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Temporal and qualitative dynamics of conditioned taste aversions in C57BL/6J and DBA/2J mice self-administering LiCl☆

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HIGHLIGHTS

- We investigated whether mouse strains differed in conditioned taste aversion learning using a self-administration paradigm.
- We did not find pronounced strain differences in taste aversion learning.
- There were strain differences in other aspects of ingestive behavior, including consumption, burst count, and lick rate.
- Mice expressing a CTA showed a dramatic decrease in lick efficiency, which reflects decreased palatability.

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ABSTRACT

Self-administration of LiCl solution has been shown to result in the formation of a conditioned taste aversion (CTA) that generalizes to NaCl in rats. This paradigm may have considerable ecological validity as it models CTA learning in natural settings, and also allows for the investigation of drinking microstructure as an assay of potential shifts in stimulus palatability. We used this paradigm to examine possible mouse strain differences in CTA acquisition, generalization, and extinction. In the first experiment, C57BL/6J (B6) and DBA/2J (D2) mice self-administered LiCl (or control NaCl) over a 20-minute free access acquisition period and were tested on the following day with a panel of taste solutions available in brief (5-s) trials delivered in random order. In the second experiment, mice again self-administered LiCl or NaCl (at low, 0.12 M, or high, 0.24 M concentrations) in a 20-minute session, and on the following day received a 20-minute free access period to equimolar NaCl. Strain differences were found for aspects of ingestive behavior, with B6 mice showing greater consumption of all stimuli, including water, while D2 mice lick faster, in less frequent but longer bursts. We did not, however, find evidence of a robust strain difference in taste aversion learning. Both strains demonstrated profound alterations in licking microstructure in the generalization session relative to controls. We suggest that a decrease in "lick efficiency" (the percentage of inter-lick intervals within a burst of short duration vs. longer duration) reflects avoidance behavior, and signals a shift in palatability of a stimulus following CTA.

1. Introduction

Conditioned taste aversion (CTA) has been commonly used as a model of Pavlovian learning and memory, as well as a tool to investigate similarity or dissimilarity of taste stimuli (for reviews, see [40,47,55]). Experimental approaches to study CTA typically involve presentation of a novel tastant (conditioned stimulus; CS), followed by an intraperitoneal (i.p.) injection of LiCl that induces gastric malaise (unconditioned stimulus; US), which results in the subsequent aversion to the taste stimulus (conditioned response). There are variations on this procedure, such as duration between CS and US, or the number of CS–US pairings, but the common endpoint is that the animals develop an aversion to the conditioned stimulus, and may generalize this aversion to other stimuli with similar sensory properties.

A more naturalistic classical conditioning paradigm involves consumption, rather than injection, of the US [14]. Nachman [43] showed that rats consuming 0.12 M LiCl formed a gradual aversion to that stimulus, which generalized to equimolar NaCl. A gradual avoidance of the ingestion of LiCl could be caused by the gradual development of nausea (i.e., unconditioned effects), but Nachman's demonstration of a generalization to similar-tasting NaCl established that rats had formed a true

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learned aversion to LiCl's taste. [41] later showed that LiCl consumption produced conditioning of a magnitude equivalent to LiCl injection. [2] estimated that this associative conditioning occurs rapidly — as early as 9 min following the start of consumption, by employing a "rapid switching" paradigm in which LiCl was replaced by NaCl 8 min into a test trial. Rats generalized the aversion to NaCl within a single, short session (consistent with other work involving injected LiCl; [52]).

In our study, we compared two well-studied inbred mouse strains, C57BL/6J (B6) and DBA/2J (D2), using this self-administration model of CTA. These strains have been shown to vary in ability in various types of learning and memory tasks, including Morris water maze, contextual fear conditioning, and trace fear conditioning, with D2 mice often showing impairments in performance relative to B6 mice (i.e. inability to find platform in water maze, or lower incidence of freezing behavior in fear learning; [3,4,32,45]). On the other hand, D2 mice showed stronger place avoidance, a type of associative learning, than B6 mice in two different paradigms [49,50]. Given these differences in learning and memory function, we hypothesized that they might differ in CTA learning as well. Conclusions from earlier CTA studies with LiCl injection were equivocal [35,49,5,22]. However, a limitation of the i.p. injection paradigm applied to strain differences is that it does not easily discriminate between sensitivity to the US vs. learning ability. Indeed, there is some suggestion that D2 mice may be more sensitive to the toxicity of the US when it is either LiCl or acetaldehyde (metabolite of ethanol) [33,35].

Most investigations of CTA formation measure, as a dependent variable, consumption of the CS at some later time point following one or more CS–US pairings. In this study we employ a microstructural analysis of licking behavior in order to examine behavior during both the acquisition as well as generalization of a CTA. Microstructural analysis (e.g., [15,40]) assesses not only the result of intake (i.e., total fluid consumption), but also the behaviors that produce consumption on a millisecond time scale. Mice engaged in a 20-minute intake trial (as in the current experiment) typically lick the drinking spout at a stereotypical rate (roughly 10 licks per second, varying by strain), and modulate intake primarily by the length of "bursts" of licking and the number of such bursts separated by pauses of varying lengths. Over the course of a meal, these bursts may be distributed preferentially during the beginning of the trial. Mice may also engage the spout faithfully (contacting the spout with each tongue protrusion) or inefficiently (extending the tongue but failing to make contact with the spout, representing a kind of avoidance behavior). Others have demonstrated that careful attention to these variables allow inferences into the ways in which, for example, taste, physiological state, and experience influence ingestion (e.g., [2,17,19,34,53,54]).

