Temporal bounds on olfactory discrimination based decisions in rats

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Abstract of the Dissertation

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Time can a play an important role in the process of deciding between competing options. Reaction time studies in humans and non-human primates have emphasized that perceptual decisions improve with longer deliberation (temporal integration of sensory evidence), especially on difficult problems. However, given the selective advantages for rapid action in natural environments, it might be expected that some types of perceptual decisions may not benefit from long temporal integration. To test this idea, we conducted a series of tests of the role of reaction time in rats performing an odor mixture discrimination task in a two alternative choice paradigm. Two pure odors were mixed in various proportions to create problems of various difficulties. The difficulty of discrimination had a modest effect on the reaction times of rats. When motivated to slow down and improve accuracy, rats slowed down but the gain in time did not translate into a gain in accuracy. Similarly, there was no effect on accuracy when rats were forced to sample odors mixtures for long durations. However, we found that the accuracy on difficult problems improved in two situations in which we manipulated the expectancy of the stimuli. In one situation we changed the expectancy by interleaving different difficulties (diffused expectancy) or by presenting only one difficulty at a time (focused expectancy). The accuracy on each of the difficulties improved when the expectancy was focused. Secondly, we parametrically varied the temporal expectancy of a response cue such that rats better expected the response cue at certain moments in time. The accuracy covaried with temporal expectancy of the response cue. Together these results highlight a dissociation of accuracy from reaction time. We find that certain perceptual problems do not benefit from temporal integration but rather demand focused attention for optimal performance. The ability to control key decision variables (time and attention) in a rodent model opens avenues for elucidating the nature of neural processing strategies that support rapid goal-directed behavior.

I dedicate my dissertation work to my parents Zainab and Abbas Zariwala.

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Chapter 1: Introduction

One of the goals of systems neuroscience is to understand the neural basis of behavior. Complex behaviors are constituted of simple decisions. There is growing interest in the role of key variables like time, reward value, attention and uncertainty in the process of decision making. Classical studies in experimental psychology have concentrated on time, more specifically the reaction time (RT) of subjects (Donders, 1869; Luce, 1986), in perception based decision making. These studies extend across a breadth of experimental subjects and sensory modalities. They have guided research that has lead to our present understanding of neural coding and computational strategies involved in perception and decision making.

Perception, decisions and reaction times

Our interests are in understanding the neural organization of olfactory processing and how olfaction is used to guide choice behavior in rodents. We undertook a behavioral approach to first identify the processing times for rats when encountered with easy and difficult olfactory problems and then find the temporal bounds when motivated to perform with high accuracy on the difficult problems.

Animal studies have been a fundamental approach in the study of neural organization of sensation, perception and behavior. Perception in a psychophysical setting in animals is studied either using an operant conditioning or decision making paradigm. A decision making paradigm is a convenient procedure for scoring behavioral parameters like choice accuracy and reaction times repeatedly over many trials and derive measure of perception and learning by the subjects with ease in a laboratory.

Rats have been shown to perform visual discrimination of light and dark cue cards (Schlosberg and Solomon, 1943) and fine auditory frequency discrimination (Blackwell and Schlosberg, 1943; Talwar and Gerstein, 1998). But the olfactory capabilities and associated behavioral repertoire of a laboratory rat are humbling. Most rodent behavioral studies of olfaction can be categorized either as natural behavior (Schultz and Tapp, 1973) or psychophysics (Nigrosh et al., 1975). Natural olfactory behaviors are intriguing and at the same time offer a method to study the role of sensation in autonomic (Pfaff and Pfaffmann, 1969) and emotional responses. On the other hand the invention of an olfactometer and computerized behavioral control (Pfaffmann, 1958; Carr et al., 1962; Slotnick and Nigrosh, 1974) in a laboratory setting has allowed us to study the limits of odor detectability (Youngentob et al., 1991) and discriminability (Nigrosh et al., 1975; Uchida and Mainen, 2003). The ability to rapidly train rats to perform detection and discrimination of many different odors, measure their sniffing pattern (Youngentob et al., 1987), make simultaneous in-vivo neural recordings (Feierstein et al., 2006) are advantages for olfactory neuroscience. The ability to combine the large repertoire of natural rodent behavior with the elaborate understanding of the odor receptors (Buck and Axel, 1991) and olfactory transduction in rodents has fuelled the inquiry into rodent olfaction.

Operant learning studies have related reaction time to the strength of response learning (Finger, 1941), to the complexity of the neural pathway involved and to the state of those pathways. Reaction times of subjects could help elucidate the neural mechanism of perception and decision making (Moody, 1970; Luce, 1986). Donders proposed that one could infer the time taken by a particular hypothetical mental stage by subjecting the subject to two procedures that differed only in whether that stage is used (Donders, 1869). It has been proposed that decision making stage could take up the bulk of the processing time. Reaction time in rats has been studied in audition for intensity and frequency discrimination (Moody, 1970; Talwar and Gerstein, 1998) and in vision (Schlosberg and Solomon, 1943). In the auditory discrimination the authors found that the reaction time increased with difficulty of discrimination (by ~10% compared to the easy discrimination) hence providing precedent for the utility of reaction time in fine versus coarse perceptual decision making. Consistent with the magnitude of this effect monkeys performing brightness judgments also show a 10% increase in response times from easy to difficult stimuli (Medin et al., 1970).

Theoretical framework for studying perception, decision making and reaction times

Signal Detection Theory: How does one separate the phenomena of improvement of perceptual sensitivity from improvement in decision making in a perceptual task? A framework to understand and separate perceptual sensitivity and decision criteria in a perceptual decision making process is provided by the signal detection theory (Green and Swets, 1988). The theory can be generalized for many task paradigms and modalities. For example, perceptual sensitivity of rats performing auditory frequency discriminations was assessed using signal detection theory (Talwar and Gerstein, 1998). Similarly signal detection theory has been successfully employed to understand the role of attention on sensitivity and decision making (McDonald et al., 2000; Correa et al., 2006). The theory allows experimenters to estimate the perceptual sensitivity of subjects known as the discriminability index (d') and the decision criteria or response bias (β). These two parameters are read off from a receiver operating curve (ROC) which is a plot of hits (ordinate) against false alarms (abscissa). If the signal is weak most values of hit rate and false alarm would lie on the diagonal (d' = 0). As the strength of the signal increase the points fall on curves corresponding to higher values of d’ (1, 2, 3 …) lying above the diagonal. By definition d’ is a ratio of the separation (median) and dispersion (standard deviation) of the signal and noise distribution (assuming normal distributions). Similarly response bias (β) is the measure of subject’s decision bias given by the ratio of hit rate to false alarm. These parameters can be used to assess the effect of attention, training and stimulus difficulty in a perceptual decision task.

Temporal integration model: Speeded decisions based on sensory experiences constitute a class of decision making which is different from choices made on the basis of economic value of one of the two competing options. Sequential sampling models (Link and Heath, 1975) have successfully provided the framework to understand the reaction times of subjects performing a perceptual discrimination and decision task. The efficiency of this model lies in its ability to average out stimulus and sensory (generated by the nervous system) noise in order to improve the quality of perception and decision making. This is an obvious advantage beyond a simple signal detection theory (Green and Swets, 1988) where decision is thought of a single comparison process between a noise and a signal.

Among different methods of sequential sampling the random walk diffusion model (Laming, 1968; Link and Heath, 1975; Smith and Ratcliff, 2004) has gained popularity in recent years by virtue of its ability to explain both the reaction time distributions and neurophysiological data from monkeys performing random dot motion discrimination task (Mazurek et al., 2003). Mathematical formulation of this model invokes temporal integration of stochastic sensory evidence in favor of or against two competing choice options. The integrating evidence resembles a random walk and modeled as a random walk diffusion process over time till the process reaches one of the two absorbing bounds (thresholds). For strong evidence the integration process is fast and reaches one of the two thresholds rapidly, but when the evidence is weak the integration process if slow and takes longer to reach the threshold.

