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13. ABSTRACT (Maximum 200 words)

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Speed-Accuracy Tradeoff in Olfaction

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Summary

The basic psychophysical principle of speed-accuracy tradeoff (SAT) has been used to understand key aspects of neuronal information processing in vision and audition, but the principle of SAT is still debated in olfaction. In this study we present the direct observation of SAT in olfaction. We developed a behavioral paradigm for mice in which both the duration of odorant sampling and the difficulty of the odor discrimination task were controlled by the experimenter. We observed that the accuracy of odor discrimination increases with the duration of imposed odorant sampling, and that the rate of this increase is slower for harder tasks. We also present a unifying picture of two previous, seemingly disparate experiments on timing of odorant sampling in odor discrimination tasks. The presence of SAT in olfaction provides strong evidence for temporal integration in olfaction and puts a constraint on models of olfactory processing.

Introduction

The central representation and integration of olfactory stimuli has both spatial (Axel, 2005; Belluscio and Katz, 2001; Buck, 2005; Leon and Johnson, 2003; Meister and Bonhoffer, 2001; Mori et al., 2006; Wachowiak et al., 2002; Xu et al., 2003) and temporal (Brown et al., 2005; Friedrich, 2006; Laurent et al., 2001; Lei et al., 2004; Mazor and Laurent, 2005; Spors et al., 2006) aspects. One approach to establishing the complementary contributions of spatial and temporal coding (Christensen, 2005; Cleland and Linster, 2005; Lledo et al., 2005; Spors and Grinvald, 2002) is to ask, "How are olfactory computations affected by greatly reducing the available spatial (Fukushima et al., 2002; Hudson, 1999; Lu and Slotnick, 1998; Slotnick et al., 2004) or temporal (Abraham et al., 2004; Jinks and Laing, 1999; Laing, 1986; Sobel et al., 2001; Uchida and Mainen, 2003) aspect of the stimulus representation?" In the mammalian olfactory system, reducing the spatial extent of the olfactory bulbs shows that 20% of a single olfactory bulb is sufficient to maintain several aspects of olfactory recognition, discrimination, and odor learning (Fecteau and Milgram, 2001; Lu and Slotnick, 1998). Limitations in the temporal domain show that a single sniff, the natural unit of active olfactory sampling (Kepecs et al., 2006; Roux et al., 2006; Schoenfeld and Cleland, 2006; Scott, 2006; Youngentob, 2005), provides sufficient information for odor identification (Mainland and Sobel, 2006; Uchida and Mainen, 2003). Understanding the computational costs of limiting the spatial and temporal extent of olfactory information processing will provide important constraints on models of olfactory information processing (Brody and Hopfield, 2003; Cleland and Linster, 2005; Hopfield, 1999; A. Koulakov et al., submitted). Here we explore computational tradeoffs in the temporal domain using new psychophysical approaches to measure speed-accuracy relationships in mice.

Can mammals achieve higher accuracy in stimulus discriminations by sampling the stimulus for a longer time before making a decision? Can the nervous system take advantage of this longer period of time by integrating information about the stimulus signal? These questions are important for understanding the mechanisms of sensory information processing and have been thoroughly investigated in vision and audition (Luce, 1986). For example, in visual discrimination tasks, clear psychophysical and neurophysiological evidence exists for temporal integration of sensory input (Shadlen and Newsome, 2001). In olfaction, the existing data (Abraham et al., 2004; Uchida and Mainen, 2003) are conflicting, and no direct experimental confirmation of speed-accuracy tradeoff (SAT) exists. (Friedrich, 2006; Khan and Sobel, 2004).

The relationship between accuracy, sampling time, and task difficulty for odor discrimination tasks was the subject of two recent studies. Uchida and Mainen (2003) trained rats to discriminate two pure odorants and their mixtures in a two alternative choice (2AC) reaction time paradigm (RTP), varying the difficulty of the task by changing the relative concentrations of the components in the odor mixtures. Rats were trained to poke their noses into a central odor delivery port, sniff an odor, and go to the left or right water port according to a previously learned association. They demonstrated that rats perform with lower accuracy on more difficult tasks; however, they still spent almost the same amount of time sampling the odor independent of task difficulty (odor sampling time increased from 270 ms for the easiest task to only 300 ms for the hardest task). Subsequently, Abraham et al. (2004) trained mice in a go-no-go (GNG) paradigm. Mice were trained to lick the water spout located in the odor sampling port in response to one odor (S+) and withdraw their heads from the port in response to another odor (S−). Correct S+ responses were reinforced by a small water reward. Abraham et al. (2004) demonstrated that when performing the task with accuracy above 90%, mice used longer odor exposures to solve harder odor discrimination tasks (270 ms for an easy task and 490 ms for the hardest task). Thus the question of whether an animal makes better discriminations if it samples odorants for longer times remains. The olfactory system may integrate its inputs over time and achieve higher odor discrimination accuracy for longer

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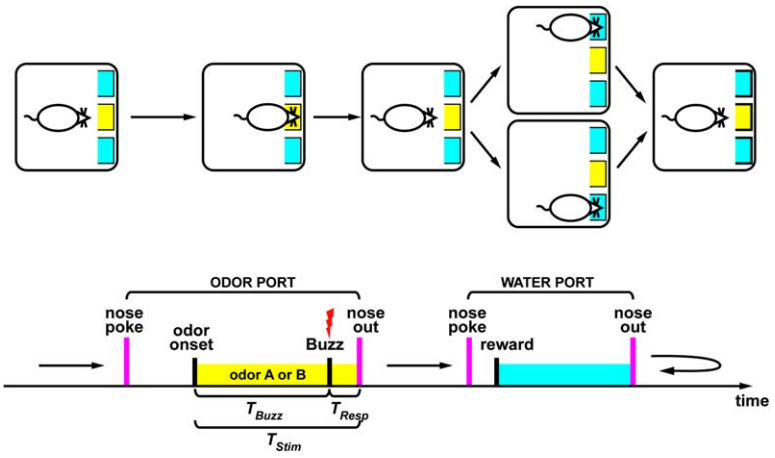

