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Odor-Sampling Time of Mice under Different Conditions

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Abstract

Response accuracy and odor sample times on positive (S+) and negative (S−) trials were recorded for mice trained on a variety of go, no-go odor detection and discrimination tasks. Odor sample time was relatively stable over extended training on the same task, increased during acquisition of difficult tasks, relatively insensitive to reinforcement magnitude, and, in some cases, provided more information regarding task difficulty and discrimination than did response accuracy. Mice generally sampled longer on S− trials in simple odor detection tasks but longer on S+ trials in odor discrimination tasks.

Key words: odor detection, odor discrimination, odor-sampling time, olfactometer

Introduction

A number of reports demonstrate that rodents are able to detect odors and discriminate between odors within a few hundred milliseconds (Slotnick 1990; Bodyak and Slotnick 1999) and, perhaps, in a single sniff (Uchida and Mainen 2003; Abraham et al. 2004; Rinberg et al. 2006). These results serve to emphasize the critical role of very early events in olfactory processing for understanding odor coding (Mainen 2006; Spors et al. 2006).

Odor-sampling time measures in the behaving rodent have been made using an olfactometer together with operant conditioning in which correct responses are rewarded. Although 3 detailed studies (Uchida and Mainen 2003; Abraham et al. 2004; Rinberg et al. 2006) have examined odor-sampling time in rodents, only mean values after extensive training were reported, and it is unclear, for example, how odor sampling may change during acquisition of simple or difficult odor detection and discrimination tasks. A closely related issue is whether a 2–response choice discrimination method or the more traditional go, no-go discrimination task may be better suited to assess changes in sampling time as a function of discrimination difficulty (Friedrich 2006). The present report examines the influence of several parameters of odor presentation and training tasks on odor sampling. The go, no-go discrimination method of Bodyak and Slotnick (1999) is used because it yields sampling time data on both positive and negative trials while maintaining the simplicity and advantages of this training procedure (Slotnick and Schellinck 2002; Slotnick and Restrepo 2005).

Materials and methods

Subjects

Eighteen albino male CF-1 strain Carworth Farm mice were housed in groups of 4 in plastic cages in a temperature- and humidity-controlled vivarium. A water restriction schedule of 1–2 ml/day begun 10 days prior to training and continued throughout the study served to maintain weights at 85–88% of prerestriction baseline weights. All experimental procedures were approved by the University of South Florida Institutional Animal Care and Use Committee.

Apparatus

Six identical Knosys LD8-1 mouse olfactometers were used. The units were similar to those described in detail by Slotnick and Restrepo (2005) except that the operant chamber was modified by inserting a water delivery tube through the front panel of the chamber wall. This tube was located 35 mm to the right and at the same height as the nose poke port of the odor-sampling tube (Figure 1). The water delivery tube within the glass odor-sampling tube was left in place but was not used. All trial procedures were computer controlled via a digital interface, and contacts between the stainless steel floor of the operant chamber and the water delivery tube were detected using a ''drinkometer'' circuit (Slotnick and Restrepo 2005).

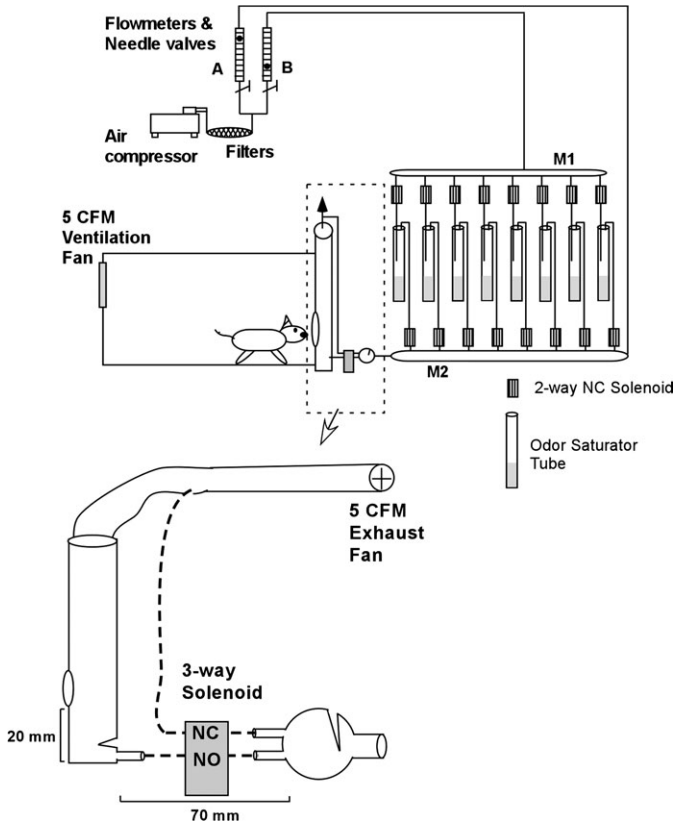

Figure 1 Schematic diagram of the 8-channel liquid dilution olfactometer.

Except where noted, odors were generated by adding a 50-cc/min stream of air from the headspace of a 240-ml PVC odor saturator bottle containing 10 ml of liquid odorant to a 1950-cc/min stream of clean air. As shown in Figure 1, the mixing of odorized and clean air in the manifold was enhanced by creating turbulence by a glass protrusion at the input of a 25-mm circular tube. The 2 output lines of the circular tube were connected to the normally open and normally closed ports of a 3-way pinch valve (final valve), respectively. The normally open port of the valve connected directly to the mouse odor-sampling tube and the normally closed port connected to an exhaust line. A glass protrusion in the base of the odor-sampling tube produced additional turbulence in the air stream.

