Effect of food restriction on acquisition and expression of a conditioned odor discrimination in mice

Catherine A. Forestell*, Heather M. Schellinck, Sarah E. Boudreau, Vincent M. LoLordo

Department of Psychology, Dalhousie University, Halifax, NS, Canada B3H 4J1

Received 6 July 2000; received in revised form 3 October 2000; accepted 13 November 2000

Abstract

Level of food restriction was manipulated in mice to assess its importance for the acquisition and expression of a conditioned odor discrimination. In training, animals were exposed to odors (either rose or lemon) presented on a piece of filter paper in a pot covered in bedding. For half of the conditioning trials, group paired received one odor (CS+) with sucrose, the unconditioned stimulus (US), under the bedding. For the remaining trials, they received the other odor (CS–) alone. Group CS-alone was also exposed to both odors, but neither odor was paired with sugar on any of the conditioning trials. During training, Group Paired mice that were food-restricted tended to dig more readily and longer in the odors, especially in the CS+ odor, than animals that were not restricted. Both restricted and nonrestricted PAIRED GROUPS dug more in the CS+ than in the CS– by the end of training, but the CS-alone mice dug very little in either. Following training, mice were exposed to both odors simultaneously in a discrimination test. Half the mice in each training food restriction condition were tested under food restriction, and half were not. Only PAIRED animals that were food-restricted in the test expressed an odor discrimination, digging only in the CS+. This occurred regardless of their previous restriction state in training. These data suggest that both food-restricted and nonrestricted mice can acquire an odor discrimination; however, expression of this odor discrimination depends on food restriction. © 2001 Elsevier Science Inc. All rights reserved.

Keywords: Discrimination learning; Odors; Mice; Food restriction; Digging

1. Introduction

Olfactory learning occurs readily in rodents, and improves rapidly over successive problems [9,25]. These characteristics of rodent olfactory memory, combined with the similarity between the rodent olfactory–limbic pathway and the primate visual–limbic pathway, make the rodent a valuable tool for the study of higher-order learning and memory [20].

The nature of olfactory learning and memory has been investigated in the rat in several laboratories using various behavioral procedures. For example, using a procedure in which rat pups learn to approach odors paired with tactile unconditional stimuli, Leon and his colleagues have shown that odor-guided learning occurs early in development (for a review see Ref. [27]). It has also been shown that adult rats can discriminate among eight different odors in a go, no-go operant conditioning procedure. Moreover, their performance improves dramatically over multiple trials [26], suggesting that adult rats may develop a strategy (or learning set) for solving a successive series of problems (but see Ref. [21]). Rats are also believed to possess declarative memory for odors that is mediated by the hippocampus [5].

Given the increasing role of transgenic strains of mice in the study of learning and memory [24], appropriate tests that assess these abilities in mice should be developed. In contrast to the abundant literature on odor learning and memory in rats, until recently there has been little research on olfactory learning in mice. Typically, the behavior of transgenic mice is examined using a battery of tests which assesses the ability of an animal to learn and retain information as an adult or throughout development [7]. Among the most popular of these tests is the Morris water maze, which measures spatial memory and hippocampal function. A range of aversive conditioning procedures, such as active and passive avoidance, contextual fear conditioning, and the conditioned eye-blink response, have also been used to assess learning capabilities in transgenic strains of mice.
Behavioral testing of transgenic strains can be difficult to interpret because sensory or motor deficits often interfere with performance, making interpretation of behavior difficult [7]. Moreover, because some of the above tests have been designed for rats, they lack ethological relevance for the mouse [12]. For example, although mice are excellent swimmers, they do not perform as well as rats in the Morris water maze [12]. This is not surprising given that rats are specifically adapted to inhabit wetlands, whereas mice typically inhabit dry fields [19].

Several groups, including ours, are assessing the effectiveness of an ethologically relevant behavioral procedure that takes advantage of the mouse’s remarkable ability to forage for food using olfactory cues [3,4,17,22,28]. In our paradigm, mice receive trials in which one of two distinctive odors is presented in a pot containing bedding. One odor (CS+) is paired with sugar hidden under the bedding, whereas the other odor (CS−) is presented with no sugar. In the test phase, both odors are presented simultaneously without sugar. Mice express their conditioned discrimination by digging more in the pot containing the CS+ odor than in CS−.