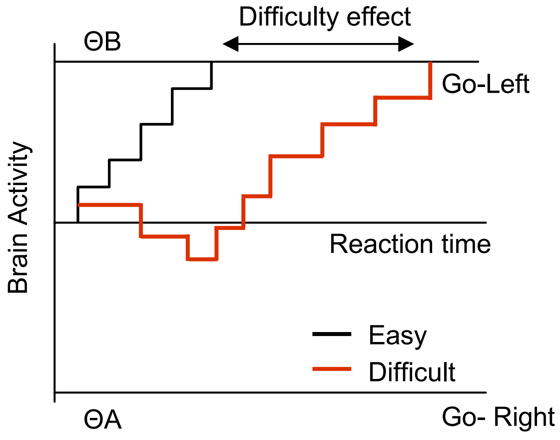


Figure 1 – 1: **A temporal integration to a threshold model for reaction time and choices in decision task.** The decision device receives stochastic input which represents evidence for two competing alternative choices (Left and Right). The device integrates evidence until it reaches one of the two thresholds (θA, θB), at which point a decision is emitted (only left decisions are shown). The stair plot shows the time course of the stochastic integration for two different stimuli of different difficulty. The integration window for the two difficulties are different and represents the reaction times. Under an accuracy instruction the thresholds are higher and therefore require longer integration (reaction) time. Accuracy would be low if thresholds are closer allowing random fluctuations to reach thresholds causing error choices.

For a noisy stimulus and sensory processing the random walk process varies from trial to trial accounting for the variance in the reaction time distributions for strong and weak sensory stimuli. The fluctuations in the noisy integration process could lead to termination on the wrong threshold on a few trials and hence an error choice. Figure 1-1 as a cartoon briefly describes the integration to threshold model. For a more comprehensive description of this model and other models for simple decision making the readers are encouraged to see other references (Mazurek et al., 2003; Smith and Ratcliff, 2004; Uchida et al., 2006)

Temporal integration and speed accuracy trade off

The temporal integration model though simple can explain the three basic reaction time effects as outlined below.

Difficulty effect: In a speeded response task where subject are free to respond voluntarily (reaction time paradigm) the reaction time varies with the difficulty of discrimination. According to the temporal integration model sensory evidence accrues slowly for weak evidence i.e., difficult stimuli before reaching threshold (as shown in figure 1-1).

Sampling time manipulation: In a direct sampling time manipulation task accuracy covaries with imposed limits on sampling duration. For short sampling times accuracy is low. According to the temporal integration model sensory evidence fails to accumulate optimally if the sampling is halted due to a response deadline.

Instruction or motivation dependent speed accuracy trade off: An interesting outcome of the role of time in sensory psychophysics is a phenomenon called the speed accuracy trade off (SAT), which highlights a cognitive capability of deciding between trading speed or trading accuracy depending upon subject’s motivation or an instruction from the experimenter. When humans are instructed to perform accurately they perform slowly and when they are instructed to perform rapidly their performance drops. This is accepted as the technical definition of speed accuracy trade off. According to the integration model under accuracy instruction the decision thresholds are high and therefore longer integration is required before reaching a decision. This in turns reduces the probability of the integration process to reach the opposite threshold, therefore, decreasing error rate (shown as deliberation effect in figure 1-1).

Behavioral studies in human (Palmer et al., 2005) and monkey (Roitman and Shadlen, 2002) making decisions based on direction of random dot motion have emphasized the effect of difficulty on reaction time. The difference in the reaction times between easy and hard discriminations in this task was in the order of hundreds of milliseconds. In addition to difficulty-dependent increase in reaction times, these laboratories have performed a viewing time experiment with monkeys (Britten et al., 1992). They found that the accuracy increased for longer viewing durations. Together these studies supported a role of integration in sensory processing and decision making.

Motivation to study SAT in rodent olfaction

Olfaction was thought of as a slow sense. Investigation of mitral cell activity in the olfactory bulb to presentation of pure odors reported temporal evolution of activity over hundreds of milliseconds. These observations were made in the bulb of the zebra fish and antennal lobe of insects to odor presentation in a non-behaving preparation (Wehr and Laurent, 1996; Friedrich and Laurent, 2001). Further analysis of the ensemble activity of recorded neurons indicated correlated activity in the early odor presentation window for two similar odors. This activity decorrelated over time ranging form 800ms to 2s. The results prompted neurophysiologists to propose that the sense of olfaction might benefit with longer processing times especially for telling apart perceptually similar odors (Laurent, 2002). Previous work measuring sampling duration in rats performing an odor discrimination task (Goldberg and Moulton, 1987) showed that rats performed this task within 1.0s of odor sampling. A human study relating natural sniffing to odor detection also reported that humans could detect pure odors within a single sniff cycle (Laing, 1983). Uchida and Mainen (Uchida and Mainen, 2003) developed an odor discrimination task in a two alternative choice RT paradigm for rats. They found that even when asked to discriminate enantiomers of an odor, rats took not more than 300ms and performed at above 90% accuracy.

The utility of sequential sampling and temporally evolving activity in the bulb may require difficult olfactory stimuli. Uchida and Mainen in their work (Uchida and Mainen, 2003), presented rats with increasing difficulty of discrimination by using binary mixture of pure odors in different proportions. Four mixture ratios (5/95, 20/80, 32/68 and 44/56) were randomly interleaved within a session. The perceptual accuracy decreased with increasing perceptual difficulty. They observed that rats performed even the most difficult stimuli within 300ms of odor sampling. The discrimination difficulty across various mixture ratios caused the rats to increase their sampling durations by just 10% or 30ms for the most difficult ratio compared to the easy one. The overall performance on the most difficult ratio was just above chance. Abraham et al (Abraham et al., 2004) developed a similar odor mixture discrimination task in mice (in a stay/no-stay paradigm) and showed that their mice readily performed the most difficult ratio at above 90% accuracy. In doing so, the mice took an additional 70ms of sampling durations (compared to 30ms increase in Uchida & Mainen study). Khan et al (Khan and Sobel, 2004) suggested a motivation-dependent speed accuracy trade off in olfaction to reconcile the results of these two studies while disregarding the differences in the behavioral paradigms. In particular, Khan et al. (Khan and Sobel, 2004) proposed that rats in the above study were not motivated to perform the difficult ratio at higher accuracy and therefore did not use longer sampling durations.

Motivating rats to trade speed for accuracy

The first goal of this study was to motivate rats to improve their accuracy on the odor mixture discrimination in the reaction time paradigm. A success in the ability to motivate rats should translate into an improvement in accuracy and increase in OSDs (speed accuracy trade off).

There has not been any instruction/motivation dependent speed accuracy trade off study in non-human subjects (for exception see a honey bee study; (Chittka et al., 2003)). To understand how motivation could affect the speed accuracy trade off, we performed the following manipulation to the standard RT version of the two alternative odor discrimination task (a) reduced the urgency to respond by introducing a 2.0s fixed delay to reward from the time of odor onset, (b) introduced a long random wait before the stimulus onset to break motor stereotypy (c) punished error choices with an air puff, (d) carefully controlled the water consumption of rats and affected their motivation by increasing the value of water reward inside the task. We then performed a direct sampling time manipulation in the same task using an auditory ‘go’ signal to instruct rats to stay in the odor port and sample odors to achieve sampling durations in the order of 300ms to 900ms.

In addition to motivating rats to perform better in this task, we studied and isolated the difference in the experimental design in our study with that of Abraham et al (Abraham et al., 2004). One of the major differences is that in our task all difficulties are randomly interleaved while in Abraham et al study mice were presented with one difficulty (non-interleaved) per day. In chapter 4, we report the results from our experiments which highlight the effects of this difference in the stimulus presentation contexts.

Stating briefly when motivated rats voluntarily slowed down by 50-100ms.

However, this increase in sampling durations did not translate into an accuracy improvement. This result was true even when we enforced long sampling durations (> 800ms). Interestingly, accuracy improved only in the context of non-interleaved stimulus presentation (100% expectancy of stimulus difficulty as compared to 25% expectancy in interleaved stimulus presentations). Surprisingly, the improvement in accuracy did not come at any cost of OSD.