Odorants were purchased from Sigma or Fisher and were the highest purities available. Odorants were diluted (v/v) with deionized water. Odor concentrations given in the text are those of the liquid dilution. Because odors were generated by mixing a 50-cc/min stream of air from the headspace of the odor saturator bottle with a 1950-cc/min stream of clean air, the odor concentration experienced by the mouse was 2.5% of the headspace concentration above the liquid odorant.

The total volume between the end of the normally open port of the final valve and the position of the mouse's nose when inserted into the sampling port was approximately 6.2 ml. Assuming plug flow, the calculation (time = volume/rate) would result in a delay of 186 ms from the time the final valve terminated to when the odor stream reached the odor-sampling position of the mouse's nose. However, it is unlikely that plug flow can be assumed and other factors, including the response time of the valve, turbulence, and the possibility that wall friction may result in a higher velocity in the center than in the periphery of the air stream, complicate calculation of how long it took for the stimulus to reach the sniff port opening.

Water delivery for correct responding on S+ trials was produced by operation of a 2-way solenoid in the tubing connecting a water reservoir to the water delivery tube. The height of the reservoir, diameter of a stainless steel flow restrictor tube within the tubing pathway, and operation time of the solenoid were adjusted to produce a volume of 3 μl. For one experiment described below, this volume was altered during a test session by changing the operation time of the solenoid.

Initial training

In general, training methods followed those described by Bodyak and Slotnick (1999). In brief, the mouse was first rewarded for licking on the water delivery tube, then for nose pokes into the odor-sampling tube, and, finally, for keeping its snout in the odor-sampling tube until an odor was presented. Operation of the final valve was introduced during this last stage of training, and valve operation time was gradually increased to 1 s over a series of 160 trials. A 5% aqueous solution of ethyl acetate (EA) provided the odor stimulus for this initial training. Training was considered complete when, after a 4-s intertrial interval, the mouse reliably inserted its snout into the odor-sampling port, remained in place during the operation of the final valve, and then withdrew and licked at the water delivery tube. All mice completed this training sequence in two or three 30-min sessions. By the end of initial training, mice had learned to insert their snout into the odor-sampling port, wait until the EA stimulus was detected, and then move to and lick at the water delivery tube. Thus, training afforded mice ample opportunity to associate the delivery of an odor with delivery of water prior to further training on odor detection and discrimination tasks.

Detection and discrimination training

In each session, one odor served as the S+ stimulus and another odor (or headspace vapor from the water solvent) as the S− stimulus. The first nose poke into the odor-sampling port after a minimum intertrial interval of 4 s initiated a trial. S+ and S− stimuli were presented in a modified random order such that there were an equal number of each in each block of 20 trials and that one stimulus was not presented more than 3 times successively.

On each trial, odor generation was accomplished by operating the upstream and downstream valves of the selected odor channel and the 3-way valve (final valve). This resulted in the mixing of the odor stream and clean air stream and having that stream directed to an exhaust path. The final valve operated for a random period varying from 0.8 to 1.2 s, and the odor control valves were turned off 1 s after the final valve terminated. Thus, upon trial initiation, the mouse experienced a sudden cessation of air flow in the odor-sampling port and then, upon termination of the final valve, a sudden onset of a stream of odorized (or nonodorized) air in the odor-sampling tube. If the mouse withdrew its snout from the odor-sampling tube within 50 ms after termination of the final valve, the trial was immediately terminated, the intertrial interval was initiated, and this trial type was repeated in the next trial. Such short samples accounted for less than 5% of trials and are not further considered in the data analysis. Stimulus sample time was defined as the interval between termination of the final valve and the first detection of the photo beam being unbroken. Although the mouse could withdraw its snout from the odor-sampling port and then return to sample the odor again within the 1-s odor-on period, such behavior was not observed. A second response measure, the interval between withdrawal from the odor sample port and the first contact with the water delivery tube, was also recorded and defines response time. A response time of 2 s was recorded if the mouse failed to respond. Also measured was the time to complete each block of 20 trials. Mean sample times over each 10 S+ and each 10 S− trials in each block of 20 trials and the percent correct responding in each block of 20 trials were the basic dependent variables used in the data analysis.

The first session after initial training, detection of 5% EA, differed from all remaining tasks in that, prior to presenting the sequence of S+ and S− trials, a series of 20 S+ trials were given. This served to insure that the operant sequence of nose poke and responding on the water delivery tube was intact prior to initiating the detection task. Mice that failed to respond correctly on more than 3 of these S+ only trials were given an additional session on the initial training program.

With a few exceptions, mice were given a 100- to 200-trial session using standard trial parameters to discriminate 0.5% EA from the water solvent (standard condition [SC]) prior to being tested on an experimental manipulation. Performance on the SC test was used to evaluate potential changes produced by the experimental manipulation.

Unless otherwise indicated, the data for the manipulations described below were based on mean values of all 18 mice. Because of occasional procedural errors and because some mice did not complete a session, data were available for 12–16 mice in some cases, and for the results shown in Figure 4D,F, only 10 mice were used. Within-group *t*-tests were used to compare performance within or between different conditions.

Results

Initial acquisition in detecting 5% and 0.5% EA

Each mouse was given four 100-trial sessions in which 5% EA served as S+ and water as S−. The first session on this task constituted the first introduction of an S− stimulus for these mice. Sixteen mice achieved criterion responding within the first session and the remaining 2 on the first blocks of the second session. Mean sample time on S+ trials, initially 347 ms in the first 100-trial session, gradually increased, and mean sample time in the last 100 trials was 392 ms ($P < 0.04$, Figure 2A). The Pearson product moment correlations between accuracy and S+ sample time across the twenty 20-trial blocks of trials was 0.85. Sample time on S− trials was consistently greater than S+ sample time and tended to decrease slightly but not significantly as performance accuracy improved.