Figure 1. Behavioral Paradigm

The top row is the sequence of consecutive behavioral states of the mouse in the apparatus with one odor port (yellow) and two water ports (cyan). Bottom row is the corresponding sequence of time events. Vertical magenta lines are events controlled by the mouse, vertical black lines are events controlled by the experimenter. The sequence of events is the following: *event 1*: the mouse pokes its nose into the odor port, and the trial begins; *event 2*: onset of odor delivery, stimulus exposure begins; *event 3*: buzz onset, the mouse is allowed to withdraw its nose from the odor port; *event 4*: the mouse withdraws its nose from the odor port, stimulus exposure ends; *event 5*: the mouse pokes its nose into one of the water ports; *event 6*: reward onset, the mouse gets a water reward if it poked its nose into the correct water port; *event 7*: the mouse withdraws its nose from the water port, intertrial interval begins; *event 8* (not shown): intertrial interval ends, mouse may initiate a new trial.

odor sampling times, which would be a manifestation of SAT in olfaction (Khan and Sobel, 2004). To answer this question, a direct manipulation of odor sampling time is necessary (Friedrich, 2006). This manipulation was performed in the present study.

Results

We developed a new behavioral paradigm in which odor sampling time was controlled by the experimenter, and we used this new paradigm to test for the existence of SAT in olfactory discrimination tasks. We systematically varied both the odor exposure time and difficulty of the odor discrimination task. To receive a water reward, a mouse had to keep its nose in the odor port until a short sound signal (buzz) occurred and then make a correct odor discrimination by going to the left or right water port according to the learned odor associations (Figure 1). The duration of odor exposure was controlled by the latency of the buzz from odor onset. The difficulty of the task was varied by changing the relative concentrations of the two components in the odor mixture, as in both previous sets of experiments (Abraham et al., 2004; Uchida and Mainen, 2003). In each behavioral session the mouse was exposed to only two odor mixtures and eight different buzz latencies in random sequence.

To make a direct link between our results and the previous results, after testing with enforced odor sampling times, mice were switched to the RTP task without the buzz, similar to the task used by Uchida and Mainen (2003). At this point the mouse was allowed to make its own decision as to how much time to spend sampling the odor stimulus before making its decision.

The total odor sampling time was determined by the buzz latency. Typical distributions of response times in the buzz (enforced odor sampling time) paradigm and in the no-buzz, RTP, odor discrimination paradigms are shown in Figure 2. For each buzz latency, T_{Buzz}, ranging from 0 to 1 s, we measured the distribution of odor sampling times, T_{Stim} (Figure 2A). The odor sampling time was defined as the time the mouse spent in the odor port from odor onset to nose withdrawal. Nose withdrawal was detected from the restoration of the photobeam spanning the entrance to the odor port. For all four tasks of different difficulty, the distributions were similar (overlapping solid lines of four different colors).

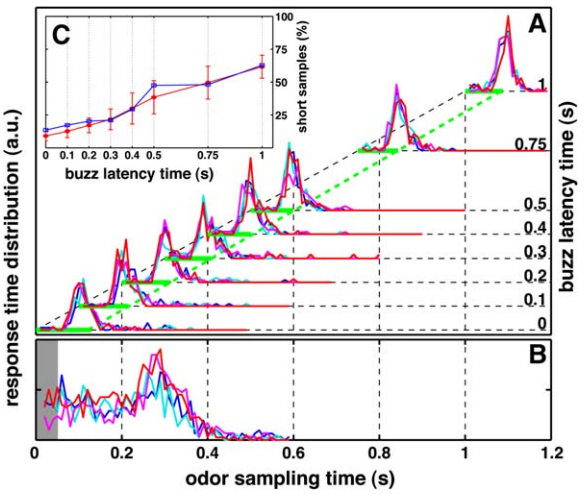

Figure 2. Response Time Distribution

(A) The distribution of odor sampling times for four different task difficulties and eight buzz time latencies for mouse #136. The tasks are discriminations between odor mixtures with different concentrations of components A ("+" carvone) and B ("−" carvone). Task 1: 100%–0% versus 0%–100% (color blue), task 2: 75%–25% versus 25%–75% (color cyan), task 3: 62%–38% versus 38%–62% (color magenta), task 4: 56%–44% versus 44%–56% (color red). Each row corresponds to one buzz time latency shown on the right axis. The line starts at the time of the beginning of the buzz signal. Horizontal green bars indicate the average response time for a given buzz latency for all task difficulties. Dashed green lines show the linear regression fit for buzz time latency T_{Buzz} (right axis) and odor sampling time T_{Stim} (horizontal axis):

$$\overline{T}_{Stim} = T_{Buzz} + \overline{T}_{Resp} = (1 - 0.0032^{(\pm 0.003)})T_{Buzz} + 0.126^{(\pm 0.002)}\text{sec}$$

Here the linear regression coefficients are given with 95% confidence intervals.

(B) The distribution of odor sampling times for RTP experiments for mouse #136, for all four task difficulties. Gray area is the time interval for short sample trials.

(C) Proportion of short sample trials for mouse #136 (blue line) and average for all mice with standard deviation error bars (red line) as a function of buzz latency.