Controlling temporal expectancy in a sampling time task

In another recent study, Rinberg et al (Rinberg et al., 2006), used the two-alternative choice task and performed a more direct sampling time manipulation. They trained mice to sample odor mixtures until an auditory ‘go’ signal indicated the time to respond out of the odor port. They randomized the delay to the ‘go’ signal for two reasons. First, with various delays to ‘go’ signal they managed to achieve a range of sampling duration within a single session and secondly they made the timing of ‘go’ signal unpredictable from trial to trial. A predictable delay to a salient ‘go’ signal could cause rats to develop a stereotypic sampling pattern concentrated around the time of the ‘go’ signal. They achieved odor sampling durations (OSD) ranging from 0 s to >1.0s in this paradigm. Mice improved their discrimination performance as a function of OSDs instructed by the ‘go’ signal delay. They then compared the best accuracy of mice in this task with that of the same mice performing reaction time task. They concluded that mice in a reaction time paradigm do not perform as well on each of the tested difficulty levels as they did when forced to sample for beyond 300 ms.

In a ‘go’ signal task rats could attempt to anticipate the time of the ‘go’ signal, somewhat similar to a pedestrian preparing to walk just before the “walk” sign. It has been shown that the anticipation (expectancy) of a randomly occurring event is given by its hazard rate (Luce, 1960, 1986; Janssen and Shadlen, 2005). The hazard rate is the probability of an event to occur given it has not occurred yet. It is calculated from the distribution from which the delay to the events is drawn. For a uniformly distributed ‘go’ signal delay, the hazard rate increases with time. In a ‘go’ signal task while the rats sample the stimulus in the odor port, two independent processes could ensue; subjects could integrate the sensory information over time to reach higher accuracy level and/or subjects increase their anticipation of the ‘go’ signal with each passing moment. The increase in anticipation or attention to a delayed moment in time could also improve discrimination performance (Nobre, 2001)

We performed experiments where we randomly interleaved the ‘go’ signal within a session. We then controlled the temporal anticipation function in the same paradigm by using an exponentially distributed ‘go’ signal delays. We found a novel effect i.e., the performance in this task is affected by temporal anticipation. The relation between discrimination accuracy and sampling durations (temporal integration) is restricted to within 300ms of odor sampling. These results could explain the accuracy effect in the Rinberg study with mice.

Certain decisions do not benefit from longer deliberation. However, SAT could occur for other class of sensory problems including other olfactory problems in which rodents may benefit from temporal integration over time scales of hundreds of milliseconds. We conclude from our results that a speed accuracy trade off is not as general as it is thought to be based on results from human studies.

Chapter 2: Olfactory psychophysics in rats

In spite of the use of rodents and odors in the study of various behaviors, it is only recently that experimenters have developed behavioral paradigms to study olfactory psychophysics. Traditionally, odor discrimination task by rats (Slotnick and Nigrosh, 1974; Nigrosh et al., 1975) have been performed in a go/no-go reaction time paradigm. Similar paradigms and stimulus delivery apparatus were later developed for mice (Bodyak and Slotnick, 1999; Abraham et al., 2004). Despite tremendous success in developing rapid training procedure, these tasks consisted of an asymmetry in the motivation of rats to make a choice. Different response times depending on whether the choice is rewarded or not rewarded have been consistently reported in these studies (Bodyak and Slotnick, 1999). In order to circumvent the confounds of asymmetric motivation on reaction times of rats performing difficult sensory discrimination tasks, Uchida and Mainen developed a two alternative choice tasks using a center odor sampling port and two symmetric choice ports for each of the two alternatives i.e. stimuli A & B. Motion discrimination task developed for monkeys have successfully used two alternative choice paradigm to study the role of reaction times on accuracy.

Two alternative choice olfactory discrimination task

We trained rats in a custom made behavioral set up. The odor delivery and behavioral event acquisition was performed in Matlab®. All data analysis and plotting was performed in Matlab®. Rats were trained to discriminate the identity of two odors in a session. Within a few days of initial training on the basic procedure rats were trained to associate the identity of each of the odor with a left or right choice port. A correct identification led to a drop of water as a reward. The two odors and two choice port combinations were balanced across the rats. The two odors were equally probable on any given trial and were randomly interleaved within a session.

Task difficulty was increased by introducing binary mixtures of the two pure odors in various proportions. Rats were rewarded when they identified correctly the component with higher concentration in the mixture.

Odor stimuli and delivery

Odor stimuli were delivered using a custom built olfactometer (figure 2-1A). The odors used throughout this study were enantiomers of 2-octanols i.e., R-(-)-2-octanol and S-(+)-2-octanol (figure 2-1 B). The mixtures of two odors in various proportions (like 5/95, 20/80 and beyond) were generated using different flow rate and mixed to give a final flow rate of 100 ml/min odorized air.