Mice in previous experiments from our laboratory have been food-restricted during the training and test phases [11,22]. However, it is not clear that restriction is necessary for them to acquire or express a conditioned odor discrimination. Indeed, the literature on rats is mixed with respect to the effects of food restriction on both learning and performance. Looking first at learning, it has been found that rats learned to prefer a flavor paired with sucrose more readily if they had less frequent access to food during training than if they had more frequent access [6]. However, neither Fedorchak and Bolles [10] nor Harris et al. [13], who used an odor rather than a flavor CS, have found an effect of food restriction during training on acquisition of a flavor preference. Turning to performance, Capaldi et al. [6], Fedorchak and Bolles [10], and Holder [14] found that rats that have less access to food prior to a preference test will display a stronger preference for a flavor previously paired with sucrose than rats that have more access to food prior to the preference test. On the other hand, Harris et al. [13] found no effect of food restriction level at test on preference for an odor formerly paired with sucrose. These experiments differ in many ways, and it is hard to make sense of the discrepant results (see Harris et al. Ref. [13] for a thorough discussion).

In the present experiment, we investigated whether food restriction affects the ability of mice to acquire and express an odor discrimination using Schellinck’s procedure [2,22]. The procedure for determining the effect of food restriction on learning involves a design in which access to food is either restricted or nonrestricted during training and test [15]. In training, the latency to dig and the time spent digging in the odor pots were measured for each trial. Training differences between the restricted and nonrestricted animals’ behavior could reflect differences in both acquisition and expression of the odor discrimination; the two are confounded. Thus, the food restriction state of half the mice in each training condition was switched prior to a subsequent test in which the time spent digging in each of the simultaneously presented odor pots was measured. In test, differences between the training restriction groups would indicate that the food restriction manipulation affected the animals’ ability to acquire an odor discrimination. A difference between the test restriction groups would indicate that the restriction manipulation affected animals’ expression of the odor association. The usefulness of this odor discrimination task would be further substantiated if mice could acquire and express a conditioned odor discrimination without continued food restriction.

2. Method

2.1. Subjects

Forty-eight male CD1 mice obtained from Charles River, Quebec were maintained on a 12:12 h reverse light/dark schedule with lights on at 1900 h. Mean weights at the beginning of the experiment were 30–35 g. Animals were weighed daily at about 1500 h. Half were fed enough Purina Rat Chow to maintain their weight at 80–85% of their free feeding level. The remaining animals received ad lib food. All animals were housed individually in plastic shoebox cages (30.5 × 6.2 × 6.2 cm) with ad lib water available 24 h per day.

2.2. Apparatus

2.2.1. Odor stimuli

Odors were 15% phenethyl acetate (Aldrich) and 15% linalool (Aldrich) diluted in propylene glycol (Caledon Chemical). The former has a rose-like odor (hereafter called “rose”), whereas the latter has a lemon-like odor (“lemon”). Odors were mixed in advance and stored in 1.5-ml aliquots at −80°C.

2.2.2. Training apparatus

Odors (0.05 ml) were presented on filter paper (Whatman) 55 mm in diameter. The filter paper was placed on the bottom of a plastic cup that had been cut to about 1.5 cm high. A cover from a standard 60 × 15-mm plastic petri dish containing ten 0.5-mm holes secured the filter paper within the plastic cup and ensured that the animals did not come into contact with the odor. Approximately 10 small fragments of sugar (cut from sugar cubes, Redpath), weighing approximately 0.05 g in total, were placed on top of the odor pot on CS+ trials and covered with pro chip bedding (PW1 Brand, St. Hyacinthe, PQ). On CS− trials, pro chip bedding was placed on top of the odor pot without sugar. Pro chip bedding was also placed on the floor of the training cages.
Animals were exposed to the odor stimuli in cages identical to their home cages. To facilitate videotaping, transparent acrylic rather than wire cage tops were used. Four training cages were placed in each of four cubicles illuminated with dim red light. In two of these cubicles, a Panasonic video camera (model wv-BL200) with a wide-angle lens was mounted approximately 1 m above the cages. The cameras were connected to Panasonic VCRs (model AG 2500) and 13-in. Panasonic monitors.

2.2.3. Test apparatus

Discrimination tests were conducted in a 30 x 30 x 90-cm transparent acrylic chamber divided into three equal compartments with transparent acrylic walls. These walls contained 2 x 4-cm openings, which enabled the mice to move freely between the middle compartment and the two end compartments. For each new animal tested, pro chip was distributed over the floor of the chamber to a depth of approximately 2.5 cm. All sessions were recorded using a video camera, similar to those used in training, mounted above the chamber at an angle so that the animal could be observed from the side of the chamber.