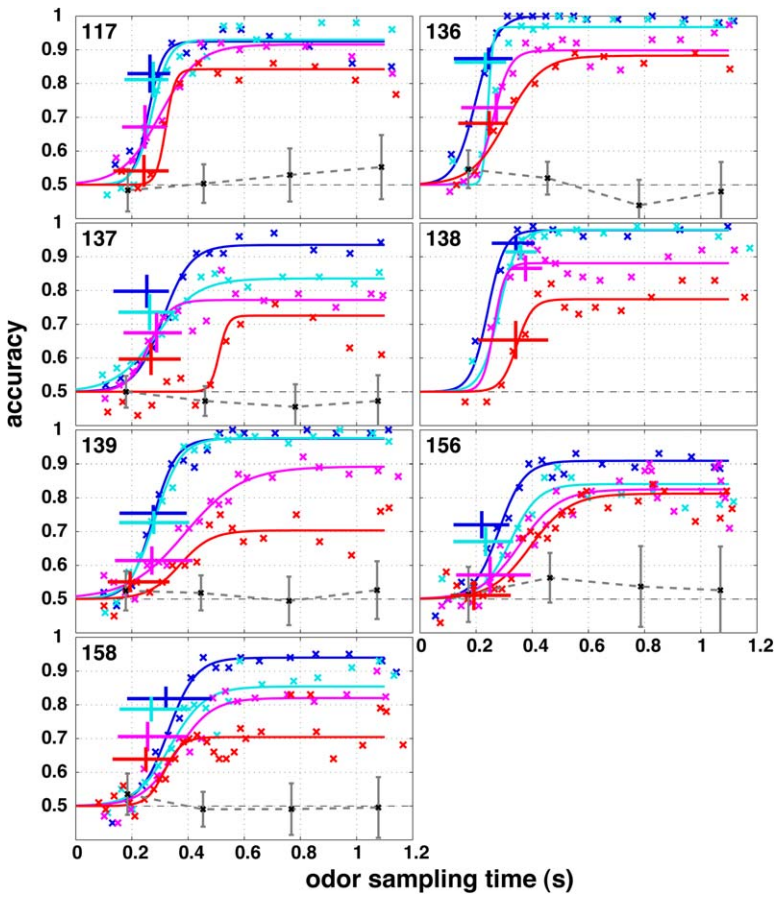

Figure 3. Accuracy as a Function of Odor Exposure Time for Different Task Difficulties for Individual Mice

Dots are averaged accuracy and odor exposure time for 100 trial windows with 50 trial steps in the sequence of trials ordered by odor sampling times. Solid lines are logistic fits of the data used as a guide for the eye. Solid crosses are averaged accuracy and timing for RTP experiments for the same mice. The horizontal bars are 20%–80% margins of response time distributions; vertical bars are 95% confidence intervals for accuracy estimations. The difficulty of the tasks is color-coded in the same way as in Figure 2. Black dots are average accuracies for control experiments with 50%/50% mixtures. Error bars are 95% confidence intervals. Each dot corresponds to control experiment trials in one out of four 0.3 s time bins. The mouse number is indicated in each panel.

For all mice the average buzz response time, T_{Resp} (the time from the buzz onset until nose withdrawal), was slightly shorter for longer buzz time latencies. The total odor sampling time was $T_{Stim} = T_{Buzz} + T_{Resp}$ (Figure 2A). Thus, there is a direct relationship between the time the mouse spent sampling the odor and the buzz latency established by the experimenter.

For longer buzz latencies mice tend to leave the odor port more often before the buzz. If the mouse withdrew its nose from the odor port before the onset of the buzz signal, the trial was considered a short sample. These trials were not rewarded and were not counted as discrimination trials. The fraction of short sample trials as a function of buzz latency for all mice (red line) and for the mouse whose data are presented in Figures 2A and 2B (blue line) are shown in Figure 2C.

From these measurements, it is clear that the buzz paradigm allowed us to control the time that the mouse spent sampling the odor in the odor port. As soon as the mouse heard the buzz it very quickly (within 90–120 ms median, for different T_{Buzz}) left the odor port. In the RTP the mouse stayed in the odor port approximately 275 ms on average (from 240 to 360 ms for different mice). For the RTP we count only trials in which a mouse stayed at least 50 ms in the port in order to eliminate trials in which the mouse was not exposed to odors at all (see Experimental Procedures). For RTP experiments the average fraction of short sample trials was about 25%.

Odor discrimination accuracy for individual mice as a function of enforced odor sampling time for four different odor discrimination difficulties is shown in Figure 3. All trials for a given difficulty were first ordered by odor sampling time, regardless of buzz time latency. Then accuracy was estimated in a 100 trial moving window with 50 trial steps. Average accuracy and time for each window is shown as a data point in the graph. A fit with a three-parameter logistic function is provided as a guide for the eye (solid lines). The difficulty of the task is color-coded.

The average accuracy for all mice (n = 7) as a function of odor sampling time for eight buzz latencies and four difficulties is presented in Figure 4. The vertical bar of each cross indicates the 95% confidence interval, and the horizontal bar of each cross indicates the 20%–80% margins of the response time distribution for a given buzz latency. To present the statistical analysis of the data, we calculated average accuracies for 20 trial bins for each mouse, each difficulty, and each buzz time latency. A two-way ANOVA test showed strong effects of buzz latency ($f = 437$, $p \ll 10^{-3}$), task difficulty ($f = 169$, $p \ll 10^{-3}$), and interaction between buzz latency and task difficulty ($f = 5.1$, $p < 10^{-3}$). We indicate statistically significant accuracy differences ($p < 0.05$, multiple comparison HSD test) between adjacent points by joining the points with a solid line. Statistically insignificant accuracy differences between adjacent points ($p > 0.05$) are indicated by a dashed line between the points.

