Performance of Mice in an Automated Olfactometer: Odor Detection, Discrimination and Odor Memory

Natalya Bodyak and Burton Slotnick

Department of Psychology, American University, Washington, DC 20016, USA

Correspondence to be sent to: B. Slotnick, Department of Psychology, American University, Washington, DC 20016, USA.
e-mail: slotnic@american.edu

Abstract

Mice were trained on a variety of odor detection and discrimination tasks in 100- or 200-trial sessions using a go, no-go discrete trials operant conditioning procedure. Odors, presented for 1 s on each trial, were generated by an air dilution olfactometer (for threshold tests) and an easily constructed eight-channel liquid dilution unit (for two- and multiple-odor discrimination tasks). Mice rapidly acquired the operant task and demonstrated excellent stimulus control by odor vapors. Their absolute detection threshold for ethyl acetate was similar to that obtained with rats using similar methods. They readily acquired four separate two-odor discrimination tasks and continued to perform well when all eight odors were presented in random order in the same session and when reinforcement probability for correct responding was decreased from 1 to 0.5. Memory for these eight odors, assessed under extinction after a 32 day rest period, was essentially perfect. Time spent sampling the odor on S+ and S– trials was highly correlated with response accuracy. As response accuracy increased, sampling time on S+ trials tended to increase and remain higher than sampling time on S– trials.

Introduction

Cloning of the olfactory receptor gene (Buck and Axel, 1990) advanced research on the molecular mechanisms of odor reception, organization of the olfactory sensory epithelium and the pattern of projections from the periphery to the olfactory bulb (Ressler et al., 1994; Mombaerts et al., 1996; Sullivan et al., 1996). This work has given rise to a number of hypotheses regarding olfactory coding that could be tested at the level of behavior using gene-targeted animals. Almost all of the mammalian research on the molecular biology of olfaction has been performed in mice, an animal that has not been widely employed in operant conditioning studies of sensory function. Olfactory guided behavior in mice has been widely studied (Passe and Walker, 1985; Alleva and Calamandrei, 1986; Wang et al., 1993; Brennan et al., 1995, 1998; Deiss and Baudoin, 1997) but, in general, the methods used (e.g. maze learning, odor preference, odor aversion learning, sexual attraction, pregnancy block) are not optimal for psychophysical analysis or other systematic studies of olfactory sensory function. Indeed, we found only one report in which contemporary olfactometric methods were used to assess olfaction in mice (Walker and O’Connell, 1986). That work indicated that mice could be successfully trained to detect odors using automated operant conditioning procedures. However, the odor generator used was somewhat complex and the behavioral task differed in a number of ways from those that have proven useful for systematic analyses of rat olfactory function. The purpose of the present study was to determine whether the relatively simple odor generator and behavioral methods used commonly with rats (Slotnick and Schoonover, 1984, 1993; Laing et al., 1989; Apfelbach et al., 1991; Eggert et al., 1994; Yougentob et al., 1997; Setzer and Slotnick, 1999) would be suitable to train mice on odor detection and discrimination tasks.

Materials and methods

Subjects

Adult male CF-1 strain mice, weighing 25–35 g, were housed individually in plastic cages in a temperature- and humidity-controlled vivarium. Fourteen mice were used for training but only four of these were tested on most of the tasks described below.

Apparatus

Odor detection threshold was assessed using a multi-stage air dilution olfactometer (Figure 1) similar to that described by Slotnick (Slotnick, 1990). Prior to use, each flowmeter was carefully calibrated using soap bubble rotometers and the system was washed with 95% ethanol and air dried. Odor discrimination tests used an eight-channel olfactometer (Figure 1) similar to that described previously.
A special feature of the current eight-channel unit was the use of pinch valves to control odor flow in the odor saturators. The valves operate by pinching close a short piece of odorless soft tubing (C-flex, Cole-Palmer, Vernon Hills, IL) connecting the saturator to the upstream and downstream air distribution manifolds. When a new odor was used in the system, all glass and Teflon connectors were washed with 95% ethanol and the pinch tubing replaced. This served to completely eliminate any potential contamination from prior odors. The olfactometers and digital interfaces were obtained from Knosys Olfactometers (Bethesda, MD).