2.3. Procedure

Three days prior to the training phase, half of the animals were put on a food restriction schedule and half were maintained on ad lib food. These animals were then assigned to one of three groups: Group R+, Group L+ (Paired groups), and the CS-alone group.

During training, subjects received 28 training trials (10-min sessions), 14 with rose and 14 with lemon, over 4 days (seven trials/day). Each day, training commenced several hours after lights off (between 0900 and 1000 h). For each trial, animals were transported from their colony room to one of the four training cubicles. There were two rooms for each odor, one in which the odor was presented with sugar (Rose+ or Lemon+), and the other in which the odor was presented without sugar (Rose− or Lemon−). Each room always contained the same four odor pots and animal cages.

After the mice were placed in their respective training cages, the odor pot containing the appropriate odor was placed near the back of each cage. For half of the trials, animals in the L+ group (n = 16) received lemon with sugar. For the remaining trials, rose was presented alone. Animals in the R+ group (n = 16) received the reverse; either rose with sugar or lemon presented alone. The CS-alone group (n = 16) also received equal exposure to both odors, but without sugar reinforcement. For all animals, different odors were used on the first two trials and thereafter, the odors were presented in a double alternation sequence (+−−+ or −+++−) throughout training. For Trials 1–7, to facilitate discovery of the sugar, a small piece of sugar was placed on top of each CS+ odor pot, and less pro chip bedding was piled on top of each pot.

At the end of each trial, animals were returned to their home cages in the colony room, where all the mice had access to water and the nonrestricted animals had access to food. During this 10-min interval, the pro chip and filter paper containing the odors were discarded and new odors were placed in the odor pots, with new pro chip on top of each pot and in the conditioning cages.

Immediately after the last training trial on Day 4, half of the animals in each food restriction condition (restricted and nonrestricted) were given ad lib access to food and the rest were food-restricted. Thus, animals in each of Groups R+, L+, and the CS-alone group were exposed to one of the following four training/test food restriction conditions: restricted/nonrestricted, restricted/restricted, nonrestricted/restricted, and nonrestricted/nonrestricted.

On Day 7, the test phase commenced. Each animal was first habituated for 2 min to the test chamber at each end of which was a pot covered in pro chip but without an odor. The animals were then removed and returned to their colony room for 5 min, while two new pots containing rose and lemon and covered with pro chip were placed on opposite ends of the chamber for the test. No sugar was hidden under the pro chip. Each mouse was again placed in the center compartment, and time spent digging in each odor pot was recorded for 3 min. The experimenter was blind to which group each mouse belonged. Testing was conducted in a room completely different from the training rooms to ensure that any training contextual cues that became associated with the sugar reinforcer would not affect behavior in the test phase.

After the first test, food was removed from all the animals that had been tested nonrestricted. They were subsequently maintained on a food restriction schedule until Day 10 when they were tested again as described above.

Animals were conditioned and tested in two equal-sized batches. For Batch 1, animals in the Lemon+ and Rose− cubicles were video-recorded in training. Thus, for this batch, L+ animals were recorded while exposed to both the CS+ and CS−, CS-alone animals were recorded while exposed to rose only, and R+ animals were not recorded. For the remaining batch, animals in the Rose+ and Lemon− cubicles were recorded. Therefore, in this batch, R+ animals were recorded while exposed to both the CS+ and CS−, CS-alone animals were recorded while exposed to lemon only, and L+ animals were not recorded.

2.4. Data analysis

Training tapes were scored by two blind observers, who recorded latency to dig, i.e., the amount of time before the first dig on each trial, and amount of time spent digging on each trial. The latency to dig measure was scored by the first observer on every trial, while the amount of time spent digging was scored on every third training trial. The second blind observer scored 50% of the previously scored training trials. Overall, there was a correlation of .85 for
latency to dig and .86 for time spent digging between the two observers.

Although animals in the Paired groups were videotaped while exposed to both odor cues, animals in the CS-alone group were videotaped while exposed to only one of the odors in training; lemon for Batch 1 or rose for Batch 2. Therefore, it was not possible to conduct a Group × Stimulus (rose vs. lemon) × Training Trials × Restriction State analysis of variance (ANOVA) for the latency to dig or the time spent digging variables. Instead, two analyses were conducted for each measure. The first analysis, which included data from only the Paired groups, was a Restriction State × Stimulus (CS+ vs. CS−) × Training Trial × Counterbalancing (Batch 1, which received lemon as the CS+ and rose as the CS−, vs. Batch 2, which received rose as the CS+ and lemon as the CS−) ANOVA.