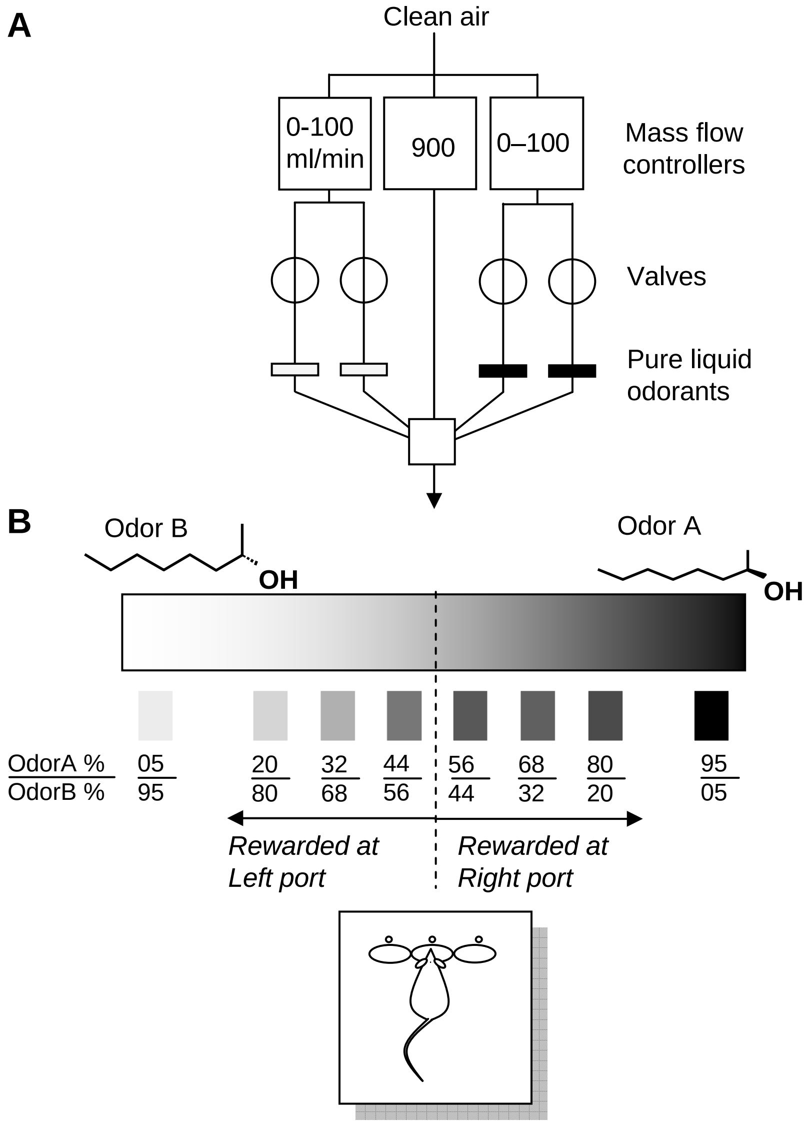


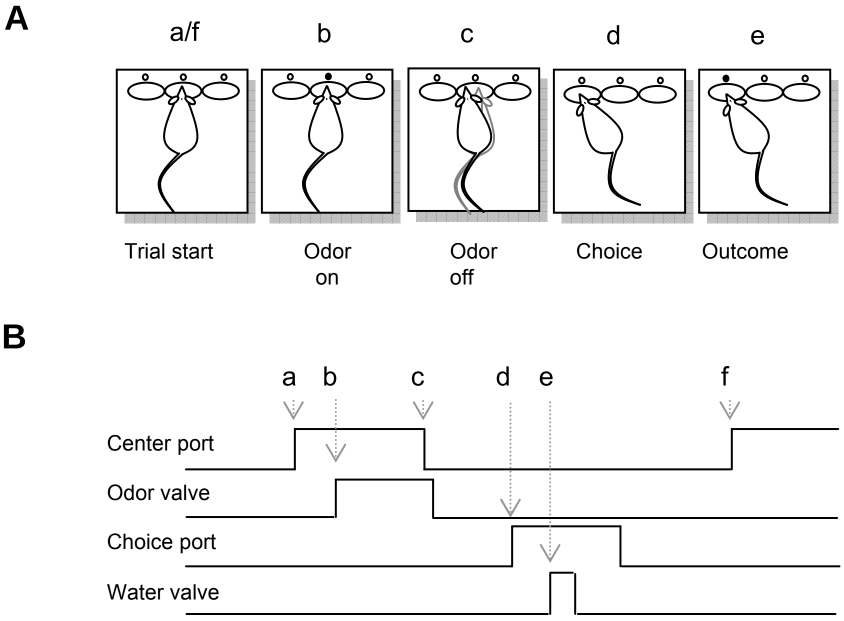
Figure 2 – 1: **Two alternative odor mixture discrimination task**.

(A) A line sketch of an olfactometer. Flow rates through the two 0 -100 ml/min mass flow controllers could be adjusted to give various mixture ratios.

(B) Odor stimuli: enantiomers of 2-octanol were mixed in various ratios. All stimuli were presented in the center port. Half the stimuli were rewarded on the left port and the other half on the right.

Task epochs and behavior times

A well trained rat initiated a trial with a poke in the center odor port (figure 2-2 A-C). The odor stimulus was delivered after a randomly chosen delay (fore period). In a reaction time paradigm rats were allowed to voluntarily pull out of the odor port any time after odor onset. A stay in the odor port beyond the time of odor onset was counted as successful odor sampling. We registered the duration between odor onset and odor port exit as OSDs. The odor delivery was programmed to cease as soon as rats exited the odor port. After completing a successful odor sampling rats initiated a movement to the choice port. The duration between odor port exit and entry into the choice port was registered as the movement time. The water delivery time was random and occurred between 0 -300ms. We subtract an odor delivery delay from the registered OSD post hoc and have reported the adjusted OSDs in all our analysis. This odor delivery delay was estimated using EOG recording in an anaesthetized rat (Feierstein et al., 2006). The variance, the median sampling durations and median movement times did not vary over days of training for these rats (fig 2-3 C, D).



C

	Standard RT task
Odor onset delay	$t_b - t_a = U\ (0.3s,\ 0.6s)$
Water Delay	$t_e - t_d \geq 0$
Inter trial interval	$t_f - t_e \geq 4$
Odor sampling time	$t_c - t_b$
Movement time	$t_d - t_c$

Figure 2 – 2: **Task epochs in a reaction time (RT) paradigm**

(A) Schematic of typical trial in a two alternative choice task. Sequential events in a typical successful trial are named with letters a-f.

(B) Time line of each recorded event along with odor and water valve on/off.

(C) Duration (range) of each behavioral epoch in a typical trial.

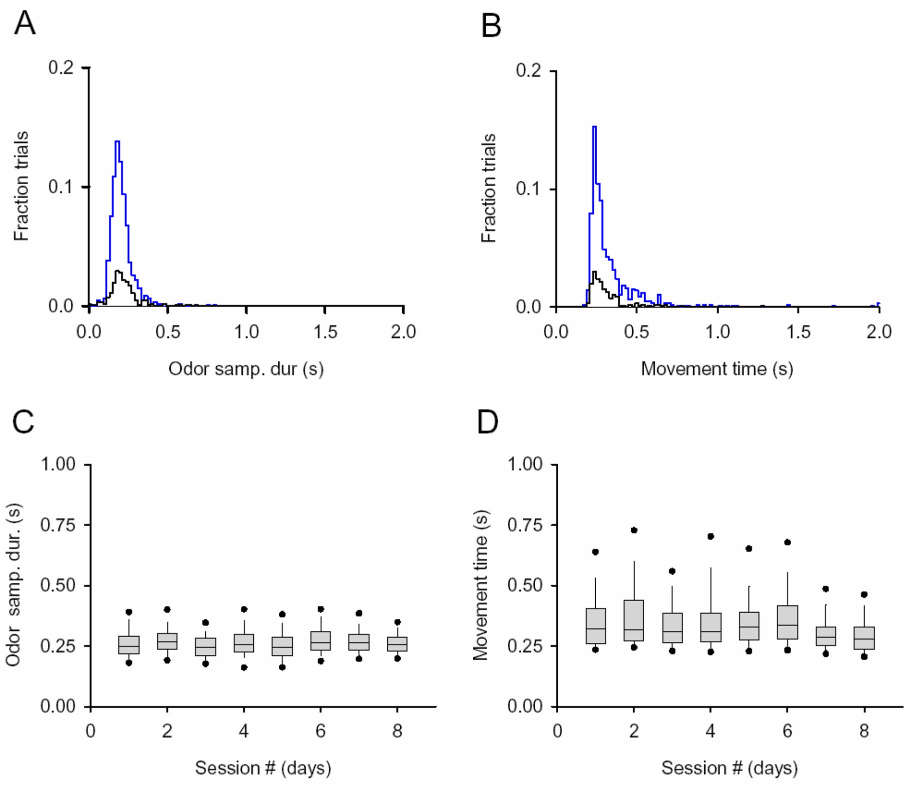


Figure 2 – 3: **Odor sampling duration and movement times in a RT paradigm.**

(A) Distribution of odor sampling time of a single rat in a single session. blue: correct trials, black: error trials.

(B) Distribution of movement time of a single rat in a single session. blue: correct trials, black: error trials

(C) Box plot of odor sampling duration over 8 consecutive sessions. Each box plot represented lower and upper 1.5*IQR (inter quartile range), lower and upper quartile (25 & 75 percentile) & median – center horizontal line. Two extreme dots represent 5th / 95th percentile.

(D) Box plot of movement time over 8 consecutive sessions. Legends same as (C).

Psychometric performance

A traditional method for studying the perceptual detectability of a stimulus by a subject is offered by signal detection theory (Green and Swets). For a perceptual detection task the theory allows a detailed method to estimate the discriminability index (d') and the response bias (β) of subjects by using a receiver operating curve (ROC) analysis. We do not use the standard d' & β measure and perform an ROC analysis in our task because the standard procedures available for a detection task are not directly applicable to a discrimination task in which the stimulus is almost always (100%) detectable. In our task we do not have a measure of false alarm (report signal for a pure noise stimulus) within the session. There is always a stimulus and rats respond by making a left or right choice. But we do estimate the d' and β of rats in the binary odor mixture discrimination using the psychometric curve. Interleaving all the different mixture ratios within a session allows us to plot the psychometric performance for individual sessions. The psychometric performance is fitted with a logistic regression (Fig 2-4A).The logistic regression fit for binomial distribution (left and right choice) is achieved using two variables i.e., the slope (discriminability) and the shift (response bias) of the fitted curve.

$\ln(P/1-P) = a + bX$ where

P = Probability to reporting left choice for left odor

b = slope of the logistic fit

$-b/a$ gives the response bias in reporting 'Left' for and odor.

We obtain the fitted slope and bias for individual day as well as for pooled for individual rat across days (sessions).

Dependence of performance and reaction time on task difficulty

The performance of rats on the binary odor mixture discrimination task varied as a function of difficulty introduced by mixing odors in various proportions. Fig 2-4 B shows the performance of an example rat on four different difficulties represented by the absolute difference in the concentration of odors in the mixture (% stimulus contrast). Also plotted is odor sampling duration (reaction times) as a function of difficulty.