The test chamber for both units was a 15 cm wide, 20 cm long, 13 cm high Plexiglas box fitted with a 15 cfm ceiling ventilation fan and stainless steel floor. A 17 mm inside diameter glass tube, mounted vertically on one wall, served...
for the delivery of odor stimuli. A 15 mm diameter hole through the Plexiglas wall and the tube allowed the mouse to insert its snout into the tube to sample the airstream. The top of the tube was connected to an exhaust line and the bottom of the tube was connected to the olfactometer. An infrared photocell was used to detect snout insertions into the tube. A 13-gauge stainless steel tube ending in a 3 mm diameter ball served to deliver water reinforcement. The reinforcement tube, located 6 cm to the right and 1.5 cm above the center of the port in the odor sampling tube, was connected to a 10 ml reservoir via a normally closed solenoid. Operation of the solenoid dispensed 0.005 ml of water. Reliable control of this small volume was aided by positioning the top of the reservoir 23 cm above the reinforcement tube and restricting flow using 0.76 mm inside diameter tygon tubing to connect the outport of the solenoid valve to the reinforcement tube.

The olfactometers and test chambers were not housed in a special enclosure but, rather, were located at one end of a room that housed other unshielded operant conditioning chambers. The room was used by a number of experimenters, and people traffic, a radio and other routine activity provided a varying background of potential auditory and visual distracters.

Procedures

Mice were maintained on a 1 ml/day water deprivation schedule for 10–12 days and then trained on a go, no-go discrimination task similar to the one that we routinely use for rats (Slotnick, 1990). All training and test conditions were automated and controlled by a 486-based personal computer. Control programs were written in QBASIC. The operant chamber was cleaned with a damp sponge after each session.

Initial training

Standard operant procedures were used to train the mouse to: (i) lick at the water reinforcement tube; (ii) insert its head in the odor sampling port; and (iii) insert its head in the odor sampling port, keep its snout there until an odor was presented and then lick at the reinforcement tube (Figure 2). Delivery of reinforcement was signaled by brief operation of a 3 kHz buzzer.

In the first stage of training, the mouse was reinforced with 0.005 ml of water for licking at the water tube. A progressive fixed ratio schedule was in effect for the first 20 reinforcements and a random ratio schedule was used to deliver the last 20 reinforcements. After completing this training stage most mice would orient and approach the reinforcement tube when the buzzer sounded.

In the next stage, reinforcement delivery was contingent upon the mouse inserting its snout into the odor sampling tube. The minimum required snout insertion duration increased progressively from 0.05 to 1 s over a series of 60 trials. In the later half of these trials, snout insertion resulted in a 1 s presentation of the S+ odor stimulus (1% ethyl acetate). A 5 s intertrial interval (ITI) was in effect during these trials.

In the last stage of training, reinforcement was contingent upon the mouse inserting its snout into the odor sampling tube, holding it there until the odor was delivered and then licking at the reinforcement tube. Timing of odor presentation was accomplished using a three-way valve (Final Valve, Figure 1) which connected the odor generator to the odor sampling tube. When the final valve was energized, airflow was diverted from the odor sampling tube to an exhaust line. When a snout insertion was detected, the odor control valve and final valve were energized. This resulted in the introduction of odor vapor into the carrier stream and the diversion of that stream to an exhaust line. The final valve was de-energized 0.1–1.5 s later, thus reintroducing the carrier stream (now containing the odor) into the odor sampling tube. The odor control valve was de-energized 1 s later and a 2 s clock was initiated (response area interval). Reinforcement was delivered if the mouse sampled the odor for at least 0.1 s and then licked at the water delivery tube during the response area interval. The operation time of the final valve was progressively increased from 0.1 to 1.5 s over 15 trials. Thus, in the final part of this stage of training, reinforcement was contingent upon the mouse inserting its snout into the sampling tube, keeping it there during the final valve period (1.5 s), sampling the stimulus for at least 0.1 s and then licking at the water tube. Responding on the water tube before the end of the final valve operation (responding before the odor was presented) or not sampling the odor for at least 0.1 s after relaxation of the final valve resulted in the trial being aborted and the initiation of a 5 s ITI. At the end of this training the mouse reliably initiated trials, sampled the odor stimulus and promptly responded on the water delivery tube.

