such as parabrachial lesions (e.g., Spector, 1995), amygdala lesions (e.g., Lasiter & Glanzman, 1985), and the Glucagon-like peptide-1 antagonist exendin (Kinzig, D'Alessio, & Seeley, 2002), may have on specific stages of CTA processing (Spector et al., 1988). Spector et al. (1988) noted that CTAs could be blocked because of impairment of taste sensation, visceral sensation, taste–visceral integration, or long-term memory processing of the CTA. To first rule out dysgeusic effects of the treatment, researchers can use brief access tests to evaluate gustatory concentration-response functions and taste thresholds (e.g., Koh & Teitelbaum, 1961; J. C. Smith, Davis, & O'Keefe, 1992; Spector, Grill, & Norgren, 1993). With the inclusion of a follow-up generalization test, the rapid generalization method of Experiment 2 could be used to evaluate whether the treatment affects any of the three remaining stages of CTA processing. If the CTA-blocking treatment renders rats insensitive to peripheral malaise, then LiCl intake should not be suppressed at all during the T1 LiCl drinking phase, as one unconditioned effect of LiCl was partial intake suppression (see Table 9). If the treatment selectively blocks taste-visceral association, then LiCl drinking should be suppressed, but this should not generalize to NaCl (relative to other tastants) offered seconds later (di Lorenzo, 1998; Trifunovic & Reilly, 2002). Finally, if long-term processing of the CTA is disrupted, rats will show generalization to NaCl in the single test but fail to show generalization when offered NaCl on a subsequent test day. Thus, this rapid generalization testing method could help to distinguish the relative contributions of pharmacological and neurologic treatments to CTA function and to taste–visceral integration in general.

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