The data in Figures 3 and 4 show clearly the increase in accuracy with increased odor sampling time for all tasks, thus indicating the presence of SAT. There is substantial variability in individual mice performance. Both the maximum level of accuracy for a given task and the approximate minimal odor sampling time, which

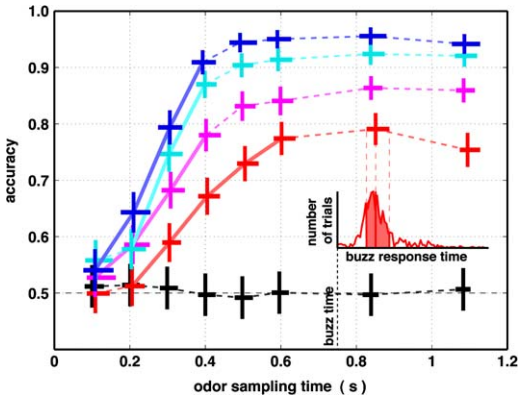

Figure 4. Accuracy for Different Buzz Time Latencies and Different Task Difficulties Averaged for All Mice

Difficulty is color-coded as in Figures 2 and 3. Each cross represents data for all mice and one difficulty and one buzz time latency. Black crosses are the results of control experiments with 50%/50% mixtures. The horizontal bars of the crosses define 20%–80% margins of response time distributions, and are positioned at the average accuracy along the vertical axis. Vertical bars define 95% accuracy confidence intervals and are positioned along the horizontal axis at the median of the response time distribution. The response time distribution for all mice for buzz latency equal 0.75 s, and the task of greatest difficulty is presented in the inset for illustration. The solid lines connecting adjacent crosses indicate statistically significant differences between accuracy levels ($p < 0.05$, multiple comparison HSD test); dashed lines indicate no statistically significant differences.

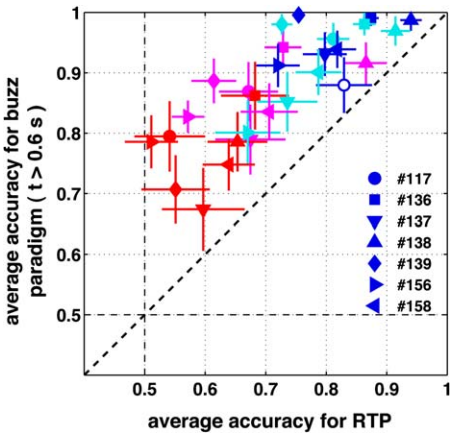

Figure 5. Comparison between the Average Accuracy in the RTP and the Average Accuracy in the Buzz Paradigm for Odor Sampling Times Longer than 0.7 s for All Mice and All Tasks

For all panels, task difficulties are coded by color; different mice have different symbols. Bars are 95% confidence intervals. Solid markers correspond to mean accuracy comparisons with p values less than 5% (open markers for p values larger than 5%) for the two-sample test for binominal statistics testing the hypothesis that both distributions have the same mean.

was necessary to reach such a level, varied for different mice. Nevertheless, the main features were the same for all mice: (1) with longer odor sampling times, the mouse reached higher accuracy for easier tasks; (2) to reach the same level of accuracy for harder tasks, the mouse needed to sample the odor for longer times; and (3) the mouse reached higher levels of odor discrimination accuracy for enforced long odor sampling times than it reached in the RTP, when the mouse was allowed to leave the odor port voluntarily (see analysis below).

To ensure that mice made discriminations only based on olfactory cues, each mouse performed two control sessions (approximately 800 trials) discriminating between two identical odor mixtures, each containing 50% odor A and 50% odor B. The results of these experiments are shown in Figures 3 and 4. The control set of vials with 50/50 mixtures was put at positions in the olfactometer regularly occupied by the pairs of odors with differing compositions (see Experimental Procedures). For each mouse and each buzz latency, the average accuracy on 50:50 mixtures equaled 50% ($p > 0.1$). Also, for short samples in the RTP, in which mice were not exposed to an odor stimulus because they left the port before odor onset, mice performed at chance level ($p > 0.1$ for each mouse averaged for all tasks). This shows that the mice did not use cues other than the composition of the odor mixture to perform the odor discrimination task.

We also examined changes in odor discrimination accuracy due to habituation during daily testing sessions. For each time interval, for each mouse, and each difficulty of the task, we compared average accuracy on the first and second halves of daily sessions. No systematic changes in accuracy were observed (x^2-test, $p \approx 0.7$). (See Figure S2A of the Supplemental Data.)

To test for changes in accuracy due to learning from session to session, we compared the performance of each mouse between pairs of consecutive sessions with the same level of difficulty and found that the mean accuracy increased by about 2%. The standard deviation estimated for different mice, different difficulties, and different buzz latencies was about 6%. Mice slightly improved their performance, presumably due to learning. However, these small changes do not alter the conclusion that under our experimental conditions, mice show SAT. (See Figure S2B).

The accuracies and average odor sampling times for RTP experiments for the same animal and the same task difficulty are shown as crosses in each panel of Figure 4. The mouse voluntarily spent approximately the same amount of time sampling odors in all four levels of difficulty, as shown by Uchida and Mainen (2003). However, the mouse reaches a lower level of accuracy in the RTP than that achieved with longer odor exposures imposed during the buzz paradigm (Figure 4). To demonstrate this point we compared the performances for long odor sampling times in the buzz paradigm ($t > t_0 = 0.7$ s) and voluntary odor sampling times in the RTP (~ 0.27 s average duration). The results are summarized for all mice in Figure 5. Most of the points reside above the diagonal ($p < 0.05$, two sample test for binominal proportion, full markers in Figure 5), showing that spending more time in the odor port beyond the voluntary response time increases the probability of correct performance.

We then examined the question whether harder odor discrimination tasks require longer odor sampling times to achieve the same level of accuracy (Abraham et al., 2004). To address this question for each mouse, we determined the average accuracy for the hardest task at odor sampling times longer than $t_0 = 0.7$ s (Figure 6, inset). Then, for all task difficulties, we evaluated the time needed to reach this level of accuracy. As evident

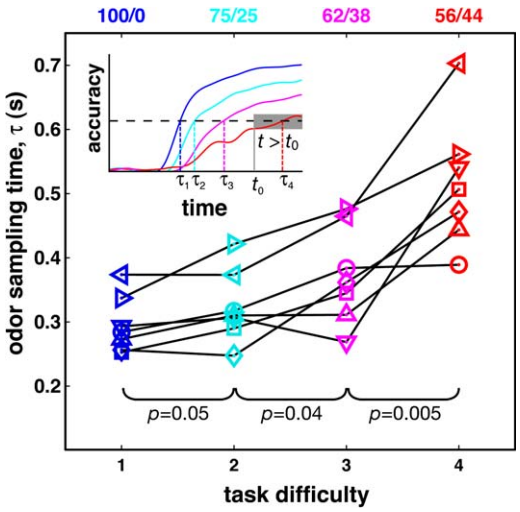

Figure 6. Dependence of Odor Sampling Time τ at which a Mouse First Reaches the Given Level of Accuracy for Different Task Difficulties

The comparison level of accuracy for each mouse was taken as its average accuracy for the hardest task at times longer than 0.7 s. The different markers correspond to different mice (see legend in Figure 5). (Inset) Cartoon showing how times τ were measured for a given mouse. Four lines correspond to accuracy-time dependencies for four tasks. Horizontal dashed line is the average accuracy for the hardest task (red line) at times longer than t_0.

