Extinction of a Conditioned Taste Aversion in Rats: I. Behavioral Effects

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NOLAN, L. J., S. A. McCaughey, B. K. Giza, J. A. Rhinehart-Doty, J. C. Smith and T. R. Scott. Extinction of a conditioned taste aversion in rats: I. Behavioral effects. PHYSIOL BEHAV 61(2) 319 – 323, 1997.—The literature is divided over whether a conditioned taste aversion (CTA) can be fully extinguished. In Experiment 1, we created a powerful aversion in 34 rats by pairing the taste of 0.0025 M NaSaccharin (CS) with intraperitoneal injections of 127 mg/kg LiCl (US) on 3 occasions. We then offered 23-h deprived rats NaSaccharin for 10 min/day to observe the course of recovery. Extinction occurred in three phases: static, dynamic, and asymptomatic. During the static phase (mean = 9.6 days), rats consumed the CS at <10% of their preconditioned rate. With dynamic recovery (6.0 days), they increased acceptance to >80% of preconditioning levels. Finally, they achieved asymptote (3.1 days) at 100% acceptance. In Experiment 2, we used 8 additional conditioned rats and 8 unconditioned controls. We followed the same 1-bottle extinction procedure and, again, obtained 100% acceptance. Then we offered both NaSaccharin and water for 8 days at 23 h/day and monitored lick patterns every 65 s to determine taste preferences. The conditioned animals consumed less NaSaccharin than controls on Day 1, and less NaSaccharin as a percentage of total fluid as late as Day 3. For the last 5 days of 2-bottle preference testing, there were no significant differences between the groups with regard to 1. volume of NaSaccharin or water consumed, 2. percentage of total fluid taken as NaSaccharin, 3. consumption of each fluid associated with a meal or taken spontaneously, 4. intake during the light or dark periods, or 5. the characteristics of ingestion, including number of drinking bouts, duration of bouts, number of licks/bout, and rate of licking. Therefore, a robust CTA is subject to complete behavioral extinction. Copyright © 1997 Elsevier Science Inc.

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<th>Rat Taste Conditioning Feeding Extinction</th>
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THE conditioned taste aversion (CTA) is a particularly robust phenomenon, learned quickly, and resistant to extinction. Indeed, there remains a controversy over whether a fully developed CTA is subject to complete extinction (14). On the one hand, when a taste (the conditioned stimulus) to which a CTA has been developed is presented to rats without visceral consequences (the unconditioned stimulus), it is eventually accepted at preconditioning levels. This process is accelerated by depriving the rat of fluid before presenting the CS (9,16) and by offering it no alternative (2,11,13,15). When rats that have undergone this protocol are subsequently given 2-bottle preference tests, they select the taste of the CS to the same degree as do unconditioned controls (5,15), indicating complete recovery from the CTA. However, others have reported that, even with lengthy extinction procedures, there is a lasting effect of the aversion that prevents the reestablishment of preconditioning acceptance levels (2,9,11,14). These investigators have used a variety of gustatory CSs and different numbers of CS-US pairings, and these may have contributed to differences in the strength of the CTA and, therefore, to its resistance to extinction.

Moreover, even in cases where a CTA has been completely extinguished, subsequent conditioning has resulted in different behavior than was shown after the first conditioning episode, implying a residual memory trace of the initial experience. There is controversy, however, over the exact nature of the difference that appears upon reconditioning, with observations of both stronger (12) and weaker (4,10) aversions than were found initially.

Our goals were to determine, first, if a particularly robust CTA could be fully extinguished, even as measured by more subtle behavioral tests than had been applied before and, if so, second, if the neural changes that have been identified in the nucleus of the solitary tract with a CTA are also reversed. We will address the behavioral issue in this paper, the neural effects in the second.

METHODS

To explore the extremes of the CTA, we sought to create an especially powerful aversion at the beginning, to continue with extinction trials over a prolonged period, and to test for even quite

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subtle vestiges of the aversion before concluding. Thus, we paired the NaSaccharin CS with intraperitoneal LiCl on 3 occasions. We then offered rats the CS under the motivational pressure of 23-h fluid deprivation, but always followed by access to water so that avoidance of NaSaccharin was not life-threatening. By this means, recovery occurred slowly enough that we were able to discern its distinct phases. We followed a large number of animals through standard extinction training, measuring number of licks to the CS in a daily 10-min period. When this gross measure of recovery (acceptance at preconditioning levels) was satisfied, we proceeded to a finer analysis of licking behavior in a second, smaller group of rats. They were offered both the NaSaccharin CS and water, and their licking patterns were monitored continuously for 23 h each day. Thus, we could determine whether there were any behavioral remnants of the CTA.