The S– stimulus was introduced in the next session. Clean air served as S– and the vapor from a 1% aqueous solution of ethyl acetate served as the S+ stimulus. The first 40 trials of this session were identical to the last trials given in stage 3 (i.e. only S+ presentation trials were given). If the mouse did not respond appropriately on at least 85% of these trials, the session was terminated and the mouse was given additional training using only S+ trials. Otherwise, the session was continued for 100 or 200 trials using a mixture of S+ and S– trials. The S+ and S– trial procedures were identical except for the stimulus presented and the absence of reinforcement for S– trial responses. The two types of trials were presented in a modified random order such that an equal number of each type occurred in each block of 20 trials and that one type of trial did not occur more than three times consecutively. Not responding on an S+ trial (miss) or responding on an S– trial (false alarm) were scored as errors. Responding on an S+ trial (hit) and not responding on an S– trial (correct rejection) were scored as correct. Trials that were aborted because the animal did not sample the stimulus for
at least 0.1 s (short sample trials) or because the mouse responded on the reinforcement tube during the final valve period (FV aborts) were scored separately. The trial procedures are shown diagrammatically in Figure 2. Accuracy was scored for each block of 20 trials. Successful completion of this session terminated the initial training procedures. Subsequent training used the same general trial procedures but the S+ only trials at the beginning of the session were not used.

**Odor detection threshold**

Two mice (M1 and M2) were trained in the air dilution olfactometer to detect successively lower concentrations of ethyl acetate. A minimum of 100 trials was given on each concentration. If the mouse achieved criterion performance of 90% correct responding in a block of 20 trials within the session, the concentration of the S+ stimulus was reduced in the next session. If criterion performance was not achieved, training on that concentration was continued until criterion...
performance was obtained or for a maximum of 200 trials. Generally, each mouse was given a single 100-trial session each day. In each session, ethyl acetate vapor served as the S+ stimulus and clean air served as the S– stimulus. The concentrations of ethyl acetate used in these tests were 1, 0.1, 0.01, 0.001, 0.0001 and 0.00005% (of vapor saturation at 20°C).

**Odor discrimination tasks**

The two mice trained on the odor threshold task and two additional mice (M3 and M4) were trained on a series of two-odor discrimination problems using the eight-channel olfactometer. Each mouse was given a minimum of 200 trials on each of the following tasks:

- **Task 1:** S+ was 1% ethyl acetate and S– was clean air.
- **Task 2:** S+ was 1% citral and S– was 1% cineole.
- **Task 3:** S+ was 1% toluene and S– was 1% benzene.
- **Task 4:** S+ was 1% butyl acetate and S– was 1% amyl acetate.
- **Task 5:** S+ was a 50% aqueous solution of REACH ACT cinnamon-flavored mouth wash (Johnson and Johnson) and S– was a 25% aqueous solution of REACH ACT bubble gum-flavored mouth (Johnson and Johnson).

If the mouse did not achieve criterion performance of 90% correct responding in two 20-trial blocks, training was continued in daily 200-trial sessions until this criterion was achieved. The odorants for tasks 1–4 were diluted in odorless mineral oil.

- **Task 6:** eight-odor discrimination.