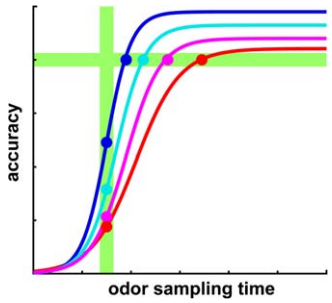

Figure 7. A Diagram of the Dependence of Discrimination Performance Accuracy on Odor Sampling Time for Tasks of Different Difficulties

The difficulty is color-coded; from easiest to hardest: blue, cyan, magenta, red. Vertical green line corresponds to results of 2AC RTP experiments, while the horizontal green line corresponds to results of GNG experiments.

from Figure 6, it takes longer for the animals to reach the same level of accuracy for the harder tasks. The p values for the paired t tests are shown in Figure 6 for each pair of difficulties.

In the experiment with random sequences of buzz latencies, when a mouse pokes its nose into the odor port, its expectation to hear the buzz increases as the time passes and no buzz occurs. This may cause variation in the attentional state of the mouse and, therefore, the mouse may be more prepared to respond to the buzz and make a correct decision on trials with a longer buzz latency—not due to sensory information integration, but due to a different attentional level. We addressed this issue by presenting blocks of 100 trials with the same buzz latency. Within each block of trials the mouse expected to hear the buzz and make an odor discrimination at the same latency from odor onset. To measure SAT in this case, we varied buzz latency between blocks of trials. The dependence of odor discrimination accuracy on odor exposure time measured in this way did not differ from that measured using the paradigm in which all buzz latencies were presented randomly interleaved on successive trials. We made this comparison for five mice presented with the hardest odor discrimination task because the hardest odor discrimination task gives the clearest demonstration of SAT, and concluded that attentional effects are negligible. (See the Supplemental Data for details and statistical tests of these data.)

Discussion

The results presented in Figures 3 and 4 directly demonstrate the existence of SAT in odor discrimination tasks. Accuracy reaches its maximal level for easy tasks after approximately 300 ms of odor sampling, and for harder tasks, after about 600 ms of odor sampling. In almost all of our experimental conditions, the level of maximal accuracy for a given complexity of the odor discrimination task using the buzz paradigm is higher than that in the paradigm when the mouse voluntarily chooses the duration of odor sampling (Figure 5). Forcing a mouse to take longer odor samples increases its odor discrimination accuracy. Presumably, in the RTP experiments, factors other than olfactory information processing determined the time a mouse chose to stay in the odor port.

Our results reconcile previous experiments dealing with SAT in rodents (Abraham et al., 2004; Uchida and Mainen, 2003). In RTP experiments (Uchida and Mainen, 2003), the accuracy for different difficulties was defined not by the best rat performance, but by the time the rat chose to spend in the odor port. This time was approximately the same for all task difficulties. The dependence of accuracy on difficulty corresponds to a roughly vertical slice of accuracy-time dependencies for different difficulties (Figure 7). In the GNG experiment (Abraham et al., 2004) mice presumably were not motivated to leave the port quickly and performed at the same high accuracy in all tasks by staying longer in the odor port for harder tasks. These results correspond to an approximately horizontal slice of accuracy-time dependencies for different difficulties (Figure 7). Our findings therefore suggest a unifying picture of speed-accuracy-difficulty dependencies, which bridges the gap between previous experiments (Khan and Sobel, 2004).

The finding that mice spend about the same amount of time (~300 ms) sniffing odors in the RTP roughly independent of the difficulty of the task (Uchida and Mainen, 2003) is usually taken as evidence for spatial encoding within the olfactory bulb (Leon and Johnson, 2003). Studies of temporal changes in odor coding (Friedrich et al., 2004; Friedrich and Laurent, 2001) suggest a mechanism of odor discrimination in which the temporal evolution of the odor representation leads to the ability to discriminate between similar patterns. According to this mechanism, the more similar a pair of odors, the longer the processing time required to discriminate between them with the same accuracy. Our data show that longer odor exposures lead to better discrimination

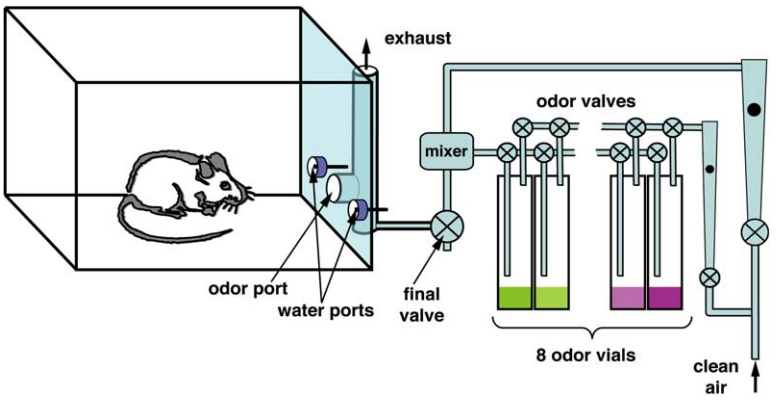

Figure 8. Computer-Controlled Olfactometer for Automated Odor Delivery and Response Measurement during Odor-Guided Learning and Odor Discrimination in the Mouse

See description in the Experimental Procedures.

and, together with the work of Abraham et al. (2004), demonstrate that the time required to reach maximal performance accuracy depends on the difficulty of the task and can be as long as 600 ms. Our observations are therefore consistent with the mechanism proposed by Friedrich et al. (2004). However, some other mechanism involving temporal integration of the signal is also possible.

