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Sex Affects the Initial Strength but not the Extinction of Poison-Based Taste Aversions in Deer Mice (*Peromyscus maniculatus bairdi*)

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An examination of the effect of sex upon taste-aversion learning in deer mice (*Peromyscus maniculatus bairdi*) found that (a) sex has no apparent effect upon either the acquisition or the extinction of a LiCl-induced aversion to sucrose solution if the animals are tested while fluid deprived, but that (b) if the animals are tested under nondeprived conditions, males exhibit a greater initial aversion than females but both sexes seem to extinguish their aversions at similar rates. These findings differ from those previously reported for laboratory rats, in which it has been found that sex affects the extinction but not the acquisition of poison-induced taste aversions. It was suggested that either (a) sex interacts with taste-aversion learning via different mechanisms in deer mice and in rats, or (b) the apparent differences in extinction rates reported for rats might conceivably reflect differences in initial aversion strength which were undetected due to the use of high doses of toxin.

Much work has shown that animals can form strong taste aversions following a single flavor/illness pairing (see extensive bibliography in Riley & Clarke, 1977), and recent work has reported sex-related differences in this phenomenon. In 1976, Chambers and Sengstake found (using 2-hr, single-bottle tests on nondeprived animals) that male Sprague–Dawley-derived rats extinguished LiCl- and delta-9-THC-induced aversions toward sucrose solution much more slowly than did similarly treated females. Since then, castration, hormone-supplement, and hormone-antagonist studies have reported that this difference is testosterone mediated, that it is an activational rather than an organizational effect of testosterone, that it is the result of the androgenic rather than the estrogenic properties of testosterone, and that it depends upon the concurrent presence of testosterone during extinction but not during acquisition (Chambers & Sengstake, 1978, 1979; Earley & Leonard, 1978).

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It has also been shown that similar results can be obtained using two-bottle tests on nondeprived animals, using Wistar as well as Sprague–Dawley rats, and using cyclophosphamide as well as LiCl or delta-9-THC as the toxin (Earley & Leonard, 1978; Sengstake, Chambers, & Thrower, 1978). The occurrence of this behavioral difference depends, however, to some extent upon the test situation, since sex-related differences have consistently not been obtained with animals which were tested when fluid deprived (Chambers & Sengstake, 1976; Sengstake et al., 1978). This consistency over strains and toxins has led Sengstake et al. (1978) to conclude that the occurrence of a sex-related difference in taste-aversion extinction and its dependence upon deprivational state "seem to be a general effect in the albino laboratory rat" (p. 1153).

The occurrence of a general, hormonally based behavioral difference in rats suggests that a similar phenomenon might be found in other species. Therefore, the present study reports a study of this difference in another taxon: mice of the native North American genus *Peromyscus*. Aversions to a 20% (w/v) sucrose solution induced by intraperitoneal injections of LiCl were employed as the test behavior, since some data bearing upon the occurrence of such aversions in deer mice are already available (Robbins, 1977b, 1978, 1979).

EXPERIMENT 1

In rats a sexual difference in extinction does not occur if the animals are tested when fluid deprived (Chambers & Sengstake, 1976; Sengstake et al., 1978). This experiment tested deer mice under similar conditions.

Methods

The animals in the study were experimentally naive, adult (100–160 days of age), male and female *Peromyscus maniculatus bairdi*—a common grassland species of deer mouse. The animals were the first generation of laboratory-born offspring from original stocks captured on or near the Michigan State University campus. During these studies the animals were housed individually in plastic laboratory cages measuring 15 × 15 × 30 cm, equipped with wire lids, and supplied with wood shavings, cotton nesting material, and lab chow.