Upon completion of tasks 1–5, each mouse was given additional training in which the eight odors used in tasks 2–5 were presented in a single 320-trial session. Stimuli were presented in a modified random order such that within each block of 40 trials each S+ and S– stimulus was presented 5 times. Mice were given 3–4 sessions on this problem.

- **Task 7:** Partial reinforcement.

To increase resistance to extinction in preparation for a memory test, mice were given 2–3 additional sessions on the eight-odor task but with the probability of reinforcement for correct responding on S+ stimuli reduced to 0.5.

- **Task 8:** Memory test.

After completing task 7, mice were maintained on their 1 ml/day water deprivation schedule for 32 days in their home cages. No water was given on day 31 and, on the next day, each mouse was given a 80-trial memory test on the multiple eight-odor problem. In this session no reinforcement was given for correct responses, hence the mouse had no feedback for correct or incorrect responses.

- **Task 9:** Odor mixture.

Two mice were trained to discriminate 1% ethyl acetate from 0.05% amyl acetate for 100 trials. Beginning on trial 101, the S– stimulus was switched to an equal parts vapor phase mixture of 1% ethyl acetate and 0.05% amyl acetate. This was accomplished by operating the valves controlling these two odors on S– trials. To insure that total airflow could not be used as a cue for correct responding, on S+ trials the S+ odor control valves and those controlling a clean air channel were operated.

**Odor concentration**

Odor concentrations generated by the air dilution olfactometer are expressed as percent of vapor saturation at 20°C and are the concentrations delivered to the mouse. For the eight-channel olfactometer the concentration of the head space above the diluted odorant is not known, hence concentration is reported as the percent dilution of the odorant material. The odor vapor, generated by passing 50 cm³/min over the surface of the odorant material, was manifolded with a 1950 cm³/min stream of clean air before being introduced to the sampling port. Thus, the concentration of all odor stimuli experienced by the mouse at the sampling port of the multiple odor system was 2.5% of the concentration of the headspace above the liquid odorant.

**Results**

- **Initial training**

Mice required 3–8 sessions on the initial training task before they would reliably insert their snout in the sampling port, keep it there until the odor appeared, sample the odor for at least 0.1 s and then respond on the water delivery tube. Most of the problems encountered in this stage of training resulted from mice not being sufficiently motivated and therefore completing only part of the training session. This was resolved by allowing mice only the water they earned in the initial training sessions. After 1–3 days of earning only 0.2–0.4 cm³ of water, mice were clearly more strongly motivated and would complete the entire initial training procedure in 1–2 sessions. However, in contrast to rats, even well-motivated mice engaged in many bouts of exploratory behavior and these probably disrupted the training sequence.

- **Ethyl acetate threshold**

As shown in Figure 3, the performance accuracy of both mice tested on the ethyl acetate concentration series was 90% or higher on all but the last (0.00005%) detection task.
Maximal accuracy on this last concentration was 75% for both mice in 400 training trials. M2 was not tested further on this task but M1 achieved scores of 80–90% correct responding in 200 additional training trials (not shown in Figure 3). Thus, detection threshold was below 0.00005% of vapor saturation (or less than 4.1×10^{-10} M).

Odor discrimination

Each mouse showed rapid acquisition of each of the four two-odor discrimination tasks (Figure 4). Note that in each case the performance accuracy dropped to chance or near chance levels when a novel pair of odors was introduced and that, in almost all cases, the new odor discrimination was acquired in a single training session. After acquiring each of the four two-odor discriminations, performance accuracy remained high when all eight odors were presented in random order within the same session (Figure 4, eight-odor task). Indeed, in the very first block of trials on the eight-odor task, the lowest accuracy score was 85% (Figure 4, mouse M1). Moreover, there was no disruption of performance when reinforcement probability was reduced to 0.5 (Figure 4, 8-Odor, 50% RF).