While the difficulty of discrimination affected the performance of rats in this task, it caused a small increase in OSD for the most difficult ratio (95/5: mean +/-SEM to mean +/-SEM for 56/44). In the first report (Uchida and Mainen, 2003) of a small effect of difficulty in this task , the two pure odors used were - Caproic acid and Hexanol.

Conditional accuracy and speed accuracy trade off functions

In order to understand the role of time in odor processing and decision making, we related accuracy to the duration spent sampling the odors. This function is called conditional accuracy function (Luce, 1986). The term was first coined by Ollman (Ollman, 1977) and later used for Wood & Jennings (Jennings and Wood, 1976) for their human study where subjects were made to identify 1000 and 1100 Hz tones under various response deadlines and pay offs for responses within the deadline. Since Wood and Jennings used different response deadlines they plotted separate conditional accuracy function (CAF) for each of the response deadlines and pieced them together to achieve the speed accuracy trade off function. In an odor mixture discrimination task, Uchida & Mainen (2003) plotted CAFs for individual difficulty (% stimulus contrast) and reported that accuracy did not benefit from sampling duration longer than 300ms. Fig 2-4D is a similar plot for the four different stimulus contrasts for rats performing the above task. In our future analysis we use an identical analysis to relate speed with accuracy. In the case where we introduce various response deadlines in our task, using a ‘go’ signals as response cues for rats, we plot the speed accuracy trade off function as plotted by Wood and Jennings.

Instruct better accuracy or discourage fast reaction times

The low performance level achieved by rats on the most difficult ratio raised a possibility that they are not able to improve their accuracy because they do not sample the stimuli long enough. The over all accuracies achieved in this task is ~0.8 fraction correct choices. The incentive of improving accuracy by another 20% might not offset their urgency to respond as fast as possible at the water port. Another possibility is that the loss of water from 20% of the incorrect choices is well compensated by the free water outside the task.

In order for the rats to improve their accuracy they need to be motivated to perform correctly on every trial. The first set of experiments is an attempt at motivating/instructing rats by manipulating various task parameters. Along with identifying a set of parameters that would instruct accuracy or discourage speed, we carefully controlled the water that the rats received per day i.e., from performing the task and free water in the cage after the task. Finally, we manipulated the OSD directly by instructing them to stay in the odor port and sample odors till an auditory ‘go’ signal.

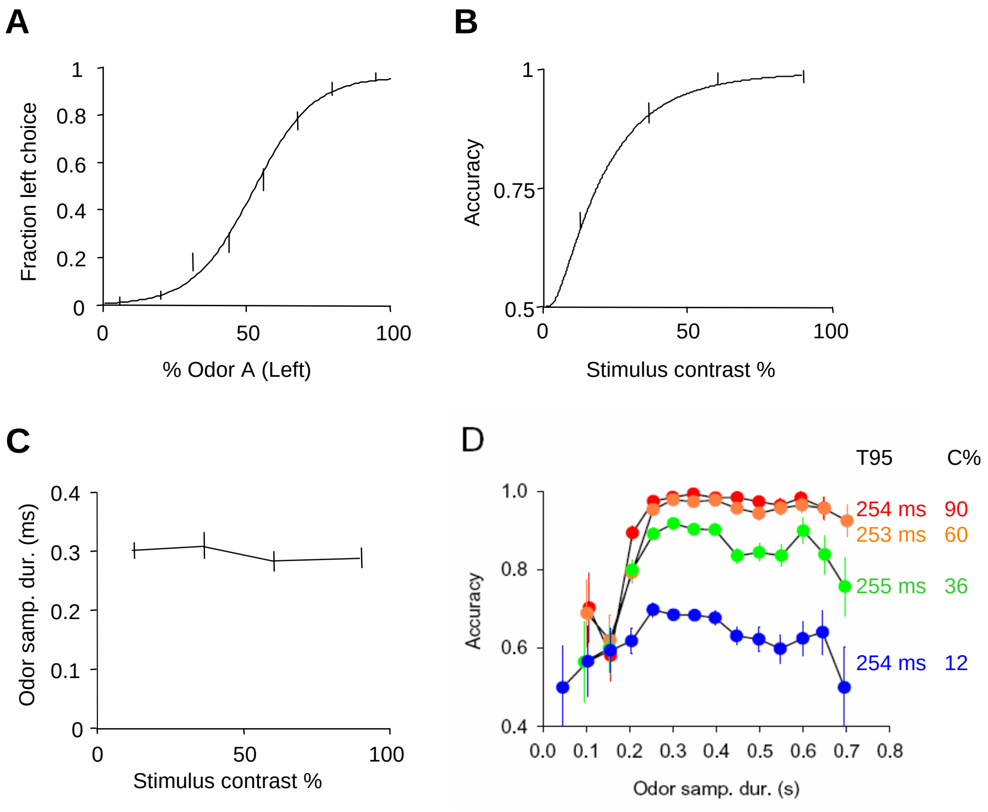


Figure 2 – 4: **Performance and odor sampling time as a function of difficulty & Performance as a function of odor sampling duration**

(A) Psychometric curve of a single rat from 8 consecutive sessions in which all 8 stimulus contrast were presented. Line represents a logistic regression fit to the data. Error bars are calculated from a binomial model.

(B) Difficulty plot: A weibull fit to the performance on individual difficulty (stimulus contrast 12, 36, 60 , 90). Error bars are calculated from a binomial model.

(C) Median odor sampling duration on individual difficulty. Error bars are mean +/- SEM (n = 8 sessions)

(D) Conditional accuracy curve: Performance on each binned odor sampling duration. Trials were pooled from all 8 sessions and binned. Colors denote stimulus contrast (%). Error bars are calculated from a binomial model.

Chapter 3: Motivating rats to trade speed for accuracy.

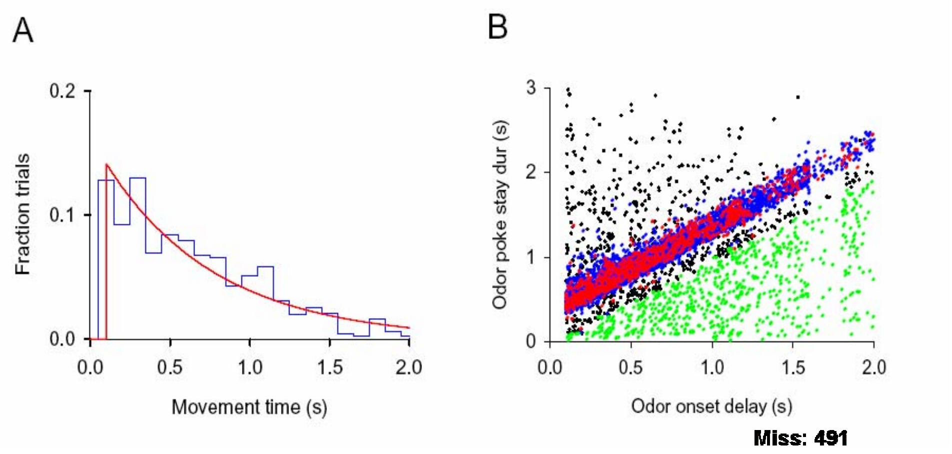
A dependence of accuracy on reaction time suggests that when a subject is motivated or instructed to perform at higher accuracy, she should be able to do so readily by trading speed. It has been (Khan and Sobel, 2004) proposed that rats performing the odor mixture discrimination task (Uchida and Mainen, 2003) tend to perform fast and do not trade speed for accuracy on the difficult problems. They suggested that experiments in which rats are motivated to perform at higher accuracy would resolve the utility of speed accuracy trade offs in this task. The two mice studies (Abraham et al., 2004; Rinberg et al., 2006) made similar proposals and interpreted their results as a sampling duration dependent increase in accuracy. Some (Kay et al., 2006; Rinberg et al., 2006) even suggested that rats do not perform optimally unless a long sampling duration is enforced using a ‘go’ signal. The suggestions of dependence of odor mixture discrimination accuracy on OSD served as a motivation for the subsequent experiments. We carefully controlled various task parameters and introduced variants to the standard task in order to motivate rats to trade speed and/or improve accuracy. In the final experiment in this section we introduce an auditory ‘go’ signal to enforce long OSD (2-3 times longer than the voluntarily achieved reaction times) in order to determined the role of long sampling duration in improving discrimination performance.