Since *Peromyscus* cannot tolerate the restriction of fluid availability to short intervals *every* day, regular drinking patterns were produced by placing the 78 subjects on the following fluid-availability schedule: On Day 0 the animals were removed from their colony cages, where water had been available ad lib, and were placed into their experimental cages. At 1300 hr, their water was removed, beginning a 24-hr deprivation. At 1300 hr of Day 1, a drinking tube filled with water was placed on each cage, left for 20 min, then removed and the amount consumed recorded. Consumption was measured to ± 0.1 ml by offering the fluid in 10-cc

plastic syringes which had been modified into calibrated drinking tubes (Robbins, 1977a). Immediately following the recording of data the tubes were refilled, replaced upon the cages, and left in position for approximately 24 hr. At 13 hr on Day 2, the tubes were removed (beginning a 24-hr deprivation) and the data recorded. This alternation of fluid availability/fluid deprivation was continued throughout the experiment. This schedule provides a regular, postdeprivation, 20-min drinking period (suitable for taste-aversion-inducing manipulations) on every odd-numbered day, while providing 24 hr of ad lib water consumption on every even-numbered day.

Water was presented to all of the animals according to this schedule for 8 days. Then the animals were weighed and assigned randomly to one of six treatment groups ($n = 13$ for each). On Day 9, Group 1 (male controls) received sucrose but no injection, Group 2 (male, low dose) received sucrose followed by an injection of 3.0 meq/kg of body wt of LiCl, Group 3 (male, high dose) received sucrose followed by an injection of 6.0 meq of LiCl, and Groups 4 through 6 were female groups treated as were Groups 1–3. Animals given 3.0 meq of LiCl were injected with 15 ml/kg body wt of 0.2 *M* LiCl, while animals in the 6.0-meq groups received similar injections of 0.4 *M* LiCl. Immediately following the injections, tubes filled with water were placed on the cages and left in place for approximately 22 hr. Then, beginning on Day 11, 20 extinction trials were administered by offering 20% sucrose solution to all animals during the 20-min drinking periods on the subsequent odd-numbered days of the schedule.

These dosages were chosen since previous work (Robbins, 1977b, 1978) indicated that they should result in strong, but not total, aversions. In studies of extinction differences in taste-aversion learning it is desirable that maximal aversions (i.e., first extinction-trial means which are not significantly different from zero) be avoided, since the occurrence of such strong aversions can easily allow real acquisition differences to be confounded with apparent extinction differences [cf. Robbins (1979); also note Elkins' (1973) demonstration of extinction differences between groups of rats pushed to apparently equal, zero-consumption aversions following different doses of toxin].

Noncontingently poisoned sensitization controls were omitted because (a) some data already exist upon poison-induced flavor sensitization in deer mice (Robbins, 1978), and (b) the present experiment is intended to test for the occurrence of sex differences in poison-based taste-aversion acquisition and extinction in deer mice, not to assess the relative contributions of learning and sensitization in these animals.

Results and Discussion

No differences were found among the groups in their sucrose consumption on Day 9 prior to injection [$F(5, 72) = 1.9063$] or in their water

consumption during the 22 hr following injection [$F(5, 72) = 1.7040$]. However, significant differences were found among the groups on the first extinction trial of Day 11 [$F(5, 72) = 37.1487, p \leq 10^{-8}$]. Planned comparisons of the pooled controls with the low- and high-dose injection groups indicated that all injected groups formed strong aversions [$F(1, 72) \geq 121.4994, p \leq 10^{-8}$]. The results for all of the extinction trials are given in Fig. 1. Examination of sex differences in aversion acquisition and extinction was made using two-sample t tests to compare the daily means of similarly treated male and female groups. [Cochran (1947) and Sokal and Rohlf (1969, p. 376) have indicated that this technique is preferred for making specific, two-mean contrasts when marked heterogeneity of variance occurs across treatment groups, as was the case here. Much power would be lost if the pooled error term (inflated by the large variance of the control groups) were used in analysis of variance to evaluate comparisons between the injected groups.] Such comparisons found no differences between male and female controls on any day [$|t(24)| \leq 1.7147$], nor between the male and female injected groups at either the low or the high dose [$|t(24)| \leq 1.5606$]. The comparisons between the male and female low dose (3.0 meq) groups are particularly informative since on the first extinction trial these two groups were not different from each other [$t(24)$