A 1–log unit decrease in odorant concentration in session 5 produced an initial modest decrease in accuracy and an initial increase in both S+ and S− sampling times (Figure 2B). Sampling time gradually decreased across sessions, and mean sample time in the last session (415 ms) did not differ from that on the last session of the 5% EA detection task (443 ms). Initially, sample times on S− trials (515 ms) were higher than on S+ trials (452 ms, $P < 0.005$) but decreased, and in the last 2 sessions of the 0.5% EA detection task, sample times on the 2 types of trials did not differ significantly. The correlation between accuracy and S+ sample times (0.42) was more modest than that obtained in the initial acquisition of the EA detection task.

Effects of altering flow rates on sample time

The effects of altering flow parameters were examined in the next set of sessions in which the S+ stimulus was 0.5% EA

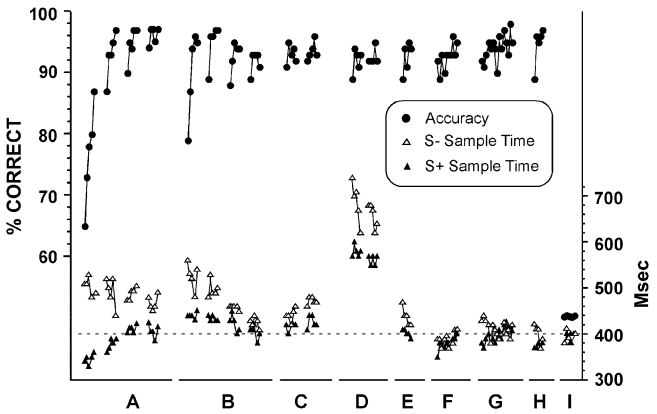

Figure 2 Response accuracy and odor sample times on S+ and S− trials. For response accuracy, each data point is the mean of 20 trials. For S+ and S− odor-sampling times, each data point is the mean of 10 trials. A, 5% EA (S+) versus water solvent. B–H, 0.5% EA (S+) versus water solvent. C, 50% increase in flows (see Results). D, 50% decrease in flows (see text). F, differential reinforcement for short sample time. G, 5× increase in reinforcement volume. I, no cues test, 0.5% EA in the S+ and S− channels.

and water served as the S− stimulus. Increasing both the odorant and clean air flows by 50% had little effect on odor-sampling time; indeed, sampling times on S+ and S− trials increased slightly (Figure 2C), but sample time on the second session of the high-flow condition did not differ from that on the last sessions of the 5% and 0.5% EA detection tasks in which standard flows were used.

The high-flow manipulation was examined again later in the test series (data not shown). Mean S+ sampling time in that session was somewhat lower (357 ms) than in prior sessions but not significantly lower than baseline sampling time on the immediately preceding SC test (mean 377 ms).

Decreasing flows by 50% produced a marked increase in S+ sampling and an even greater increase in S− sampling but no change in response accuracy (Figure 2D). Mean S+ sampling time (572 ms) represents a 43% increase from the approximate 400-ms S+ sampling time characteristic of terminal performance on the preceding 5% and 0.5% EA detection task at the standard flow rate. Mean S− sampling time in the low-flow tests was significantly greater than that for S+ ($P < 0.001$) and decreased sharply within each low-flow test session (Figure 2D). Recovery to the approximately 400 ms odor sample time occurred in the subsequent session on the 0.5% detection task using standard flows (Figure 2E).

Differential reinforcement of short sample times and increasing reinforcement magnitude

Mice were given a 200-trial SC session in which the number of reinforcements obtained for responding on an S+ trial in trial blocks 3–10 was contingent upon deviation from the mean S+ sample time calculated from the first 2 blocks of trials. On each correctly completed S+ trial in trial blocks 3–10, one reinforcement was given if sample time was equal to or not greater than 75 ms of mean S+ sample time. S+ sample times that were 25–49, 50–74, or more than 75 ms less than mean S+ sample time were reinforced with 2, 3, or 4 water deliveries, respectively. Trials on which sample times were more than 75 ms above mean sample time were not reinforced.

Differential reinforcement of sample time had no marked effect on performance accuracy or on S+ or S− sample times (Figure 2F). Indeed, sample times tended to increase slightly over the course of the session.

Because differential reinforcement of short sample times could have no effect if mice made little or no contact with the sampling time/reinforcement contingency, the outcomes were reanalyzed with regard to the number of extra reinforcements obtained in each block of 20 trials. The 16 mice tested obtained an average of 29.6 extra reinforcements for fast responding (range 2–64). The results of the 4 mice with scores closest to the 29.6 group mean were discarded, and the remaining mice were divided into 2 subgroups: group Hi, ($n = 6$) with the highest number of extra reinforcements (mean 47.2, range 36–64), and group Low ($n = 6$), with the lower number of extra reinforcements (mean 12.2, range 2–18). Mean S+ sample times over the 8 blocks of trials during which differential reinforcement was in effect was 413 ms for group Hi and 358 ms for group Low ($P > 0.1$). However, with regard to the change in S+ sample times from the first 2 blocks of trials in the session (prior to differential reinforcement for fast sampling), the mean sample time of group Hi decreased by 21 ms and that of group Low increased by 52 ms ($P < 0.01$). Thus, differential reinforcement for fast sampling on S+ trials produced, for those mice that made more contact with the contingency, a small but reliable decrease in sample time.

The effect of increasing reinforcement volume on sample time was also assessed by abruptly increasing reinforcement size from 3 to 15 μl in the third block of trials during a 0.5% EA detection task (Figure 2G). The increase in reinforcement magnitude had no significant effect on odor sample time. Indeed, there was a small increase in S+ sample times over the course of this session.