Odor memory

When tested under extinction after a 32 day rest period, performance accuracy for each mouse was 90% or higher (Figure 4, Memory Test). Thus, each of the four mice showed essentially perfect retention for each element of the eight-odor discrimination task.

Odor mixture

The two mice trained with ethyl acetate and amyl acetate acquired the discrimination by the second block of trials (Figure 5). Performance dropped to chance levels when the S– stimulus was abruptly shifted to a vapor phase mixture of the two acetates, but both mice rapidly acquired the new task (Figure 5). All errors made during this session were false alarms.

Control tests

To assess whether the system generated non-vapor cues that could be used for discriminative responding, it was washed and three mice were given a single 200-trial session in which one channel was designated as S+ and another as S–. Saturator bottles in both channels contained deionized water. Both mice performed at chance (40–60%) in each block of 20 trials. Initially, mice did not respond on either the S+ or the S– trials. After being reinforced by hand on several S+ trials, they began responding and then responded on all trials.
Stimulus sampling

Stimulus sampling on each trial was defined as the time the mouse's snout interrupted the photobeam during the 1 s stimulus period. Mean stimulus sampling time per trial over all trials for the four mice was 0.62 s. However, sampling time varied as a function of response accuracy and type of trial (S+ or S–). Figure 6 shows percent correct performance and average sampling time on S+ and S– trials on each block of 20 trials for the first and fourth two-odor discrimination task for three mice. The figure illustrates a pattern that was observed during the acquisition phase of almost all of the two-odor discrimination tasks for each mouse. Initially, when performance accuracy was at chance levels, the time spent sampling on the S+ and S– trials was essentially identical. As performance accuracy increased, mice spent more time sampling the S+ stimulus than the S– stimulus. During criterion performance, mean sampling time was 0.71 s on S+ trials and 0.52 s on S– trials.

Discussion

The present results demonstrate that mice are readily trained on odor detection and discrimination tasks using a go, no-go discrete trials operant conditioning procedure. Like rats trained using similar methods, mice learn to respond by licking on the water delivery tube when presented with odors associated with reinforcement (S+ stimuli) and to inhibit responding when presented with odors not associated with reinforcement (S– stimuli). In general, our results serve to confirm and extend the findings of Walker and O’Connell (Walker and O’Connell, 1986) that mice can be trained in an automated olfactometer and can perform at high levels of accuracy on a simple odor detection task.

Under optimal conditions of motivation, initial training on the discrete trials operant task could be completed in one or two 1 h sessions. However, most mice required 3–4 such training sessions. Still, learning was faster than that in the Walker and O’Connell study, where approximately twelve 1 h training sessions were required before a regular S+/S– trials procedure was introduced. Walker and O’Connell used a 30 s ITI and a 1 min time-out punishment for incorrect responding, and required their mice to make a relatively complex sequence of responses on each trial. In general, our experience with the four mice reported on here and with 10 additional mice indicates that mice require about twice as much training as do rats to acquire the basic operant repertoire of initiating a trial, sampling the odor and responding. This may reflect the inadequate control of the motivational level and/or the use of trial parameters (e.g. ITI, type of reinforcement, minimum required sampling time) that were devised for the rat but may not be optimal for mice.

Once mice had completed their initial training, further acquisition of odor detection and discrimination tasks was efficient. With two exceptions, their performance on these tasks was similar to that we have reported for rats. The exceptions concern the time required to complete a 200-trial
session and the pattern of sampling on S+ and S– trials. Adult male rats maintained on a 10 ml/day water deprivation schedule are highly efficient and complete a 200-trial session in 40–55 min. Thus, on average, they complete a trial every 12–15 s. Because ~8 s of each trial period is fixed (ITI, stimulus presentation and response time), rats spend, on average, only ~4–7 s after the end of the ITI before initiating the next trial. In contrast, mice often engage in a long bout of exploratory behavior during the ITI and hence take 90 min or more to complete a 200-trial session.