GENERAL METHODS

Subjects were 70 female Wistar rats, 54 of which were used in the first experiment in which we measured the time-course of extinction, and 16 in the second where we monitored the fine details of licking patterns. The conditioned stimulus was 0.0025 M NaSaccharin (Aldrich); the unconditioned stimulus, 127 mg/kg LiCl, injected intraperitoneally.

Conditioning Procedures

All rats were maintained on a 23-h deprivation schedule, and trained to lick rapidly during a 10-min exposure to water in the test chamber. This was followed by 50-min access to water to ensure adequate hydration. The conditioning process was initiated when a rat exceeded 1800 licks during the 10-min trial for 3 consecutive days.

In the conditioning phase, both CTA and control subjects were offered 0.0025 M NaSaccharin for 10 min in the test chamber. Ten minutes later, they were injected intraperitoneally with 127 mg/kg LiCl (CTA) or physiological saline (control). There were 3 conditioning trials at 48-h intervals to ensure a robust aversion in the CTA animals.

Daily extinction trials began 48 h after the final conditioning session, and served to verify the strength of the aversion in CTA rats. Each trial consisted of a 10-min exposure to the NaSaccharin CS under 23-h water deprivation. This was followed by 50 min of access to water. Extinction trials continued until each rat's daily lick rate reached its preconditioning level.

EXPERIMENT 1. CHARACTERIZATION OF EXTINCTION USING A ONE-BOTTLE TEST

METHODS

Subjects

We used 54 female Wistar rats with a mean weight of 251 ± 3 g at the onset of conditioning. Rats were kept in pairs except during conditioning, when they were housed individually. The housing room had lights on from 0800–2000 h daily. Food was available ad lib.

Procedures

Groups of 3 rats were trained in a staggered fashion, with one group's training beginning as the previous group entered the conditioning phase. Training took place in a Plexiglas test chamber that measured 22 × 21 × 19 cm. In one wall was a shutter that exposed a 15-mm opening through which rats were offered access to a single spout in an adjacent chamber (56 × 56 × 56 cm) with its tip at a distance of 4 mm from the opening. This latter chamber was evacuated by a small fan that insured an air flow away from the rat and prevented its detecting the NaSaccharin by smell. Each rat was run at the same time every day, in the midst of the light cycle. It spent 10 min in the test chamber, beginning with the first lick, and was then returned to its home cage for 50 min of access to water.

Test for Spontaneous Recovery

The day after criterion had been reached, the CS was replaced by water for 2 daily sessions. On the third day, NaSaccharin was returned, and acceptance was measured to assess spontaneous recovery of the aversion. If lick rate fell below 80% of preconditioning level, extinction trials were re instituted. Eight rats fulfilled the criterion for spontaneous recovery, and were given additional extinction trials.

Data Analysis

We measured daily progress toward extinction and the period for total recovery for each rat. Extinction occurred in discrete phases (see below), and we performed separate analyses of licking behavior during each phase.

RESULTS

Training and Conditioning

Forty-eight rats completed the entire conditioning and recovery regimen. The mean period for lick training was 21.9 ± 0.7 (SEM) days. The 3 CS-US pairings produced a suppression of 99.4% of NaSaccharin licking (Fig. 1). The range of suppression was 94.0–99.9%.

Extinction

A mean of 18.7 ± 1.0 (SEM) daily trials was required to reestablish preconditioning lick rates to NaSaccharin (range = 5–35 trials). The mean recovery function for all rats is displayed in Fig. 1. However, this does not accurately represent three apparent stages of recovery (see below), because individual rats were in different stages on any one day. To reduce this variabil-

![Graph](image)  
FIG. 1. Mean (± SEM, indicated by shaded area) licks during 10-min sessions in Experiment 1. □, water; ☆, NaSaccharin on conditioning days; ○, NaSaccharin during extinction testing.
FIG. 2. Mean 10-min licks of NaSaccharin during extinction in the groups of rats with the shortest, intermediate, and longest times to reach criterion in Experiment 1. Each mean value was placed into 1 of the 3 stages of recovery, as described in the text, using the same criteria as were applied to individual rats. S, static phase; D, dynamic phase; A, asymptotic phase.

ity, we divided the rats into those with the shortest, middle third, and longest extinction periods, and plotted the mean recovery function for each subgroup (Fig. 2). We define the three stages of recovery apparent in Fig. 2 as static, dynamic, and asymptotic. Rats avoided the CS assiduously for a period that was quite variable, during which their lick rates were low and static. Eventually, they progressed to a stage of dynamic recovery, during which lick rates increased from <10% of preconditioning levels to >80%. Finally, there was a period during which they approached 100% asymptotically, with pronounced individual differences.