Sample time and extended training on simple detection and discrimination tasks

To determine whether extended training on simple tasks would produce a progressive decrease in sample time, mice were given two 400-trial sessions in which S+ was 0.5% EA and S− was the water solvent (1 session per day). This was followed by three 400-trial sessions on a simple discrimination task in which 0.1% pyridine served as S+ and 0.1% benzaldehyde served as S−. The 2-odor discrimination task was preceded by 3 blocks of trials in which the S+ and S− stimuli were 0.1% pyridine and water, respectively. Mice maintained high levels of accuracy on the EA detection task, and as shown in Figure 3A, extended training resulted in a slight but not sustained or significant decrease in sampling time. Differences between mean sampling time on S+ and S− trials in the first session (396 and 400 ms, respectively) and in the second session (370 and 384 ms, respectively) did not differ ($P > 0.1$). Changes in S+ or S− sample time over the first or second session did not vary with accuracy (all product moment corrections were less than 0.2). Of the 12 mice trained in this manipulation, 4 had mean S+ sample times less than 300 ms, and among these mice, S+ sample times on several blocks of trials were less than 250 ms. However, neither group means nor inspection of individual animals revealed a trend for sample time to decrease with continued training on this simple detection task.

A different pattern in the dynamics of sample time occurred during training on the novel 2-odor discrimination (Figure 3B1). Mice acquired the pyridine detection task within the first 2 blocks of trials (Figure 3B) and continued to perform at high levels of accuracy when 0.1% benzaldehyde replaced water as the S− stimulus (Figure 3B1). In contrast to their prior performance on the EA detection task, the new detection and discrimination tasks resulted in a sharp

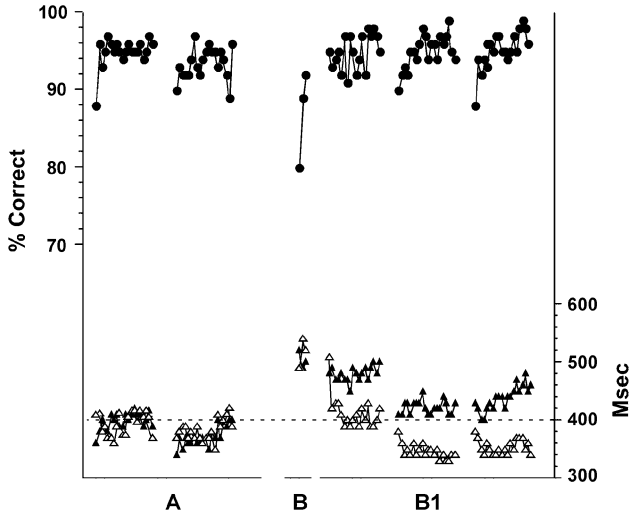

Figure 3 Response accuracy and odor sample times on S+ and S− trials. For response accuracy, each data point is the mean of 20 trials. For S+ and S− odor-sampling times, each data point is the mean of 10 trials. A, 5% EA (S+) versus water solvent (S−), extended training. B, 0.1% pyridine (S+) versus water solvent. B1, 0.1% pyridine versus 0.1% benzaldehyde, extended training.

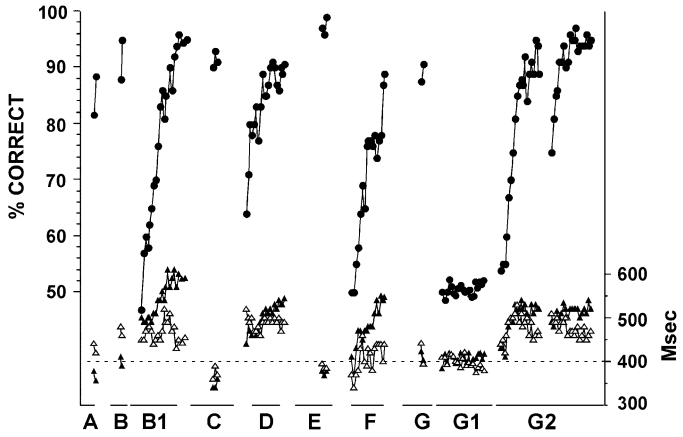

Figure 4 Response accuracy and odor sample times on S+ and S− trials. For response accuracy, each data point is the mean of 20 trials. For S+ and S− odor-sampling times, each data point is the mean of 10 trials. A, C, and E: 0.5% EA (S+) versus water solvent (S−). B, 0.1% propyl acetate (S+) versus 0.1% amyl acetate (S−). B1, 0.1% propyl acetate (S+) versus mixture of 99 parts of 0.1% propyl acetate plus 1 part of 0.1% amyl acetate (S−). D, 0.5% EA (S+) versus 0.375% EA (S−). F, 0.0005% EA (S+) versus water solvent (S−). G, 0.1% propyl acetate (S+) versus 0.1% valeric acid (S−). G1, 0.1% propyl acetate (S+) versus mixture of 99 parts of 0.1% propyl acetate and 0.1 parts of 0.1% valeric acid (S−). G2, 0.1% propyl acetate (S+) versus mixture of 80 parts of 0.1% propyl acetate and 20 parts of 0.1% valeric acid.

increase in sampling time and consistently higher sample times on S+ than on S− trials in each session on this task ($P < 0.03$, each comparison), and extended training on the discrimination was not accompanied by a progressive decrease in sample time.