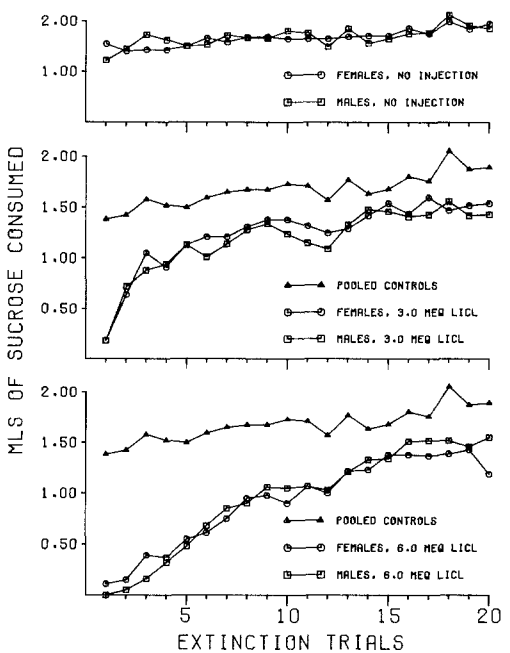

FIG. 1. Mean sucrose consumption (measured as actual ml consumed) for all groups in Experiment 1. No differences were found between any equivalently treated pair of male and female groups on any day of the experiment.

= .4713], but both were significantly greater than zero [$|t(12)| \geq 2.3819, p \leq .05$], ensuring that their extinction results can be interpreted with the knowledge that they are in no way confounded with acquisition effects masked by very strong initial aversions.

EXPERIMENT 2

Experiment 1 found that under fluid deprivation deer mice show behavior similar to that reported for laboratory rats (i.e., no sex-related differences in either taste-aversion acquisition or extinction). This experiment examined the behavior of deer mice when tested under nondeprived conditions. Under these conditions, laboratory rats have been reported to exhibit sex-related differences in taste-aversion extinction, but not in taste-aversion acquisition.

Methods

Experimentally naive animals, reared and housed as in Experiment 1, were used to replicate the previous experiment as closely as possible while also ensuring that the subjects were not fluid deprived when tested. Accordingly, for 8 days water was presented to the animals following the same fluid-availability/fluid-deprivation schedule as used in Experiment 1, except that two tubes of water were placed on each cage. On Day 8, all animals were weighed and assigned to one of six treatment groups. As before, the groups were male controls (sucrose/no injection), male low dose (sucrose/3.0 meq LiCl), and male high dose (sucrose/6.0 meq LiCl), and similarly treated female groups ($n = 15$ for all male groups, $n = 12$ for the female control and high-dose groups, and $n = 13$ for the female low-dose group). On Day 9, each animal was offered a single bottle of 20% sucrose solution during the 20-min drinking period following which it received its assigned injection. Immediately following the injections, two tubes of water were placed on each cage and left in place for approximately 22 hr. At 1300 hr of Day 10, the water tubes were removed and the 22-hr ad lib water consumption was recorded. Then two tubes (one containing water, the other 20% sucrose solution) were replaced on each cage, beginning a 24-hr ad lib two-bottle choice test. The next day consumption was recorded and all tubes were refilled with water or sucrose and replaced immediately. This was repeated until the results of 36 consecutive 24-hr ad lib two-bottle choice tests had been accumulated.

To guard against the possibility of a between-groups, systematic intrusion of position bias, each animal was offered sucrose only in its "preferred" position, as determined by that animal's water consumption during the 8 preinjection training days.

Results and Discussion

As in Experiment 1, no differences were found among the groups in their sucrose consumption on Day 9 prior to injection [$F(5, 76) = 1.2066$],

but significant differences were found in their total water consumption during the 22 hr following injection [$F(5, 76) = 2.9311$, $p \leq .05$], with injected animals drinking less. This direct effect of lithium toxicosis upon water consumption is frequently found. Obviously, it is desirable that the test animals not be under the direct effects of the toxin at the time of testing. However, since data exist which indicate that deer mice recover even from 9.0 meq/kg of LiCl within 24 hr of injection (Robbins, 1977b, 1978), the results of the first extinction trial in this experiment (which was not initiated until 24 hr after injections of only 3.0 and 6.0 meq/kg of LiCl) should be free from the direct effects of LiCl poisoning.