Not much is known about the relationship between sampling time, task difficulty, and accuracy of odor discrimination in human psychophysics. According to Laing (1986), humans can identify dissimilar odors in one sniff. Larger numbers of sniffs or longer sniffs do not improve their performance. In rodents, this observation may correspond to our results for an easy task, in which mice quickly (<300 ms) achieve the highest accuracy. Presumably, in humans, as in rodents, to observe SAT at extended time intervals (more than one sniff) requires harder odor discrimination tasks. As in rodent experiments (Abraham et al., 2004), the study of Wise and Cain (2000) showed a relationship between the difficulty of the odor discrimination task and the latency of the behavioral response. Both of these studies suggest the presence of SAT in olfaction.

The natural unit of olfactory timing in mammals is the sniff (Schoenfeld and Cleland, 2006) or its central neural correlate, the theta rhythm of local field potential oscillation (2–8 Hz) in the olfactory bulb that is phase-locked to sniffing (Gelperin, 2006; Hayar et al., 2004; Kay, 2005; Macrides et al., 1982; Margrie and Schaefer, 2003). Our results imply that more sniffs are required to obtain sufficient information to make harder odorant discriminations than are required for easier odorant discriminations. Recognizing the importance of making measurements in the awake behaving animal (Rinberg and Gelperin, 2006), several methods have been employed to directly measure active odor sampling rate (sniffing) in the awake behaving animal, involving implantation of thermocouples or pressure sensors in or near the nasal passages of the animal (Bensafi et al., 2003; Buonviso et al., 2003; Kepecs et al., 2006; Pager, 1985). It will be very interesting to determine directly how enforced durations of odor sampling in the buzz paradigm translate into changes in sniff frequency, intensity, and number during odorant sampling.

We directly demonstrate the basic psychophysical principle of SAT in rodent olfaction. The presence of SAT suggests that olfactory systems integrate odor stimuli in time to achieve higher performance from longer sampling times. SAT experiments reveal the time window of olfactory information processing needed to make an odor discrimination and provide temporal boundaries within which to focus future neurophysiological studies.

Experimental Procedures

Seven male C57BL6 mice were used in this study. Subjects were 6–8 weeks old at the beginning of behavioral training and were maintained on a 12 hr reversed light-dark cycle in isolated cages in a temperature- and humidity-controlled animal facility. All behavioral training was conducted during the dark half of the mouse's light/dark cycle. Mice had free access to food but were on a water restriction schedule designed to keep them at 80%–85% of their baseline body weight. All animal care and experimental procedures were in strict accordance with an animal care protocol approved by the Monell Chemical Senses Center Institutional Animal Care and Use Committee.

Mice were trained in a modified Knosys olfactometer (Bodyak and Slotnick, 1999) (Knosys, Washington, D.C.) (Figure 8). The combined odor-water port originally supplied with the instrument was replaced by an odor port and two separate water ports as shown in Figure 8. A mouse nose poke into an odor or water port was monitored by photo emitters and detectors spanning the entrance to each port.

Prior to odor exposure all mice were trained to maintain a nose poke into the odor delivery port, in the absence of odor delivery, until the occurrence of a short auditory signal (buzz). If the mouse withdrew its nose from the odor port before the buzz, no water reward was available. If the mouse stayed in the odor port until the buzz signal, water reward was available in both water ports. When the mouse stayed in the odor port for 1.5 s in 50% of the trials, the buzz response was considered robust and odor training started.

In the odor discrimination paradigm, the mouse nose poke into the odor port triggered the activation of the final valve, which diverted the main air flow away from the odor port, and caused simultaneous opening of one of the eight pairs of odor valves (Figure 8). The air flow then went through the odorant source vial and the headspace odor in the chosen vial was mixed with clean air (1:10 air dilution). 0.5 s later the final valve released and odorant from the selected vial was delivered to the mouse's nose. The time lag between final valve release and odorant reaching the mouse's nose was constant for all stimulus presentations and equaled approximately 50 ms, which was estimated based on flow velocity and channel geometry. In the buzz odor discrimination paradigm, the mouse had to keep its nose in the odor port during odor exposure until the buzz signaled availability of the water reward. One odor mixture (e.g., 75/25) signaled water reward available on the left while the complementary mixture (25/75) signaled that water was available at the right port. If the mouse withdrew its nose from the odor port before the buzz, the trail was considered a short sample and was not counted as a discrimination trial.

We used (+) and (−) carvone diluted 1:10 in mineral oil as a pair of odorants. Enantiomers were chosen because they have identical

physical properties and are easily mixed in liquid phase. (+) and (−) carvone have different odor percepts that make the discrimination task between pure chemicals relatively easy, but discrimination between similar mixtures very hard. Pilot experiments with (+) and (−) limonine and nondiluted carvones did not show any qualitative differences.

Initially the mouse was trained on the simplest odor discrimination task, discrimination between two pure odorants A and B. Then the difficulty of the task was increased by presenting mixtures: 75%–25% versus 25%–75%, 62.5%–37.5% versus 37.5%–62.5%, and 56%–44% versus 44%–56% A-B mixtures. The last pair is the most difficult one.

Data collection started after each mouse was exposed to at least two full sessions for the hardest task. A typical session lasts ~1 hr and consists of 400–500 trials with eight different buzz timings from 0 to 1 s after the final valve release. For data collection, each mouse performed the tasks in descending order of difficulty; the task for each difficulty was presented for at least two consecutive sessions, totaling more than 800 trials, at least 100 trials per each buzz time latency. After data collection experiments with the buzz paradigm were over, each mouse was tested with the RTP (without buzz). During the first 10–15 min (50–100 trials) of the RTP, the mouse tended to stay in the odor port for longer times, perhaps waiting for the buzz, then the average odor sampling time shortened and stabilized. The data presented for the RTP exclude this initial interval.