Random stimulus delay breaks any stereotypic motor behavior

A rat repeatedly performing large number of trials might develop a stereotypy (rhythmic behavioral pattern), that might constraint the OSD restricting an increase in sampling duration demanded by difficult problems. To break this potential motor stereotypy we introduced a random delay before the odor onset so that rats had to stay in the odor port for a randomly selected time before they are presented with an odor.

Temporal expectancy and stimulus delay: Psychophysicists have used both fixed and random stimulus delay in various experiments (Niemi and Naatanen, 1981; Luce, 1986). In order to control for a fixed expectation of stimulus onset a random stimulus delay is generally chosen. With a randomly interleaved stimulus delays experimenters control the temporal expectancy i.e., expectancy of upcoming stimulus onset, to affect performance and reaction times. Even with randomly interleaved delays the temporal expectancy could vary as a function of the hazard rate calculated from the underlying stimulus delay distribution (see chapter 5). We used an exponentially distributed and randomly interleaved stimulus delay to control for the hazard rate (Luce, 1986). For exponentially distributed delays the hazard rate of stimulus onset is flat. Because the hazard function is flat, the time elapsed since the warning signal does not affect at all the momentary tendency for the reaction signal to appear. And because the reaction signal can occur at any time, it is very difficult to see how the subject can make any successful use of time estimates, therefore, controlling temporal expectancy.

The mean of the chosen exponential distribution was 0.5s and ranged between 0.1 – 2.0s (figure 3-1A). Rats were therefore forced to wait in the odor port for a randomly selected long time before odor onset. In trials where they failed to stay through the fore period, the trials were aborted and rats had to wait out the 4.0s inter-trial interval. Figure 3-1B shows that the total odor poke duration scaled with the stimulus delay preceding the odor onset.



C

	Standard RT task	Low urgency RT task
Odor onset delay	$t_b - t_a = U\ (0.3s, 0.6s)$	$t_b - t_a = \lambda e^{-\lambda x}$; $1/ \lambda = 0.5s$
Water Delay	$t_e - t_d \geq 0$	**$t_e - t_d \geq 2$**
Inter trial interval	$t_f - t_e \geq 4$	$t_f - t_e \geq 10$
Odor sampling time	$t_c - t_b$	
Movement time	$t_d - t_c$	

Figure 3 – 1: **Low urgency task & control of motor stereotypy**

(A) A random odor onset delay was chosen from an exponential distribution with mean 0.5s and clipped between 0.1s and 2.0s. Blue: experienced odor onset delay Red: λe-λx; 1/ λ = 0.5s

(B) Breaking motor stereotypy: The odor port stay duration of 4 rats Eat dot represents a single trial. Green: invalid short sampling trials, Blue: correct valid trials, Red: incorrect valid trials, Black: No response valid trials

(C) Comparison of task parameters in the standard task and a task with low urgency. Urgency to respond was reduced by delaying the water reward by 2.0s from the time of odor onset (in bold).

Accuracy did not improve for naïve rats trained on reduced urgency

In a standard task, rats were rewarded with water on successful trials as soon as they made a choice at one of the two choice ports. The urgency to respond at the choice port could constraint the OSDs. We introduced a 2.0s fixed delay to water delivery starting from the time of stimulus onset in order to reduce the urgency to respond and encourage longer sampling durations.

We trained four naive rats with a 2.0s fixed delay to water reward on the first day they ever experienced odors. The delay was fixed from the time of odor onset. This was followed by training on two odor discrimination task and the odor mixture discrimination task with a 2.0s fixed water delay.

The results in figure 3-2A&B for example rat and 3-2C&D for average across all four rats show the discrimination performance and the OSD. Early training with a 2.0s fixed water delay had an effect on the OSDs of rats. The OSDs increased on an average by 50 ms compared with the rats in the standard task. The accuracy of the same rats was not significantly different from those of rats trained on the standard task.

These results for the first time indicated that rats responded to the instruction of reducing their speed. The increase in reaction time did not affect accuracy suggesting dissociation between reaction time and discrimination performance.

Air puff punishment increased sampling duration but not the accuracy

Punishments for a response affects behavior in many ways like unconditioned fear response, response suppression or escape response. Mild punishments in the form of electric shocks can drive learning (Warden and Aylesworth, 1927) and has been shown to improve discrimination by decreasing the error rates in white rats (Muenzinger, 1934). See Church 1963 for a review of this literature. We introduced an immediate and aversive punishment in the form of an air puff to the snout of the rats for an error choice. Air puff are mild aversive punishments and are known to decrease the value of response (Paton et al., 2006). The air puff punishment was delivered using a thin opening inside the reward port just above the water delivery tube. The air puff punishment was introduced after four new rats learned the basic two alternative discrimination task using pure odors. In case of a correct choice the water reward was available after a randomly delay of 500 - 800 ms from the time of the choice port entry but for the error choices the rats were puffed with air immediately following a choice port entry. The air-puff lasted only 100ms. The airflow rate was maintained at 2 liters per minute using a regulated pressure valve.

An air puff punishment successfully provided a strong motivation for rats to trade speed. Fig 3-3 A, B shows the comparison of the performance accuracy and OSD for the four rats in this task with the rats in the standard task. The averaged median OSD in this task increased on average by 100ms for all the mixture ratios tested. Interestingly, there was no increase in performance accuracy.

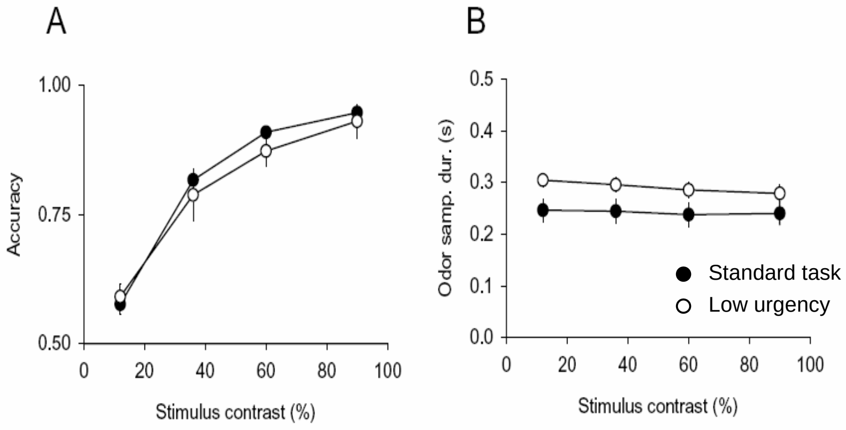


Figure 3 – 2: **Effect of low urgency on the performance accuracy and odor sampling duration.**

(A) Mean fraction performance accuracy of rats on each of the four difficulties. Filled circle for rats tested on the standard task and open circle for rats trained with low urgency. Error bars are mean +/- SEM (n = 4 rats).

(B) Mean across rats of the median odor sampling duration on each of the four difficulties. Filled circle for rats tested on the standard task and open circle for the rats trained with low urgency. Error bars are mean +/- SEM (n = 4 rats).