In the second analysis, performance of the Paired group was compared with that of the CS-alone controls. Separate Restriction State × Group × Training Trial × Counterbalancing (Batch 1 vs. Batch 2) ANOVAs were conducted for the comparison of CS+ with control stimuli and the comparison of CS− with control stimuli.

Since the discrimination test data did not meet all of the assumptions for the parametric ANOVA, data from each of the two discrimination tests were analyzed using a two-way nonparametric nonspecific ranks sum analysis [16].

Fig. 1. Mean amount of time before the first dig on each training trial in the paired and CS-alone groups in the nonrestricted food condition (A) and the restricted food condition (B).
3. Results

3.1. Acquisition: comparisons including only Paired groups

3.1.1. Latency to dig

As shown in Fig. 1A and B, regardless of their restriction state, animals in the Paired groups were faster to dig in the CS+ than the CS−. As training progressed, latency to dig in the CS+ decreased, while that for the CS− increased. Food-restricted animals in the Paired group dug more readily in the odor stimuli than nonrestricted animals. These assertions were supported by a repeated-measures Restriction State × Stimulus × Training Trials × Counterbalancing ANOVA, which revealed main effects of Stimulus (CS+ vs. CS−) [F(1,12) = 31.9, P < .001], and Restriction State [F(1,12) = 6.0, P < .05], and a Stimulus × Training Trial interaction [F(13,156) = 5.3, P < .001]. There was also a Stimulus × Counterbalancing interaction [F(1,12) = 5.0, P < .05], which occurred because animals in Batch 2 (for which lemon was CS−) were slower to dig in the CS− than animals in Batch 1 (for which rose was CS−). Although the difference between the latency to dig in the CS+ and in the CS− was larger when lemon was CS− than when rose was CS−, animals in both counterbalanced halves of the experiment were significantly slower to dig in CS− than in CS+ (P < .001 for both batches).

3.1.2. Time spent digging

As shown in Fig. 2A and B, Paired animals spent more time digging in the CS+ than in the CS− during training, and this difference increased as training progressed. Time spent digging in the CS+ was higher in the restricted condition than in the nonrestricted condition. These assertions were supported by a repeated-measures Restriction State × Stimulus × Training Trials × Counterbalancing ANOVA. This yielded a significant effect of Restriction State [F(1,12) = 22.8, P < .001], Stimulus [F(1,12) = 133.1, P < .001], and Trials [F(4,48) = 18.8, P < .001]. These main effects were qualified by a Stimulus × Trials interaction [F(4,48) = 16.5, P < .001] and a Stimulus × Restriction State interaction [F(1,12) = 21.3, P < .001].

3.2. Acquisition: comparisons of Paired and CS-alone groups

3.2.1. Latency to dig

As shown in Fig. 1A and B, the mean latency to dig in the CS+ odor of mice in the Paired group was significantly shorter than the mean latency to dig in the control odors of mice in the CS-alone group. This difference grew as trials progressed. Mice in the first batch had shorter latencies overall than mice in the second batch, but batch did not interact with any other variable. These assertions are supported by a repeated-measures Restriction State × Group × Trials × Counterbalancing ANOVA, which yielded significant effects of Group [F(1,24) = 83.45, P < .0001] and Counterbalancing [F(1,24) = 4.48, P < .05], and a significant Group × Trials interaction [F(13,312) = 5.84, P < .0001].

For food-restricted mice, the Paired groups’ latencies to dig in the CS− odor were shorter than the CS-alone groups’ latencies to dig in the control odors, whereas this was not so for the nonrestricted mice. Latencies increased as training progressed. Mice had longer latencies to dig in lemon than in rose, regardless of their group assignment. These assertions were supported by an ANOVA that yielded significant effects of Trials [F(13,312) = 3.00, P < .0004] and Counterbalancing [F(1,24) = 13.85, P < .002], as well as interactions of Group × Restriction State [F(1,24) = 4.76, P < .04] and Counterbalancing × Trials [F(13,312) = 2.18, P < .02].