We defined the static phase as the number of daily trials before a rat exceeded 10% of preconditioning lick rate for 2 consecutive days. This phase had a mean duration of 9.6 ± 0.7 days, and the rate of increased acceptance for those rats in it was 18.8 licks/day (0.8% of preconditioning level per day).

The dynamic phase lasted from the end of the static period until the rat surpassed 80% of preconditioning levels for 2 consecutive days (frequently exceeding 100%). This phase lasted a mean of 6.0 ± 0.5 days, during which rats increased acceptance at a rate of 342 licks/day (15.4% of preconditioning level per day).

The asymptotic phase, for those rats that remained below preconditioning levels, extended from the end of the dynamic period to the achievement of 100% recovery. It had a mean duration of 3.1 ± 0.6 days.

The period spent in the static phase of recovery had no relationship to the time in the dynamic phase. The correlation between the lengths of these periods across the individual rats was just +0.03 (Fig. 3). Thus, after a rat had made the commitment to sample the CS, it entered a separate and unrelated phase of recovery.

EXPERIMENT 2. CHARACTERIZATION OF EXTINCTION USING A 2-BOTTLE TEST

METHODS

Subjects and Apparatus

Subjects were 16 female Wistar rats with a mean weight of 299 ± 5 g on the first conditioning day. They were divided into equal Control (saline-injected) and CTA (LiCl-injected) Groups. One control rat's behavioral data was subsequently dropped when its apparatus failed to monitor intake (the rat was still included in the neural experiment, however, as it underwent the complete procedure required of control subjects). Each rat was housed individually in a cage of 18 × 18 × 24 cm where it was also tested (3). Each cage contained 2 drinking spouts and a food jar. An infrared LED emitted a beam that was sensed by a phototransducer that, when interrupted, indicated the initiation of a feeding bout. Food was available ad lib. Light and dark phases of the day were signaled by a sensor in the housing room.

Conditioning and Testing

The rats acquired a CTA and underwent extinction in the same manner as in Experiment 1, except that all training took place in the home cage. After they had recovered from the CTA according to the criterion in Experiment 1, they were given free access to water for 24 h. They were then offered continuous access to 2 bottles, one with water, the other containing the CS. The positions of the bottles were reversed each day. Licks from each spout, time spent feeding, and amount ingested were recorded in consecutive 6-s time bins, for a total of 13,800 bins in each 23-h period (3). Behavior was monitored until there were no statistically reliable differences between the groups.

RESULTS

One-Bottle Testing

All subjects underwent 52 consecutive days of 1-bottle testing, the period required for the last rat to achieve its preconditioning level of licking to saccharin. The intake of control rats on test Day 1 was 28.7% higher than on the first conditioning day (reflecting a minor degree of neophobia on first exposure). CTA rats showed 99.8% suppression of licking to the CS on the first test day, followed by a gradual recovery. The 2 CTA rats that had the highest levels of pre-illness NaSaccharin licking never regained those levels during extinction. We considered their recovery complete when they reached 90% of preconditioning rate.

FIG. 3. Scatterplot of length of the static phase vs. the length of the dynamic phase in Experiment 1 (r = +0.03).
The mean number of days to reach criterion was 26.3 ± 3.9. This was significantly longer than in Experiment 1 (t = -2.54; df = 54; p = 0.05), a difference that was attributable to a longer mean static phase (16.7 vs. 9.6 days in Experiment 1; t = -3.47; df = 54; p = 0.001). The mean durations of the dynamic and asymptotic phases (8.5 and 1.0 days, respectively) did not differ from those of the previous experiment.

**Two-Bottle Testing**

Two-bottle testing was carried out for 8 days, during which the consumption patterns of the CTA group became indistinguishable from those of controls, by the following measures: 1. Volume of NaSaccharin, water, and total fluid consumed across 23 h; 2. Intake of each fluid associated (postprandial) or divorced from (spontaneous) a meal; 3. Intake of each fluid consumed postprandially or spontaneously during the light and dark phases of each 23-h period; 4. The proportion of total fluid consumed as NaSaccharin; and 5. The characteristics of ingestion, including number of bouts, duration of bouts, number of licks per bout, and rate of licking.