Data Analysis

To demonstrate speed-accuracy tradeoff, we collected data for all mice, for each of four task difficulties, and each buzz time latency (Figure 3). Each trial was characterized by odor sampling time t_i and behavioral result r_i ($r_i = 1$ for a correct decision, $r_i = 0$ for an incorrect decision). The estimate of average performance accuracy is

$$\hat{A} = \frac{1}{n}\sum_{i=1}^{n} r_i$$

with n as the total number of trials for all mice for a given task difficulty and buzz time latency. The 95% confidence interval of the accuracy estimation is calculated based on the assumption that each trial is an independent Bernoulli trial (Rosner, 1999):

$$\hat{A} \pm 1.96\sqrt{\hat{A}(1-\hat{A})/n}$$

To present the data for individual mice (Figure 4), we collected all trials from two or three sessions to accumulate at least 800 trials for a given task difficulty, and ordered the trials in ascending order of t_i. We took the first window of $n = 100$ trials, estimated the average odor sampling time

$$\tau_1 = \frac{1}{n}\sum_{i=1}^{n} t_i$$

and average accuracy, or probability of the correct response

$$\hat{A}_1 = \frac{1}{n}\sum_{i=1}^{n} r_i$$

and considered this pair of values to be the first data point in the accuracy-time dependence graph $(\tau_1, \hat{A}_1)$.

Then we moved our analysis window by 50 trials and repeated the procedure. So each data point $(\tau_k, \hat{A}_k)$ in Figure 3 is an average accuracy and time for a series of 100 trial windows with 50 trial overlaps. The density of dots τ_k reflects the envelope density of odor sampling times. Sets of data points for each mouse and each difficulty were fitted by a logistic equation $A(\tau) = 0.5 + b/[1 + \exp(-(\tau - \tau_0)/\delta)]$ with three parameters τ_0, δ, and b as a guide for the eye.

To compare the performances in the RTP and buzz paradigms with long odor exposure times, we estimated average accuracies and confidence intervals in the same way as described above for all samples (excluding short samples) from the RTP and all trials with odor exposure times longer than 0.7 s for the buzz paradigm. The two-sample test for binominal proportions (Rosner, 1999) for the hypothesis that distributions in both cases have the same mean was used to establish statistical significance of the differences between averaged accuracies in these two cases.

To estimate the timing τ at which the accuracy reaches a certain level for a given task, we used the same set of ordered trials and made the same windowed accuracy-time calculation, but for window size equal to 100 trials and for step size equal to 5 trials. The first average time for which average accuracy exceeds a certain level was considered the time of interest τ (see Figure 6). This estimation has a bias toward smaller times. The bias for harder tasks may be stronger because the accuracy-time dependence is usually flatter (see Figure 3). This means that the real dependence of how much time is required to solve a harder problem may be even steeper than the one shown in Figure 6. The paired t test was used to establish the statistical significance of the finding that on average across all mice, the harder tasks take longer times to reach the same accuracy.

Supplemental Data

The Supplemental Data for this article can be found online at http://www.neuron.org/cgi/content/full/51/3/351/DC1/.

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Supplemental Data
Speed-Accuracy Tradeoff in Olfaction

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Attentional effects

The relationship between time of odor exposure and accuracy of mice performance may be influenced by stimulus context effects (Zariwala et al., 2006). The strongest effect is expected for the hardest odor discrimination task, for which mice need the longest odor sampling time to reach maximum accuracy. To check the role of stimulus context effects on SAT we compared the accuracy of performance in two different paradigms. In both paradigms mice were performing the hardest odor discrimination tasks with different buzz latencies. For paradigm **R** (random), buzz latencies were chosen randomly on each trial, so the mice did not know when to expect the buzz signal on each trial. For paradigm **B** (block), mice performed blocks of 100 trials with the same buzz latency. The sequence of buzz latencies between blocks was random. During one block, the buzz occurs with the same delay, and mice may develop an expectation as to when the buzz would occur and therefore may be more prepared to respond. As proposed by Zariwala et.al. (2006) changing the hazard function of buzz expectation may change the SAT relationship. In our experiments, we see very little change, as outlined below.

For both R and B paradigms we collected data from all trials and binned them by 200 ms windows of odor exposure times. For each window, we compared the average accuracy for paradigm R and B (Fig. S1). Results for mouse #156 are presented in Fig. S1A. The p-values for the hypothesis that both distributions for the same time window have the same mean value are presented above the bars (two-sample test for binomial proportion). The data for all tested mice for both paradigms are presented in Fig. S1B. The data points for one mouse for all time bins have the same markers as in Fig. 3. The data points for pairs of distributions which have statistically significant differences in their mean values ($p < 0.05$) are shown as solid markers, the other data points ($p > 0.05$) are shown as open markers. The distribution of differences of average accuracies for all

mice and all time widows is shown in inset C. The average difference between mice performances on the two paradigms for all mice and all time windows is equal to zero (p = 0.3). The most interesting temporal interval for this effect is between 0.2 and 0.5 sec of odor exposure time. The average increase of accuracy for B paradigm in this time window is only 1.5 %.

We conclude that stimulus context effects play an insignificant role in our experimental demonstration of SAT.