Figure 2 gives the results for all extinction trials. The data are shown, and were analyzed, as a preference score defined as milliliters of sucrose consumed divided by total milliliters of water and sucrose consumed. Before an analysis of sex-related differences in extinction were made, the initial aversions shown on the first extinction trial were examined to see (a) if any of the groups show near-zero consumption (thus requiring caution in their interpretation) or (b) if there are any sex-related differences in initial aversion strength (thus making unambiguous extinction comparisons between similarly injected male and female groups impossible).

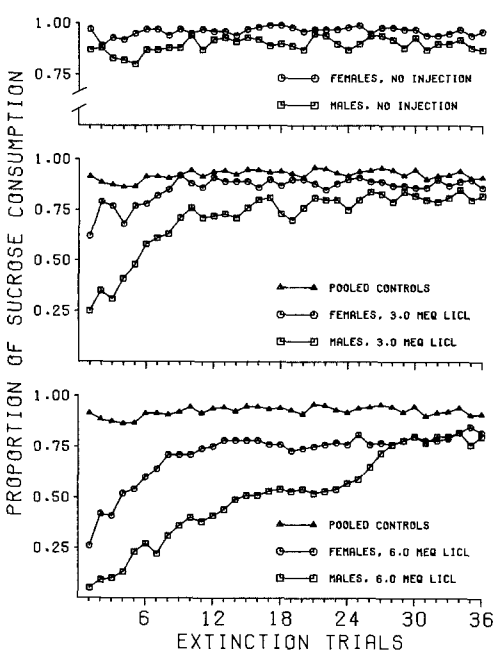

FIG. 2. Mean sucrose consumption (measured as a preference score defined as ml of sucrose consumed divided by the total ml of water and sucrose consumed) for all groups in Experiment 2.

The female high-dose group and both the male and female low-dose groups all showed nonmaximal initial aversions; that is, their first-extinction-trial means were significantly greater than zero ($|t| \geq 2.3681$, $df \geq 11$, $p \leq .05$). However, the mean of the male high-dose groups was not significantly greater than zero [$t(14) = 1.4126$].

There were no differences between the male and female control groups on the first trial [$t(25) = 1.3800$]. However, on trials 1–3 and 5, the female low-dose group exhibited significantly weaker aversions than did the similarly treated male group [$|t(26)| \geq 2.4659$, $p \leq .05$]. The male and female high-dose groups did not show a significant difference on the first trial [$t(25) = 1.9615$], but this result must be regarded with caution since (a) the t value is nearly significant and (b) the near-zero consumption of the male group but not of the female group has most probably compressed the real difference between the two groups. This assumption of compression is supported by the observation that as soon as the compression was relieved by the beginning of extinction on the next trial, a significant difference appeared between the groups, with the females showing weaker aversions on trials 2–9 [$|t(25)| \geq 2.1427$, $p \leq .05$]. Therefore, if primary emphasis is placed upon the unconfounded results of the low-dose groups, it appears that in deer mice sex affects the initial strength of poison-based taste aversions—a finding in contrast to those previously reported for laboratory rats.

Unambiguous comparisons of the extinction behavior of the similarly injected male and female groups cannot be made, because the low-dose (3.0 meq of LiCl) groups showed initial differences in aversion strength and the high-dose (6.0 meq of LiCl) groups were confounded by the near-zero consumption of the males. However, a useful extinction comparison can be constructed from the data. The male low-dose group had a first-trial mean of .2491 while the female high-dose group had a first-trial mean of .2586. Since these means are not different from each other [$t(25) = .0710$], but both are greater than zero [$|t| \geq 2.3681$, $df \geq 11$, $p < .05$], these two groups may reasonably be considered to have acquired equivalent, nonconfounded aversions. Hence, a comparison of their extinction behavior may be meaningfully interpreted. Figure 3 gives this comparison. As is apparent, these groups showed virtually identical extinction curves. The t tests comparing their daily means found no significant differences on any of the 36 trials [$|t(25)| \leq .7499$], indicating that (when an unconfounded comparison is made) sex does not appear to affect the rate of extinction of poison-based taste aversions in deer mice, even if the animals are tested under nondeprived conditions.