Effects of increasing detection and discrimination difficulty

Task difficulty was abruptly changed in 4 experiments. The first 3 manipulations produced the same pattern of changes: an initial decrease in accuracy and a progressive increase in sampling time as accuracy improved. In the first such manipulation, mice were given 2 blocks of trials in which 0.1% propyl acetate served as S+ and 0.1% amyl acetate as S− (Figure 4B). In the next 20 blocks of trials, a mixture of 99 parts of 0.1% propyl acetate and 1 part of 0.1% amyl acetate served as S−. As shown in Figure 4B1, introduction of the mixture S− stimulus produced an immediate decrease in response accuracy and, even within the first 2 blocks of trials, an abrupt increase in S+ sampling time ($P < 0.01$). Discrimination accuracy and S+ sampling time gradually increased over the remainder of the session. The product moment correlation between accuracy and S+ sampling time over these 17 blocks of trials was 0.88. S− sampling time increased initially but was more variable.

The second manipulation was an intensity discrimination task: after 4 blocks of training under SCs (S+, 0.5% EA; S−, water; Figure 4C), the S− stimulus was changed to a 0.375% solution of EA. This resulted in a more modest decrease in accuracy and an increase in S+ and S− sample times (Figure 4D). As in the mixture discrimination task, S+ sample time gradually increased as performance accuracy increased.

In the third manipulation, water served as the S− stimulus in all trials, but the S+ stimulus was changed from 0.5% to 0.0005% EA on the fourth block of trials. Changes produced by this sudden decrease in odorant concentration (Figure 4F) were essentially identical to those obtained in the mixture discrimination task (Figure 4B1).

The fourth manipulation was an attempt to replicate the propyl acetate/amyl acetate mixture task (Figure 4B1) but using 2 unrelated odorants: propyl acetate and valeric acid. Mice were first given 2 blocks of trials in which 0.1% propyl acetate served as S+ and 0.1% valeric acid served as S− (Figure 4G). In the next 18 blocks of trials, a mixture of 99 parts of 0.1% propyl acetate and 1 part of 0.1% valeric acid served as S−. Mice rapidly acquired the propyl acetate versus valeric acid discrimination (Figure 4G), but contrary to expectations, all mice failed to acquire the mixture task within the 360 trials allowed and neither accuracy nor sample time increased over the course of the session (Figure 4G1). Training was continued in two 400-trial sessions in which 0.1% propyl acetate served as S+ and a mixture of 80 parts of 0.1% propyl acetate and 20 parts of 0.1% valeric acid served as S−. Twelve of the 18 mice achieved criterion performance on this task in the first session, and the performance of these mice is shown in Figure 4G2. As shown, odor sample time increased during the acquisition phase of the session and remained elevated in the second session on this task.

Of the 6 mice that performed largely at chance on the 80/20-mixture task, only 2 improved but still did not achieve criterion performance in the second session. Interestingly, despite near-chance performance, differences between sampling

time on S+ and S− trials gradually emerged, and sample time on S+ trials increased in both sessions (Figure 5A,A1). Differences between S+ and S− sample times in the last 5 blocks of trials in the first session (Figure 5A) and between mean sample times over all blocks of session 2 (Figure 5A1) were significant ($P < 0.01$).

Variability in odor sample time

The mean standard deviation (SD) for odor sample time over all S+ and S− trials for all problems was 101 ms. Variability in sampling time was lowest for the 0.5% EA detection task and decreased with repeated presentations of the task. Thus, mean SDs for sample time on the first and last sessions on the EA detection task were 104 and 80 ms, respectively ($P < 0.04$).

Relative to the preceding SCs test (0.5% EA detection task), variability in mean sample time increased on each introduction of a new detection or discrimination task. Thus, from the preceding SC test to the test with low air flows, SD increased from 101 to 153 ms ($P < 0.005$). Similar increases in variability of sample time occurred with the introduction of each of the discrimination tasks.

For simple or well-practiced tasks, that is, for those in which performance accuracy was relatively high in all trial blocks, variability in odor sampling across the session was not related to accuracy: the mean product moment correlation between accuracy scores and SDs of odor-sampling time for such sessions was 0.19 (range 0.1–0.3). However, on more difficult tasks, those resulting in a clear acquisition function, variability in odor sampling tended to decrease as performance improved. Thus, product moment correlations between SDs of mean odor sample time and accuracy scores across the blocks of trials on the amyl acetate/propyl acetate odor mixture discrimination (Figure 4B1), the EA intensity discrimination (Figure 4D), and the low-concentration EA detection task (Figure 4F) were −0.61, −0.81, and −0.65, respectively.

Response and trial times

Response time and trial time were monitored in most tasks, and typical results are shown for two 400-trial sessions on the 0.5% EA detection task (Figure 6). Response time was defined as the time between withdrawing from the odor sample port and making the first contact on the water delivery tube. Because mice performing at high levels of accuracy generally did not respond on the water delivery tube on S− trials, response times on S− trials were consistently high and are not further considered. As shown in Figure 6, mean response times on S+ trials were consistently shorter than sample times on either S+ or S− trials. On the familiar 0.5% EA detection task, response times were reasonably stable (Figure 6) and did not change progressively in one direction over the course of each session.

Trial time was the time taken to complete a block of 20 trials and, hence, yielded a measure of the average rate of initiating trials. Three of the 12 mice used in this analysis stopped working for 5 or more minutes during the session, and because these high and nonrepresentative total time scores unduly influenced both means and medians, they were not used in the analysis. As shown in Figure 6, except for characteristic longer trial times in the first 1–3 blocks of trials in each session, there was no progressive increase or decrease in the rate of initiating trials over the sessions on the 0.5% EA