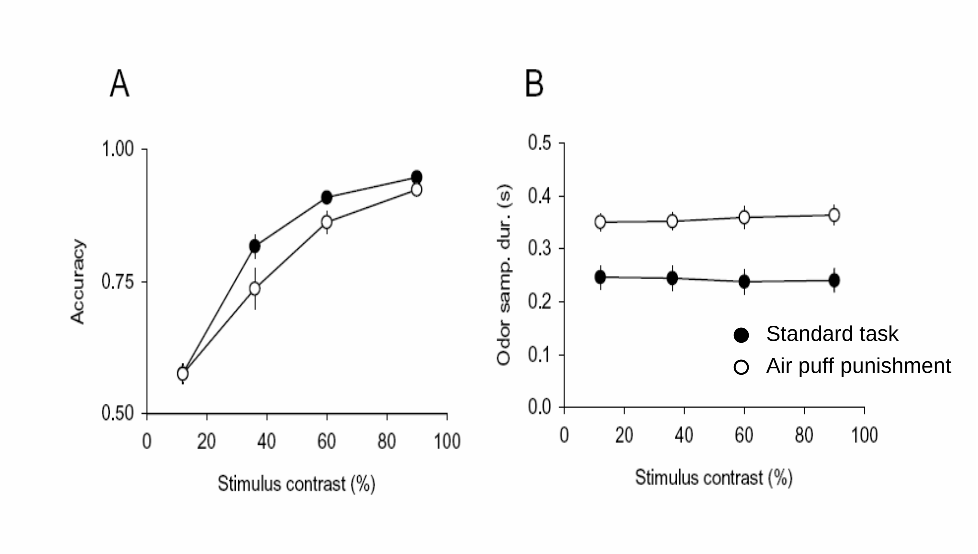


Figure 3 – 3: **Effect of air puff punishment on the performance accuracy and odor sampling duration.**

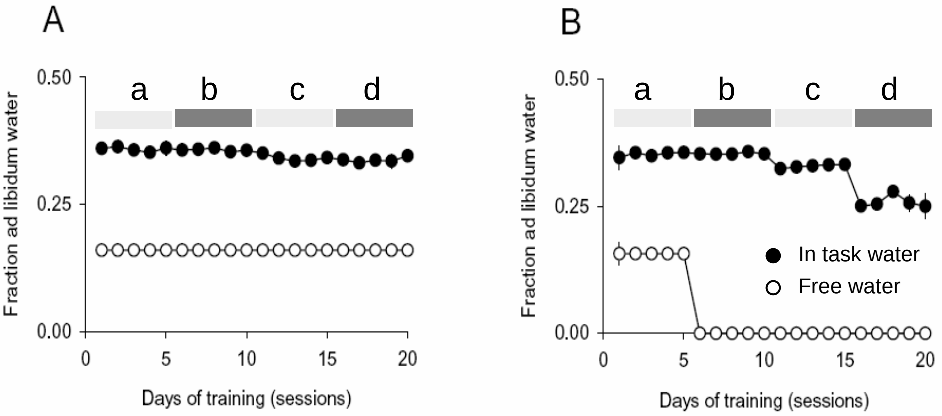
(A) Mean fraction performance accuracy of rats on each of the four difficulties. Filled circle for rats tested on the standard task and open circle for the rats trained with an air puff punishment for errors. Error bars are mean +/- SEM (n = 4 rats).

(B) Mean across rats of the median odor sampling duration on each of the four difficulties. Filled circle for rats tested on the standard task and open circle for rats trained with an air puff punishment for errors. Error bars are mean +/- SEM (n = 4 rats).

Careful regulation of water intake of rats

The speed accuracy trade off function of rats in this task could be affected by the expectation of total reward that the rats receive in the task as compared to outside the task. In addition, other factors like the value of a single reward received per trial, the loss of water due to errors and the cost benefit trade offs of improving performance on the difficult stimuli. In order to affect these contingencies we carefully monitored the water intake of each of the rats and manipulated the water inside and outside the task in a systematic manner (see methods for the protocol for controlling water intake in this task).

Testing procedure: All the rats were divided equally into two groups, a test group and a control group. Both groups went through four test phases as outlined in Fig 3-5 A, B. After training on five successive sessions in the baseline condition (phase ‘a’) we took away the free water from the test group. This constituted the phase ‘b’ of testing. In phase ‘c’ the number of hard stimuli were increased to twice as many keeping the total number of trials constant. Since this manipulation itself can affect accuracy (for reasons emphasized in the next chapter) we made this change for the control group rats too, except that the control group continued to receive free water outside the task. In the final phase ‘d’, we introduced an error time out punishment for the test group and reduced their total session time per day to 30 minutes or 256 trials, which ever came first. The water reduction in the phase ‘c’ due to increase in the number of difficult stimuli was calculated to be 10% of their in-task water if the rats maintained their error rate. In the third step the water amount reduced further because of the long error time out and reduced session duration.



C

	Control group	Test group
Phase 'a'	Free & task water	Free & task water
Phase 'b'	Free & task water	Only task water
Phase 'c'	Free & task water + 2X difficult stimuli	Only task water & 2X difficult stimuli
Phase 'd'	Free & task water + 2X difficult stimuli	Only task water + 2X difficult stimuli 15.0s Error ITI + 30 minute session

Figure 3 – 4: **Increasing the value of water reward inside the task.**

(A) Control group of rats: fraction of ad libidum water consumption provided in task (filled circle) and outside the task (open circles). Error bars are mean +/- SEM (n = 4 rats). Error bars are smaller than symbols

(B) Test group of rats: fraction of ad libidum water consumption provided in task (filled circle) and outside the task (open circles). Error bars are mean +/- SEM (n = 4 rats).

(C) Four phases in which the control and test groups were tested.

Choice of error time out: To determine the length of error time out in phase ’d’, we calculated the averaged median experienced inter trial interval for the test group in phase ‘a’. An experienced inter-trial interval was the duration between odor onsets on two successful trials. Rats learned the enforced 5.0s inter trial interval in the baseline condition and attempted to initiate the next trial roughly around 5.0 s after odor onset. Their median experienced inter-trial interval in phase ‘a’ was 7.55s +/- 0.3s. We also measured how soon these rats were ready to initiate their next trial by measuring the duration between odor onset and the next “attempted” i.e., both successful and unsuccessful trial initiations (odor port entry). The median of this duration was 5.4s +/- 0.3s for the test group. Given how reliably rats learned their imposed ITI, we chose a 15.0s error time out, i.e., three times the enforced inter-trial interval in the baseline condition. We compared the performance and reaction times of test and control groups in the three test phases.

Effect of taking away free water: Test group lost weights (as shown in fig 3-5 A) when the free water was taken away. The loss of weight and free water should increase the value of water rewards inside the task and motivate rats to perform each stimuli more accurately. We compared the discrimination accuracy and OSDs of these rats with that of the control group that continued to receive free water (figure 3-5 B, C). The discrimination performance and the OSD did not change ($P > 0.05$, Kruskal Wallis test).

Effect of taking away free water and increasing the number of hard stimuli: The motivation for the test group was increased by introducing twice as many hard stimuli (56/44 mixture ratios) compared to the baseline condition. Therefore, rats had to perform 128 trials of hard stimuli and 128 trials of other stimuli (68/32, 80/20 & 95/5) out of 256 total trials. Changing the number of stimuli in a task could affect the performance independent of the motivation to perform better. Therefore, the control group also faced the same task except that they were given free water.