3.2.2. Time spent digging

As shown in Fig. 2A and B, time spent digging in the CS+ odor by mice in the Paired group was significantly greater than time spent digging in the control odors by mice in the CS-alone group. Digging increased over trials, as did the difference between the Paired group and controls. Food restriction enhanced digging in CS+ in the Paired group more than it enhanced digging in the odors by the CS-alone controls. These assertions were supported by an ANOVA that yielded significant effects of Group [F(1,24) = 140.36, P < .0001], Restriction State [F(1,24) = 20.30, P < .0001], and Trials [F(4,96) = 17.93, P < .0001], and Group × Trials [F(4,96) = 17.95, P < .0001] and Group × Restriction State [F(1,24) = 24.24, P < .0001] interactions. A comparable analysis of digging in the CS− odor by the Paired groups and digging in the control odors by the CS-alone groups...
revealed no effects of group or restriction state. The ANOVA yielded only an effect of counterbalancing. The mice spent more time digging in rose than lemon, both when they were control stimuli and when they were the CS – \[F(1,24)=5.47, P<.03\].

3.3. Discrimination test

3.3.1. Test phase 1

Only one animal in the CS-alone group dug in the test phase, and he dug for only 1 s. In the Paired groups (see Fig. 3), expression of the odor discrimination depended on whether the animals were food-restricted for the test. All of the mice that were food-restricted during the test dug in the CS+, and only in CS+, except for two animals that did not dig in either odor pot and one that dug for just a few seconds in CS –, though much less than it dug in CS+ (see Table 1, which presents discrimination ratios for individual mice; discrimination ratio = 100 × [time spent digging in CS+/time spent digging in CS –]). In contrast, less than half the animals that were not food-restricted during Test 1 dug in the CS+.

A 2 × 2 (Training Restriction × Test Restriction) nonspecific ranks sum analysis was conducted [16] on the difference between time spent digging in the CS+ and CS – pots during the test. This revealed a main effect of test restriction only. Animals that were food-restricted during the test showed a stronger odor discrimination than those which were not restricted during test \[H(1,3)=10.5, P<.01\]. There was no main effect of training food restriction \[H(1,3)=1.1, P>.05\], nor a Training Restriction × Test Restriction interaction \[H(1,3)=0.2, P>.05\]. Thus, animals in the same restriction state during test showed similar behavioral levels of odor discrimination regardless of their restriction state during training.

3.3.2. Test phase 2

In the second test (Fig. 3), in which all of the Paired animals that were not food-restricted for Test 1 were tested while food-restricted, all but two of the animals dug exclusively in the pot containing the CS+ odor (Table 1). A Wilcoxon matched pairs analysis conducted on CS+/CS – difference scores for Tests 1 and 2 confirmed that these animals did dig significantly more in Test 2 than in Test 1 \[T(17)=19, P<.05\]. Moreover, of the animals that were included in the second test, those that had been restricted during training did not differ from those that had been nonrestricted according to a Mann–Whitney U test \[U(4,4)=4, P>.05\]. This result further indicated that restriction during training did not affect acquisition of the conditioned odor discrimination.

4. Discussion

In the present study, animals were trained and tested on an odor discrimination task while either food-restricted or nonrestricted. Both food-restricted and nonrestricted Paired animals learned that the CS+ odor was paired with sugar. In Test 1, Paired animals that were food-restricted all displayed a conditioned odor discrimination regardless of their level of restriction during training. However, mice that were not food-restricted during test failed to show a reliable odor discrimination. However, in Test 2, when the nonrestricted mice from Test 1 were tested while food-restricted, they did display an odor discrimination, indicating that these animals had indeed acquired the odor association. Behavior during training was consistent with these results. Restricted animals initially dug longer in both odors than the nonrestricted animals and continued to dig longer in the CS+ even late in training.