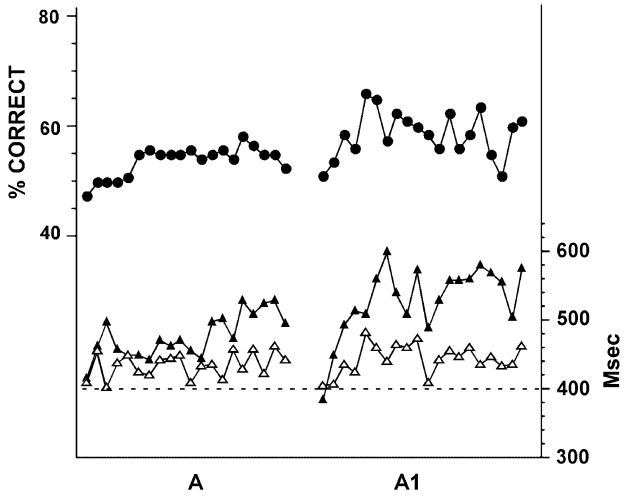

Figure 5 Response accuracy and odor sample times on S+ and S− trials over 2 training sessions (A and A1) for 6 mice that failed to discriminate between 0.1% propyl acetate (S+) and a mixture of 80 parts of 0.1% propyl acetate and 20 parts of 0.1% valeric acid. For response accuracy, each data point is the mean of 20 trials. For S+ and S− odor-sampling times, each data point is the mean of 10 trials.

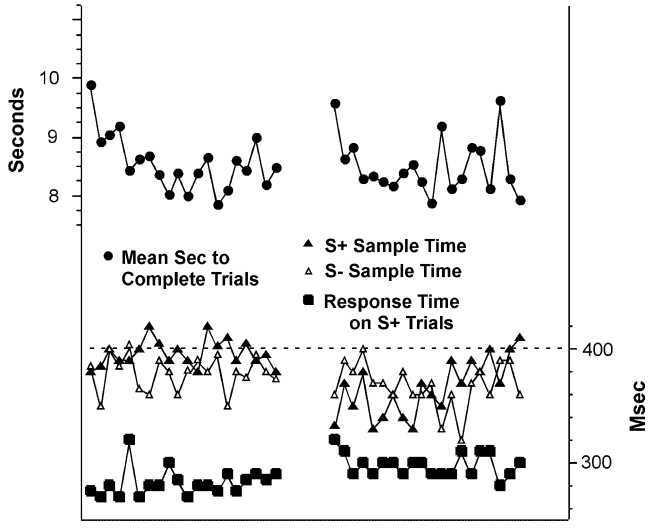

Figure 6 Mean seconds to complete trials, S+ sampling time, S− sampling time, and response times for 16 mice tested in 2 sessions on 0.5% EA (S+) versus water solvent (S−). For seconds to complete trials and response time, each data point is the mean of 20 trials. For S+ and S− odor-sampling times, each data point is the mean of 10 trials.

detection task. The minimum time to complete a trial consists of the 4-s intertrial interval plus the final valve time (1 s on average) and the sampling and response times on the next presentation of the stimulus. Not responding on an S− trial adds the sampling time (0.376 s on average) plus the 2-s response period time or a total of 7.4 s. On S+ trials, the average response time (0.35 s) during the response period would result in a minimum trial period of approximately 5.4 s. Because there were 10 S+ and 10 S− trials in each block, a mouse that responded correctly on every trial could, theoretically, complete trials in, on average, 6.4 s. The median trial time for the 800 trials on the 0.5% EA detection task (Figure 6) was 8.9 s. Thus, on average, trial time was only 28% longer than the minimal possible trial time. These results demonstrate that mice were strongly motivated to perform and that there was no progressive decrease in that level of motivation over either of the 400-trial sessions on the EA detection task.

Response time on S+ trials was consistently shorter and less variable than odor sample time. Response time was largely unrelated to odor sample time and, hence, was a poor predictor of problem difficulty. The shortest mean response time for an individual mouse was 190 ms but most often was in the range of 250–325 ms.

Discussion

The present results provide the first descriptive study of changes in odor-sampling time in mice during training on a variety of odor detection and discrimination tasks. The main contributions of this study may be summarized as follows:

1. Odor sample time exhibits considerable plasticity and varies as a function of task novelty, discrimination difficulty, and performance accuracy.

In virtually all cases, introduction of a new and/or more difficult detection or discrimination task resulted in an increase in odor sample time. This occurred, for example, when the concentration of the EA stimulus was reduced (Figures 2B and 4F), when a novel odor was used in the detection task (Figure 3B), and when a clearly more difficult task (as indexed by accuracy) was introduced (Figure 4B1,D,F,G2). An increase in sample time was not necessarily accompanied by a decrease in response accuracy; thus, a change in an odor detection task produced little or only a brief perturbation in accuracy but a marked or sustained change in odor-sampling time (e.g., Figure 2B,D) as did the initial introduction of the pyridine detection task and the pyridine versus benzaldehyde discrimination task (Figure 3B,B1).

That an increase in problem difficulty produces an increase in odor sample time serves to confirm similar findings of Abraham et al. (2004) and Rinberg et al. (2006). Indeed, using a particularly elegant design, Rinberg et al. (2006) demonstrated that a trade-off between sampling speed and accuracy (speed–accuracy trade-off), characteristic of psychophysical functions in other modalities, occurs also for olfaction. However, in both the studies of Abraham et al. (2004) and Rinberg et al. (2006), the effect of problem difficulty was shown by mean sample time values over numerous training trials. The present results extend these observations by demonstrating that, in the acquisition phase of a difficult task, sample time gradually increases as accuracy improves and, further, that after asymptotic performance accuracy is approached or achieved, sample time remains at a high level (e.g., Figures 2D, 3B1, and 4D). The sudden increase in S+ sample time upon the introduction of the PA/AA mixture (Figure 4B1) and the odor intensity difference (Figure 4D) tasks are of particular interest. Because, in those problems, there was no change in the S+ stimulus and virtually all errors in the first blocks of trials were false alarms (responding on an S− trial), the increase in S+ sampling time must reflect the effects of not receiving an expected reinforcement after responding on S− trials. In brief, in response to the demands of the detection or discrimination problem, mice can rapidly adjust the time spent sampling the stimulus.