Microstructural analysis has only recently been applied to the acquisition, generalization, and extinction of CTA. In general, following the formation of a CTA, rodents alter their drinking behavior of the CS by engaging in smaller bursts of licking [2,23,25,26,28,37–39,56] which are paradoxically more numerous in some [2,56] but not all [28] studies. Initial lick rate to the CS also declines [2,25,39,56] which, along with reduction in burst size, is suggestive of a decrease in CS palatability, a perceptual inference more commonly made by the assessment of orofacial behaviors [7,30,31,52]. Some studies have also examined "lick efficiency," our terminology for the percentage of short interlick intervals during an intake session. Licking behavior to the CS becomes less efficient after conditioning [2,25,56], a behavior also characteristic of intake of unconditionally avoided substances like quinine [34,54].

Because microstructural analysis provides inferences about the organization of behavior beyond that offered by simple intake measures [24,40,51], we examined the possibility that B6 and D2 mice might differ behaviorally on these measures during the acquisition, generalization, and extinction of CTA. To our knowledge, this is the first experiment to examine the microstructure of the licking behavior of mice during the formation and expression of CTA.

2. Method

2.1. Animals

A total of 119 naïve adult mice (mean age = 92.2 days old) from inbred strains C57BL/6J (B6; n = 60) and DBA/2J (D2; n = 59) were used in all experiments. Similar numbers of mice of each sex (52 males, 67 female) were used. Prior to testing, mice were group housed according to sex in standard plastic shoebox cages (28 × 17.5 × 13 cm) with ad libitum chow and water. Approximately 24 h prior to testing, mice were weighed and individually housed in new cages with ad libitum chow, but no water. Mean initial mean weights (g) were as follows: B6 males (26.02), B6 females (19.26), D2 males (24.56), D2 females (22.18). Each mouse was tested on the subsequent 4 days, during which time they received all of their fluid either during the test session, or during a 15-minute supplemental access period with water several hours after testing on days 2 and 3. The Animal Care and Use Committee at UTHSC approved this study, and all experiments were carried out in accordance with the National Institute of Health Guide for Care and Use of Laboratory Animals (NIH Publications No. 80-23), revised 1996.

2.2. Lickometer

All brief-access tests were conducted using MS-160 computer-controlled lickometers (DiLog Instruments, Inc., Tallahassee, FL), using procedures similar to those previously described [59]. Briefly, mice were placed in a plastic test chamber (30 × 14.5 × 16 cm) with a stainless-steel mesh floor, and could access taste stimuli or water via a small opening at the front of the chamber. A trial began when a shutter opened to allow access to a stainless steel drinking tube, and ended after a defined period (beginning with the mouse's first lick) when the shutter closed. Licks were counted via a high-frequency AC contact circuit, imperceptible to the mouse. Stimuli were presented in inverted glass bottles affixed with stainless steel drinking tubes with an orifice measuring ~3 mm diameter.

2.3. Experiment 1: CTA generalization with short trials

A total of 42 naïve mice were tested using a one-week protocol to examine acquisition and generalization of a CTA to a salt (0.12 M LiCl). We have previously measured stable licking and taste-guided behavior using a short protocol such as this (e.g. [12][9,11]). Mice were divided into CTA (n = 11 B6, 11 D2) and control (n = 11 B6, 9 D2) groups, individually housed, and placed under water restriction 23 h prior to the first training session. Mice were then given one training or test session per day for the next 4 days. The first training session consisted of a single 20-minute trial with distilled water. For the second session, mice were given 24 5-s trials with distilled water; 4 bottles containing water were presented in random order during the session, with a 7.5 s intertrial interval. On day 3 (acquisition), mice were given a single 20-minute trial with either 0.12 M LiCl (CTA group) or 0.12 M NaCl (control group). The following day (generalization), avoidance was measured using a standard brief-access tested (5-s trials) with water and four stimuli: 0.12 M LiCl and 0.12 M NaCl, as well as a mildly avoided bitter tastant (0.01 M $MgCl_2$), and a preferred sweet tastant (0.1 M sucrose). A single presentation of each stimulus plus two presentations of water was randomly presented in 4 blocks, for a total of 24 trials. 4 h after training or testing on days 2 and 3, mice received supplemental access (15 min) to water in the home cage. On days 1 and 3, bottles were weighed before and after the session to calculate total fluid intake.

2.4. Experiment 2: CTA generalization in a 20-minute test

In Experiment 2, we sought to examine possible effects of CTA on microstructure during the generalization session by using a longer trial similar to that used in acquisition. A total of 78 naïve mice were