Table 1

<table>
<thead>
<tr>
<th>Discrimination ratio (%)</th>
<th>Discrimination ratio (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group</td>
<td>Test 1</td>
</tr>
<tr>
<td>R/R</td>
<td>100</td>
</tr>
<tr>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>100</td>
<td>did not dig</td>
</tr>
<tr>
<td>83</td>
<td>did not dig</td>
</tr>
<tr>
<td>100</td>
<td>did not dig</td>
</tr>
<tr>
<td>100</td>
<td>did not dig</td>
</tr>
<tr>
<td>100</td>
<td>dug in CS – only</td>
</tr>
<tr>
<td>100</td>
<td>dug in CS – only</td>
</tr>
<tr>
<td>100</td>
<td>dug in CS – only</td>
</tr>
<tr>
<td>100</td>
<td>dug in CS – only</td>
</tr>
<tr>
<td>100</td>
<td>dug in CS – only</td>
</tr>
</tbody>
</table>

Fig. 3. Median time spent digging in the CS+ minus time spent digging in the CS – in Tests 1 and 2. Data points represent data from individual animals.
These data are consistent with those of Fedorchak and Bolles [10], who reported that when rats received flavor–ethanol pairings during training, they were more likely to express flavor preferences if they were food-restricted in test than if they were not restricted. This occurred regardless of whether they were food-restricted in training. This result suggests that food deprivation in this situation was a performance and not a learning variable. The absence of an effect of food restriction during training is in contrast to the results of Capaldi et al. [6] who found that food-restricted animals more readily learned and expressed flavor preferences during training and test than nonrestricted animals when consumption of the CS was equated between groups. However, it is unclear as to how these results relate to the present experiment since the food restriction parameters used in Capaldi et al. [6] were quite different: Animals were either exposed to high restriction (42 h without food) or moderate restriction (21 h without food).

It is noteworthy that the ability of nonrestricted animals to acquire flavor preferences has also been reported for rats that were intragastrically infused with calorific reinforcers [1,8,23]. Since the reinforcer did not provide a reinforcing taste in these experiments, it has been suggested that these preferences are primarily a function of flavor–calorie associations. In our study, sucrose, which has both a sweet taste and post-ingestive effects, was used as the reinforcer. Thus, it is possible that preferences could form as a function of odor–taste and/or odor–calorie associations.

In all of the above studies, animals were conditioned to prefer flavor or taste rather than odor stimuli. Thus, in some respects, the data reported by Harris et al. [13], in which restricted and nonrestricted rats were conditioned to prefer aqueous odors, are most comparable to the present study. In Harris et al. [13] all rats showed preferences regardless of whether they were food-restricted or nonrestricted in test. Further, their results indicated that the odor preferences expressed by the nonrestricted animals were based on an association between the odor and the sweet taste of sucrose, whereas preferences in the restricted animals were based on both odor–taste and odor–calorie associations. If deprivation does in fact enhance expression of nutrient-reinforced and not taste-reinforced preferences, then our data are inconsistent with the Harris et al. [13] hypothesis that nonrestricted animals acquire only odor–taste associations. In our study, if the mice had acquired only odor–taste associations, then their level of food restriction during the test phase should not have affected the expression of the odor–taste association. This suggests that the mice were capable of forming an odor–calorie association regardless of whether they were food-restricted or not in training. It is also possible that sated mice are more sensitive than sated rats to changes in energy state, and so are better able than rats to monitor the contingency between particular sensory characteristics of a food and the calories it provides.

One of the most obvious differences between our study and those discussed above is that learning in conditioned flavor preference studies is assessed in terms of consumption of the CS flavors, whereas discrimination learning in this study is reflected by appetitive, searching behaviors, i.e., digging. These behaviors, which differ markedly in their topography and function, may well be controlled by different neural substrates [2]. Thus, it is reasonable to suspect that manipulations such as food restriction might affect these processes differently. However, Myers and Hall [18] have shown that in flavor preference conditioning both the sweet taste of sucrose and its post-ingestive effects serve to condition not only increased consumption and increased palatability of the CS+ flavor, which was mixed with sucrose, but also increased orienting to the odor component of that flavor.

Finally, this simple behavioral procedure is a reliable method for generating and testing olfactory learning and memory capabilities in mice. Mice will readily learn to dig in odorized bedding where sucrose has been found and in a subsequent test will discriminate the sucrose-paired odor from one that had been presented without sucrose. Moreover, this procedure requires food restriction only during the test phase. Thus, the use of this odor conditioning procedure with nonrestricted mice should induce minimal stress, while tapping an association which is readily learned by mice in relatively few trials. Given the importance of the olfactory system in rodent learning and memory, the present test should prove to be a valuable addition to test batteries currently employed with transgenic strains of mice.

Acknowledgments

This research was supported by grants from the Natural Science and Engineering Research Council of Canada. We would like to thank Jennifer Stapleton and Mary Beth McIsaac for scoring behavior. Thanks also to Bob Boakes and Fred Westbrook for commenting on an earlier draft of the manuscript.

References