On Day 1, these analyses revealed that CTA rats consumed less NaSaccharin spontaneously during the dark phase [F(1, 13) = 11.24, p = 0.01]. The difference was sufficiently robust to result in a significantly lower 23-h spontaneous NaSaccharin consumption [F(1, 13) = 5.94, p = 0.05], 23-h total NaSaccharin consumption [F(1, 13) = 5.90, p = 0.05], and 23-h total fluid consumption [F(1, 13) = 4.68, p = 0.05]. The lower intake resulted from significantly fewer bouts of NaSaccharin consumption [F(1, 13) = 12.01, p = 0.01], not from differences in the duration of the bouts, mean licks per bout, or rate of licking. Postprandial intake of NaSaccharin did not differ between the 2 Groups on Day 1.

The percentage of total intake devoted to NaSaccharin in the CTA Group was lower as late as Day 3, but not thereafter. The difference on Day 3 arose from significantly greater water consumption [F(1, 13) = 9.80, p = 0.01], not less NaSaccharin, in the experimental Group. We found no significant differences between the Groups after Day 3.

The CTA Group showed a steady increase in spontaneous consumption of NaSaccharin across the 8 days (Fig. 4), with a correlation between amount of NaSaccharin consumed and day of testing of +0.90 (p = 0.01). Although this progression was regular, it was not large enough to result in significantly greater spontaneous intake on day 8 than on day 1 (t = -1.57; df = 7; p = 0.16). Control rats did not show any consistent change in NaSaccharin consumption over the 8 days (r = +0.02). Therefore, the 2-bottle test revealed a continuing change in the experimental rats' behavior that culminated in their being indistinguishable from that of controls after 3 days.

**DISCUSSION**

A conditioned taste aversion is subject to total behavioral extinction. We have explored the extremes of the phenomenon. On the one hand, with 3 CS-US pairings, we created a more robust aversion than is likely to happen in the wild. On the other, we measured its decay and disappearance using behavioral methods more subtle than those previously applied to this task. These included the time of day licking occurred, its association with feeding, and its bout characteristics. Moreover, conditioning and testing were conducted in the same location, which is the condition under which the aversion is most resistant to extinction (1). It is possible that some residue of the aversion would have appeared with retraining. However, this was not an option in this study because our intent was to record the neural activity from CTA-extinguished rats.

The sequence of recovery proceeded from a mean of 10 days of near total rejection, even under the motivational pressure of 23-h fluid deprivation (the static phase), through a week-long period of rapidly increasing acceptance (the dynamic phase), to total acceptance over 3 additional days (asymptotic phase). Then, with all thirst-induced motivation relieved in the 2-bottle test, vestiges of the aversion were revealed on the first day by a lower level of NaSaccharin consumption in conditioned rats. The difference was not attributable to NaSaccharin consumed for hydration (i.e., postprandially) but, rather, for that chosen spontaneously, where taste is presumed to provide the reinforcement.

This difference eroded over the course of 3 days of 2-bottle testing, after which there were no significant differences between Control and CTA rats with regard to how much NaSaccharin they ingested, the periods over which they did so, or the manner of their licking.

Although some studies have failed to find complete extinction of a CTA, the aversions created were not stronger than those of the present study, as measured by initial rejection of the CS. Instead, the discrepancy most likely relates to the extinction method employed. In the previous experiments, animals were given greater access to water (6–9) and, thus, were under less pressure to consume the CS for hydration purposes, or were tested for fewer days than in the present experiment (2,11,14). Our extinction procedure, which initially involved access only to NaSaccharin after 23-h fluid deprivation, has resulted in full acceptance of the CS when employed by others (6,9). Moreover, we also included a second stage of extinction training, in which subjects had access to both the CS and water and, eventually, found that extinction was complete using criteria more stringent than mere acceptance. Although this stage of 2-bottle testing likely would have been ineffective immediately after conditioning, it was effective in causing complete extinction in rats that had already received extensive 1-bottle training. Furthermore, the results of 2-bottle testing suggested that a persistent
suspicion of NaSaccharin may be masked by fluid deprivation. Thus, both 1- and 2-bottle tests were necessary to effect total extinction.

With the behavioral effects of the aversion fully extinguished, we were in a position to determine if the neural consequences of a CTA were also abolished.

REFERENCES