2. Obtained sample times were probably the shortest congruent with accurate responding.

Sampling time was relatively insensitive to reinforcement magnitude and to an increase in flow rate. The failure of reinforcement magnitude to influence sample time on a simple detection task suggests that mice were making a sampling decision as quickly as they were able while still maintaining high levels of accuracy. The failure of an increase in flow rate to influence sample time is of interest as it relates to physical and potential cognitive factors governing this measure. Odor-sampling time as measured in this study has 2 or, possibly, 3 major components: respiratory motor acts to bring odorant molecules in contact with the olfactory epithelium, the time to withdraw from the sampling port, and possibly, a cognitive component in the mouse deciding whether to respond on the water delivery tube prior to withdrawing from the odor port. Over sessions on the well-practiced 0.5% EA detection task, mean sampling times on S+ and S− trials were 383 and 379 ms, respectively. The contribution of the delay in odor delivery from the time the final valve terminated to the time at which it is available to be sampled to this latency is unclear because a 50% increase in flow rate failed to produce a decrease in sample time, whereas a decrease in flow rate produced a substantial increase in sample time. By calculation, the delay in stimulus arrival on each trial would be approximately 200 ms (assuming a 20-ms delay due to operation of the final valve and the plug flow time required for a 2000-cc/min stream to transverse a 6.2-ml volume of space). It is likely either that this calculation does not represent the true delay or that the delay in the arrival of the stimulus beyond some minimal time is a negligible factor in

determining sample time. If the calculated delay is correct, then the time to withdraw from the odor-sampling port from the arrival of the stimulus is approximately 200 ms. Further, because an increase in reinforcement magnitude or differential reinforcement of faster sampling had little influence on sample time, 200 ms may approximate the shortest sample time required for accurate responding. If, on the other hand, odor arrival time is less than 200 ms and a further decrease (i.e., increase in flow rate) does not shorten sample time, then the minimum required sample time exceeds 200 ms and, above some minimal value, the delay in odor arrival constitutes a relatively minor contribution to the factors that determine sample time. If so, then the 3 other factors producing odor sample time (as measured in this and related studies), the time required to detect or identify the odor, the proposed cognitive component (time to make a decision to respond or not respond), and the time required to withdraw from the odor-sampling port, would constitute the major contributions to sample time. Clearly, the time needed to detect or identify the odor is only one component of measured "odor-sampling time" in this study and those of Bodyak and Slotnick (1999), Slotnick and Schellinck (2002), Uchida and Mainen (2003), Abraham et al. (2004), and Rinberg et al. (2006).

3. S+ and S− sample times reflect different processes.

There was considerable variability in differences between S+ and S− sample times across conditions. For some problems, there was essentially complete overlap in S+ and S− sample times (e.g., Figures 2F,G and 3A), whereas in others, S− sample time was clearly different than S+ sample time. Whereas frequent changes in test conditions make it difficult to identify patterns, several outcomes appear related to particular conditions. For one, S− sampling time was notably and consistently higher than S+ sampling time in the first EA detection task (Figure 2A), upon introduction of a lower concentration of EA (Figure 2B) and when flow rate on the EA detection task was decreased (Figure 2D). With repeated presentations of the standard 0.5% EA detection task, there was a reduction in differences between S+ and S− sample times. This trend is apparent in the repeated sessions in the first 0.5% EA detection task (Figure 2B) and in the extended training on this task (Figure 3A).

In contrast, when odorant concentration was abruptly decreased in a detection task and in each of the 2-odor discrimination tasks, mice most often had longer sample times on S+ trials. Abrupt introduction of a difficult discrimination or detection task consistently resulted in higher S+ than S− sample time (Figure 4B1,D,F,G2). Among these later tasks, the magnitude of differences in S+ and S− sample times were related to task difficulty, as indexed by performance accuracy. Thus, the largest difference in S+ and S− sample times occurred for the task with the lowest rate of acquisition (Figure 4B1,F) and the smallest difference with the task having the highest rate of acquisition (Figure 4D).

Why were sample times on S+ and S− trials, most often, not equivalent? In some cases, there appears to be an obvious explanation. In an easy odor detection task, there is no signal on S− trials, and it is reasonable to assume that the mouse may require a somewhat longer sample on those trials to determine that a signal is not present. This explanation is supported by the generally longer S− sample times in all but the more difficult low-concentration EA detection task (Figure 4F) in which S− sample time was clearly shorter than S+ sample time. However, shorter S− sample times also characterized performance on the pyridine versus benzaldehyde task (Figure 3B1), the odor mixture discrimination tasks (Figure 4B1,G2), and the intensity difference discrimination task (Figure 4D). Why, in these tasks, should the mouse require a longer sample to determine that the positive stimulus is present than to determine its absence? One explanation proposed for a similar phenomenon in rats (Slotnick 1990; Slotnick and Schellinck 2002) is that sample time on S+ trials is governed not by detection but by recognition and that in low-concentration detection tasks or 2-odor discrimination tasks, detecting that an odor is present takes less time than determining the identity of the odor.

4. Odor-sampling time may provide a more sensitive measure of discrimination than does response accuracy.

Except at near-threshold stimulus concentrations, rats and mice generally achieve similar high levels in response accuracy in a variety of detection and discrimination tasks. Indeed, even psychophysical functions on well-practiced detection problems tend to be relatively flat except at perithreshold levels (e.g., Slotnick and Schoonover 1984). However, as indexed by the number of errors made in acquisition, odor-sampling time may continue to reflect discrimination difficulty even after asymptotic levels of performance on a task are achieved. Thus, even after achieving levels of accuracy similar to those on a simple odor detection task, sample time on odor mixture discriminations (Figure 4B1,G2) remained appreciably higher than on the detection task. Odor sample time also provided a sensitive index of changes in detection and discrimination requirements even when the new tasks produced little or no change in response accuracy (e.g., Figures 2D and 3B1). Finally, the analysis of odor-sampling times of mice that failed to acquire the acetate/acid mixture task (Figure 5) suggests that differences between sampling on S+ and S− trials may provide a better index of discrimination than does response accuracy.