The two species also differ sharply in their patterns of odor sampling. They both sample the stimulus, on average, for ~0.5–0.6 s/trial but they differ with regard to amount of time spent sampling the S+ and S– stimuli at asymptotic levels of performance. When performance accuracy is at chance levels (in initial trials of a new discrimination task or in acquiring a particularly difficult task), sampling time on S+ and S– trials is essentially identical for both species. But, as response accuracy increases, rats spend more time sampling on S+ trials while sampling on S– trials either decreases or remains unchanged (Slotnick, 1990). Indeed, the increase in S– sampling time often precedes an increase in accuracy and, for rats, may provide a particularly sensitive index of odor detection or discrimination (Slotnick, 1990). The opposite results were observed for most mice we have tested: as accuracy improved, mice increased their sampling of the S+ stimulus while sampling on S– trials decreased or changed in an irregular fashion. In almost all sessions with mice, high levels of performance accuracy were accompanied by longer S+ than S– sampling. It is unclear why rats and mice differ in their sampling patterns, but its occurrence suggests that the two species may employ rather different strategies in identifying the stimulus.

In most other respects, including odor sensitivity, acquisition functions on two-odor discriminations, ability to maintain high levels of accuracy when multiple S+ and S– stimuli are presented within a session and their memory for such odors, the performance of mice appears comparable to that of rats. The absolute detection threshold for ethyl acetate is similar to that reported by Apfelbach et al. (Apfelbach et al., 1991) for rats trained using a similar trials procedure and odor generator. In the Apfelbach et al. study, the mean threshold for seven 140- to 360-day-old adult rats was 0.000014% of vapor saturation. Of the two mice tested with ethyl acetate in the present study, both were able to detect the odor at 0.00005% (Figure 3). Had we used the more extensive training procedures and sequential dilution steps of 0.1–0.2 log units employed by Apfelbach et al., it is likely that the mouse would prove as sensitive as the rat for this odor. In the Walker and O’Connell study (Walker and O’Connell, 1986) mice were given extensive training (~30 sessions) on n-amyl acetate using ascending, descending and staircase psychophysical procedures and step dilutions of 0.5 log units. The threshold obtained for a 75% accuracy criterion was ~10^{-12} M, which is among the lowest values reported for macrosmatic mammals tested with this odor (Slotnick and Schoonover, 1984). Of course, cross-species comparisons are difficult to make: a standard procedure for animal olfactory psychophysical testing has not been established, and methods for generating stimuli and training animals vary widely across different studies (Passe and Walker, 1985). Nevertheless, on the basis of our experience, we are in agreement with Walker and O’Connell’s conclusion that the olfactory sensitivity of the mouse is similar to that of the rat.

The performance of mice on odor discrimination tasks is also similar to that of rats. In almost all cases, mice acquired each of the four two-odor discrimination problems within a single training session and often within the first 40–80 trials. On average, mice made somewhat more errors than did rats trained on these same tasks (N. Bodyak and B. Slotnick, unpublished data), but, once each task had been acquired, retention for these discriminations was excellent. Thus, when the pairs of odors used in these tasks were presented in random order within a single session, a high level of accuracy was evident in the very first block of trials (Figure 4). That each mouse responded almost without errors when tested under extinction on these odors after a 32 day rest period further attests to an excellent and long-term odor memory.

In summary, no significant problems were encountered when mice were trained using an apparatus and test procedures that have been used successfully with rats. Mice took more time than rats to complete odor detection and discrimination tasks but their odor sensitivity, acquisition of two-odor discrimination tasks and memory for odors were all quite comparable to those obtained in rats in a variety of studies. The present results, together with those of Walker and O’Connell, demonstrate the feasibility of using automated test procedures for detailed studies of olfaction in mice. These procedures should prove useful in studying olfactory function in gene-targeted strains and those with specific abnormalities of the olfactory system.

References


Accepted June 4, 1999