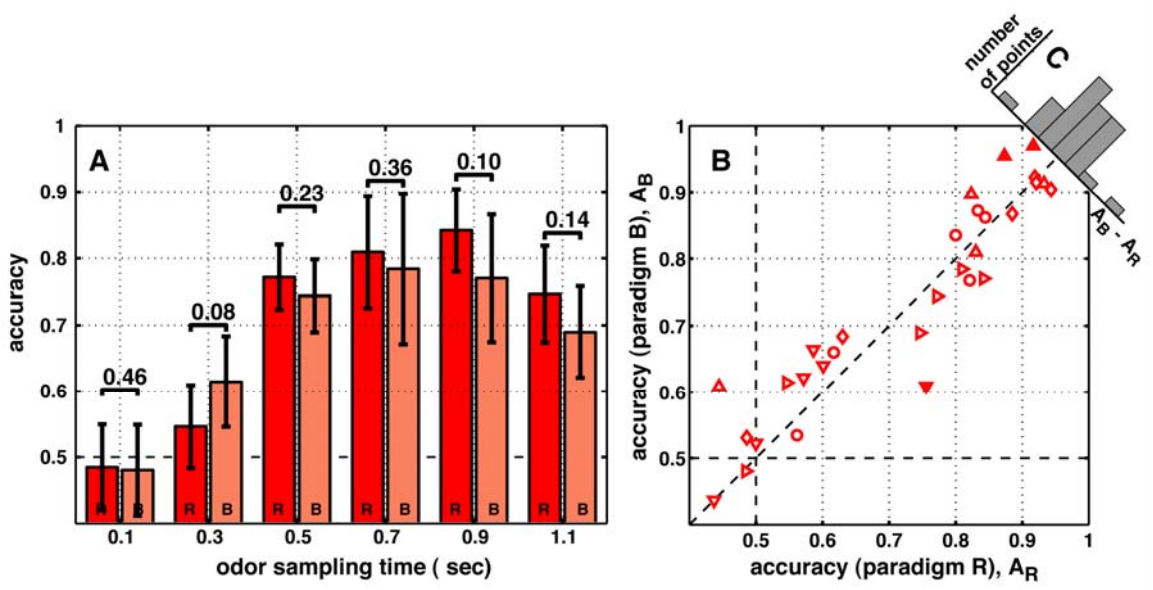

Figure S1. A. Accuracy of performance on the two odor discrimination tasks for mouse #156 for two paradigms B and R (see text), for odor exposure time windows of 200 ms, centered as shown on the labels on the horizontal axis. The data were obtained for the most difficult odor mixture (56/44%). Vertical bars are 95% confidence intervals estimated for binary distributions. Numbers above each pair of bars indicate p-values for the hypothesis that the two distributions have the same mean values (two-sample test for binomial proportion). **B.** Comparison of accuracy for five mice (#117, #137, #138, #139, #156) and all time windows. Mice are coded by markers as in Fig. 3. Statistically significant differences ($p < 0.05$) in average accuracies for pairs of distributions are marked by solid markers. Statistically insignificant differences ($p > 0.05$) are indicated by open markers. **Inset.** The distribution of mean accuracy differences for all mice and all time windows.

Habituation and Learning Effects

To address the issues of habituation during one test session and learning between test sessions we performed the following tests. For each task and each mouse the data were binned in 4 time intervals between 0 and 1.2 sec of odor exposure time. For each bin we compared 1) the average accuracy for the first and second halves of the session and 2) for the first and the second consecutive sessions. Results for habituation and learning tests (see main text) are presented in Fig. S2. We observed no systematic changes in accuracy due to habituation (χ^2-test, $p \approx 0.7$). Comparison of the accuracy between subsequent sessions shows an average 2% increase in performance, presumably, due to learning, as described in the main text.

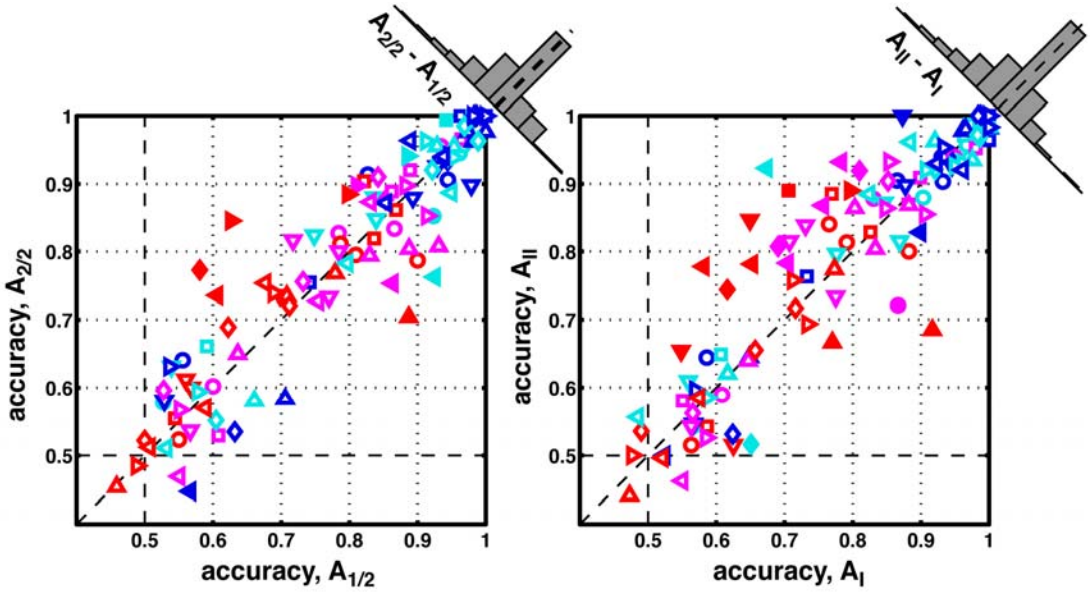

Figure S2 **A.** Comparison of average accuracies for the first and the second halves of the same test session. Each data point represents averaged accuracies for one mouse (marker coded), one difficulty of the task (color coded) one 300 ms time bin of odor exposure for the first ($A_{1/2}$) and the second ($A_{2/2}$) halves of the testing session. Sessions of the same task are averaged together. **Inset.** Distribution of the average accuracy differences. **B.** Comparison of average accuracies for the first and the second session. Each data point represents averaged accuracies for one mouse (marker coded), one difficulty of the task (color coded), one 300 ms time bin of odor exposure for the first (A_I) and the second (A_{II}) sessions of the same difficulty. **Inset.** Distribution of the average accuracy differences.

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