5. Advantages and disadvantages of different discrimination methods to measure sample time.

Of the 3 detailed reports quantifying sample times of rodents trained using an olfactometer and operant conditioning, 2 used an alternate choice, symmetrical reinforcement procedure (Uchida and Mainen 2003; Rinberg et al. 2006),

and one (Abraham et al. 2004) used a go, no-go discrimination task, but because reinforcement was provided within the odor-sampling tube, only the speed of head withdrawal on S− trials could be used to index sample time. In the 2–alternative choice procedure, 2 response ports or keys are provided, and a correct response (i.e., respond on the right key for odor A and on the left for odor B) produces a reward on each trial. Friedrich (2006) has suggested that the alternate choice method may promote speed of responding over accuracy because each response could be reinforced and, thus, the animal would be strongly motivated to respond on each trial. In support of this speculation, it appears that, on difficult discrimination tasks, rats trained using alternate choice (Uchida and Mainen 2003) made more errors but largely maintained response speed, whereas mice trained using the go, no-go discrimination method achieved higher accuracy on comparable problems but did so at the expense of response speed. However, the 2 studies differed in many details, and despite the potential resolution of this issue by Rinberg et al. (2006), it remains unclear what advantages might accrue from the use of the alternate choice method used by Uchida and Mainen (2003) and by Rinberg et al. (2006). Odor-sampling time of mice trained using symmetrical reinforcement is relatively insensitive to changes in problem difficulty, and Rinberg et al. (2006) were able to demonstrate a speed–accuracy trade-off only with the use of special training to control odor sample time. A practical disadvantage of alternate choice is that it requires far more training than does the go, no-go method and that side or key preferences may prevail when a new discrimination task is introduced or the task is made more difficult. A more relevant disadvantage is, of course, that alternate choice does not provide a measure reflecting the animal's recognition that response to a particular stimulus will not be reinforced. On the other hand, the go, no-go method used by Abraham et al. (2004) does not provide a measure of sample time on S+ trials. Because an increase in problem difficulty, particularly in 2-odor discriminations, is most often accompanied by an increase in S+ sample time, outcomes based only on S− trials would underestimate mean sample time. As shown in this study, both positive and negative trials may provide different and complimentary information on factors that govern sample time. The method described in this report allows sample time to be measured on both types of trials and may provide a useful compromise between alternate choice and the go, no-go method of Abraham et al. (2004).

6. Differences in odor sample time reported among studies.

Odor sample times on discrimination tasks in this study were, on average, about 100–150 ms shorter than those reported for similar tasks by Bodyak and Slotnick (1999). Both studies used the same or similar trial procedures and methods of generating and delivering odors, but the Bodyak and Slotnick (1999) results were based on only 4 mice. Whereas the sample times for these mice were relatively long, the values fall well within the range of sampling times for comparable problems in the present study. Also, for reasonably comparable (2-odor discrimination) problems, similar patterns of odor sampling were found in both studies: initially, when performance was at or near-chance levels, there was little or no difference in S+ and S− sample times, and during acquisition, S+ sample time increased, exceeded, and remained higher than S− sample time.

Although some mice in the present study performed at near-perfect accuracy while having sample times below 250 ms, average sample time was appreciably longer than the shortest mean sample times reported by Abraham et al. (2004) (269 ms) and Rinberg et al. (2006) (275 ms) for relatively simple discrimination tasks. The odor generators used by these investigators were essentially identical to ours, but there are differences in response requirements and the amount of training given to mice prior to obtaining the reported sample times. Although Abraham et al. (2004) used only S− trials to measure sample time, the sampling times they reported are similar to those obtained by Rinberg et al. (2006) who used an alternate choice task. Thus, it is unlikely that differences in response requirements account for the longer sample times found in this study. It is more likely that the amount of training is a critical variable: the 269-ms value reported by Abraham et al. (2004) was obtained from mice that had been trained for several months on simple and difficult discrimination tasks. The mice in the study of Rinberg et al. (2006) also appeared to have received intensive training on a series of related odor discrimination problems. Odor sample times for tasks reasonably similar to ones used in this study and for performance accuracy in the 90–100% range are shown in Figure 3 of Rinberg et al. (2006) and Figure 3B of Abraham et al. (2004). In the study of Rinberg et al. (2006), mean sample time was approximately 385 ms for 7 mice trained on a simple odor detection task. This value is essentially identical to the mean sample of 383 ms for mice given extended training on the 0.5% EA detection task (Figure 3A). The mean (S−) sample time reported by Abraham et al. (2004) for mice trained on a simple odor discrimination task was approximately 360 ms (their Figure 3B), and this compares favorably with the mean of 357 ms for S− sample time in the last session on the pyridine versus benzaldehyde task (Figure 3B1). Thus, to the extent that comparisons can be made across studies, odor sample times obtained in this report are comparable with the studies using mice of Abraham et al. (2004) and Rinberg et al. (2006).

In conclusion, the present study demonstrates that odor sample times for mice trained on a go, no-go discrimination task provide a sensitive index of several parameters of odor detection and discrimination tasks including novelty, difficulty, and amount of training. Because responses on both S+ and S− trials can provide separate and complimentary information on odor-sampling strategy, it is advantageous to record sample times on both types of trials. The methods

used in the present report provide such measures, avoid the complications of using 2-choice procedures, and should prove useful in future studies of odor-sampling time.

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