GENERAL DISCUSSION

Although the results obtained in Experiment 1 under fluid deprivation are consistent with those previously reported for laboratory rats, the

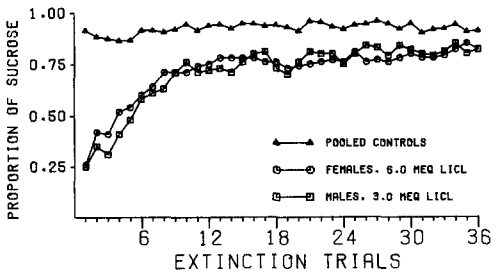

FIG. 3. A comparison of the extinction results of the high-dose injected females and the low-dose injected males of Experiment 2. Note that these groups (which showed equal, non-zero initial aversions on the first extinction trial) exhibit virtually identical extinction curves.

results obtained in Experiment 2 under nondeprived conditions are the converse of those in rats. Although one might attempt to offer a facile, adaptive explanation for this apparent taxon-specific difference in the effect of sex upon taste-aversion learning, it is also desirable to ask whether procedural differences may have generated the discrepancy. There are at least two such possible sources: (1) The deer mice were tested with continuous 24-hr tests while the rats have been tested with briefer (15 min–2 hr) daily tests, and thus, it is conceivable that the sex difference seen on the first trial with the deer mice actually represents an extinction difference that developed during the first 24 hr. This notion is supported somewhat by the occurrence of apparently weaker first-trial aversions in the normally more sensitive two-bottle test. On the other hand, deer mice usually restrict the bulk of their fluid consumption to the period just following lights off and just preceding lights on. Thus, their first 24-hr test is more comparable to the summation of only two 2-hr tests than to the summation of twelve 2-hr tests. (2) Experiment 2 investigated the effect of sex upon taste-aversion learning in deer mice using strong, but not maximal, aversions, while apparently all of the studies on laboratory rats have employed aversions involving virtually zero first-trial consumption. [See Figs. 1 and 3 in Chambers & Sengstake (1976) and Figs. 1–3 in Earley & Leonard (1978), and note the following toxin dose: 6.0 meq of LiCl/kg of body wt in Chambers (1976), Chambers & Sengstake (1978, 1979), and Sengstake et al. (1978); 50 mg/kg of cyclophosphamide in Sengstake et al. (1978).] Therefore, it is at least possible that there really is no taxon-specific difference in the effect of sex upon taste-aversion learning and that the previous studies on laboratory rats which reported extinction differences may actually have been detecting initial strength differences which were masked by the occurrence of too strong initial aversions.

However, it has been demonstrated that testosterone does not affect the *acquisition* of taste aversions by rats: Chambers and Sengstake (1979)

manipulated the availability of testosterone during acquisition and extinction and found that "testosterone slows the rate of extinction . . . by acting during the extinction process. Testosterone does not prolong extinction by affecting some process during acquisition" (p. 55).

Thus, sex may in fact interact with taste-aversion learning via different mechanisms in rats and in deer mice. At the same time, it is possible to offer a single, tentative hypothesis which might explain the differing results. Suppose that in both species testosterone does not affect acquisition but does affect the expressed strength of an aversion. Then the findings on deer mice would be expected, since low enough toxin doses were used to permit the detection of the effect on the first trial, while the results reported for rats would be expected since those studies used high doses of toxin which would have permitted the detection of the effect only upon later trials—thus apparently indicating an effect upon extinction.

The present study has in no way established, nor even tested, this hypothesis. All it has shown is that for deer mice (tested under nondeprived conditions) sex affects the initial strength of a taste aversion but does not seem to affect its extinction. Since these results differ from those previously reported in laboratory rats, and since the present results were obtained using aversions of a strength which permitted the detection of first-trial differences, while such was not the case in the rat studies, it would seem reasonable if some reconsideration were directed toward the analysis of the phenomenon in rats. In particular, it would be desirable to see the phenomenon studied using low enough toxin doses to permit the potential detection of sex-related differences on the first test trial.

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