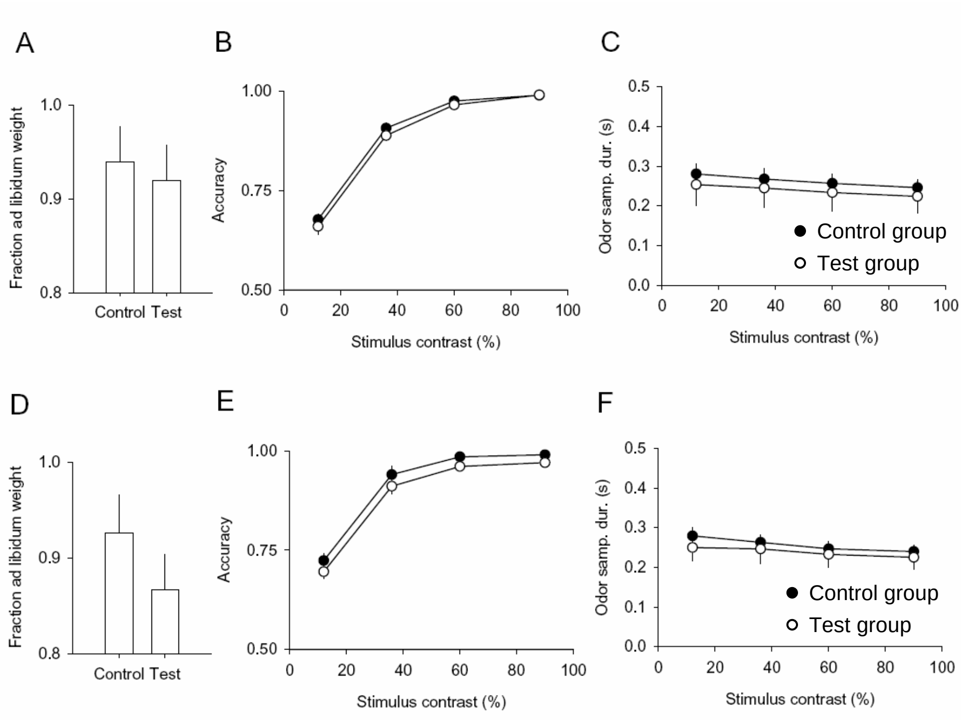


Figure 3 – 5: **Effect of taking away free water and punishing errors with long time outs on the accuracy and OSD.**

(A) Phase ‘b’: Mean weights are represented as fraction of the weights with ad libidum food and water. The weights of test group rats decreased more than that for the control group when the free water was taken away from the test group rats. Error bars are mean +/- SEM.

(B) Phase ‘b’: Mean fraction performance accuracy of rats on each of the four difficulties. Filled circle for control group rats and open circle for the test group rats (no free water). Error bars are mean +/- SEM (n = 4 rats).

(C) Phase ‘b’: Mean across rats of the median odor sampling duration on each of the four difficulties. Filled circle for control group rats and open circle for the test group rats (no free water). Error bars are mean +/- SEM (n = 4 rats).

(D),(E) & (F) are the same as (A),(B) & (C) respectively but for Phase ‘d’ of training.

We compared the performance accuracy and OSD of test group against the control group in this phase. The accuracy and the OSD across the two groups was not significantly different ($P > 0.05$, Kruskal Wallis test). We also compared the performance and OSD of test group across phase ‘b’ and ‘c’ and found no significant difference across these groups (data not shown). The same analysis was done for the control group (data not shown).

Effect of introducing long time outs for error choices: The error rates of rats in both groups in phase ‘c’ were identical to the baseline condition. The effect of introducing higher frequency of hard problems in phase ‘c’ affected the in-task water by small fraction (~3% of their ad libidum water consumption). In this phase of testing we introduced a long time out for error choices only for the rats in the test group. This was in addition to no free water and the new stimulus distribution introduced in previous phase. The total session duration for each rat in the test group was also reduced to 30 minutes to further affect the total number of trials the test group rats could perform in a session.

Rats in the test group continued to lose weight in this test phase. We terminated the data collection after 5 sessions in this phase as the average weights reduced to around 87% of their weights on ad libidum food and water. The performance accuracy and OSD did not improve significantly (Fig 3-8 A, B) across the control and test group in this phase. The total water received in a session for the test group reduced markedly as shown in Fig 3-5 A. This manipulation therefore affected the water received by rats in the task due to fewer available trials brought in effect by the long error time outs and shorter sessions. This should have increased the value of each water reward and provided strong motivation to trade speed for accuracy.

In order to test if the rats have a sense for error time out punishment, we measured the duration between a choice port exit and the attempted initiation of the next trial following a correct or an error choice. The significant difference in the distribution of these durations for error and correct choices in presence of long error time outs indicate that the rats learned the long time outs. The distribution of these durations was not significantly different in the control case (figure 3-6).

The absence of any improvement in the performance accuracy and the inability to increase the OSD by the rats in the test group while they continued to lose free water, in-task water and weight argues strongly that the speed accuracy trade off function for rats in this task is not dependent on the value of water reward in this task. Even with depleting energy and metabolism rats failed to improve their accuracy or show any change in OSDs.

The results from the experiments so far suggest that the accuracy in this task is not dependent on motivation to perform the task. Rats did trade speed when punished with an air puff or when the urgency to respond was reduced by a fixed water delay. But these OSD increases did not translate into an increase in accuracy. Together, the data suggest a dissociation of speed and accuracy in the reaction time version of odor mixture discrimination task.

In the next experiment we directly controlled the OSD of rats by training them to continuously sample odor stimuli and respond only after a ‘go’ signal. We wanted to test if rats can improve accuracy of discrimination when forced to sample odors for a long duration (say up to 1.0s) not normally obtained in a RT task when rats voluntarily cease odor sampling.

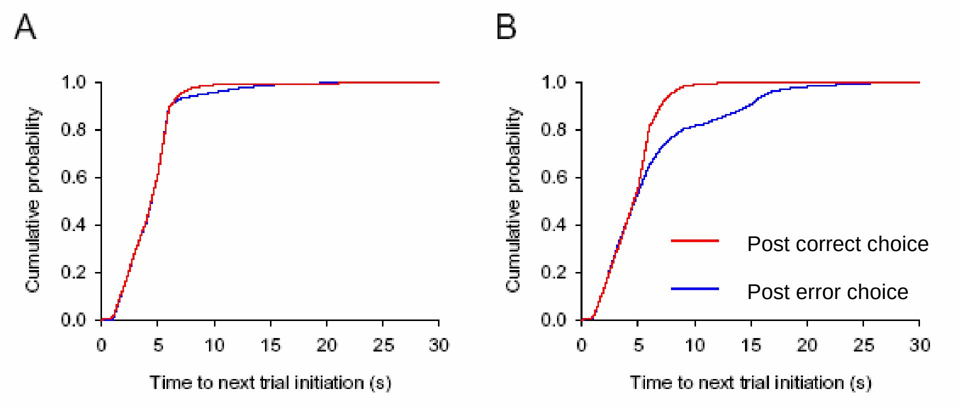


Figure 3 – 6: **Rats in the test group learned the error time out and delayed their attempted next trial initiation in case of error choice.**

(A) Sessions with no error time out: Cumulative probability distribution of duration between attempted trial initiation and previous trial's odor onset. Red line for trials with correct choices and blue for error choice trials.

(B) Sessions with 15s error time out: Cumulative probability distribution of duration between attempted trial initiation and previous trial's odor onset. Red line for trials with correct choices and blue for error choice trials

‘Go’ signal experiment: effect of directly manipulating the OSD using a ‘go’ signal

Training: Four naive rats were trained on a ‘go’ signal paradigm. A typical trial is as outlined in fig 3-9A. Rats learned to respond after an auditory ‘go’ signal which was delayed by a fixed time. Once achieving sufficient training on the ‘go’ signal response, rats were trained to discriminate the two pure odors. Rats were then trained on a complete mixture discrimination task using three different pairs of mixture ratios.

Test procedure: The test procedure included repeating the mixture discrimination task using a single fixed delay to the ‘go’ signal over a few days. After a few days of training the delay was switched to another value and training continued for another few days till rats reliably learned the new fixed delay. The sequence of switching delays to ‘go’ signal is outlined in figure 3-7B.

Results: The data for each delay to the ‘go’ signal was pooled over all identical sessions. We plotted the performance accuracy and OSD for individual ‘go’ signal delay (figure 3-7C, D). The performance accuracy and OSD from standard RT version of the task is also plotted for comparison. The median OSD of rats in this task ranged from 400ms – 900ms but the performance accuracy did not change with long sampling duration ($P > 0.05$, Kruskal Wallis test).

We also plotted the conditional accuracy function pooled across all rats for each of the delay to the ‘go’ signal overlapping on a single plot (Jennings and Wood, 1976) to study the speed accuracy trade off (Figure 3-8 C). Accuracy did not improve as a function of odor sampling durations ranging from 0.1 – 1.0s.

We wanted to test if accuracy improves with longer sampling durations in the early half of the sessions before rats learn to anticipate the ‘go’ signal after a fixed delay. Well anticipated fixed ‘go’ signal can cause a stereotypic sampling

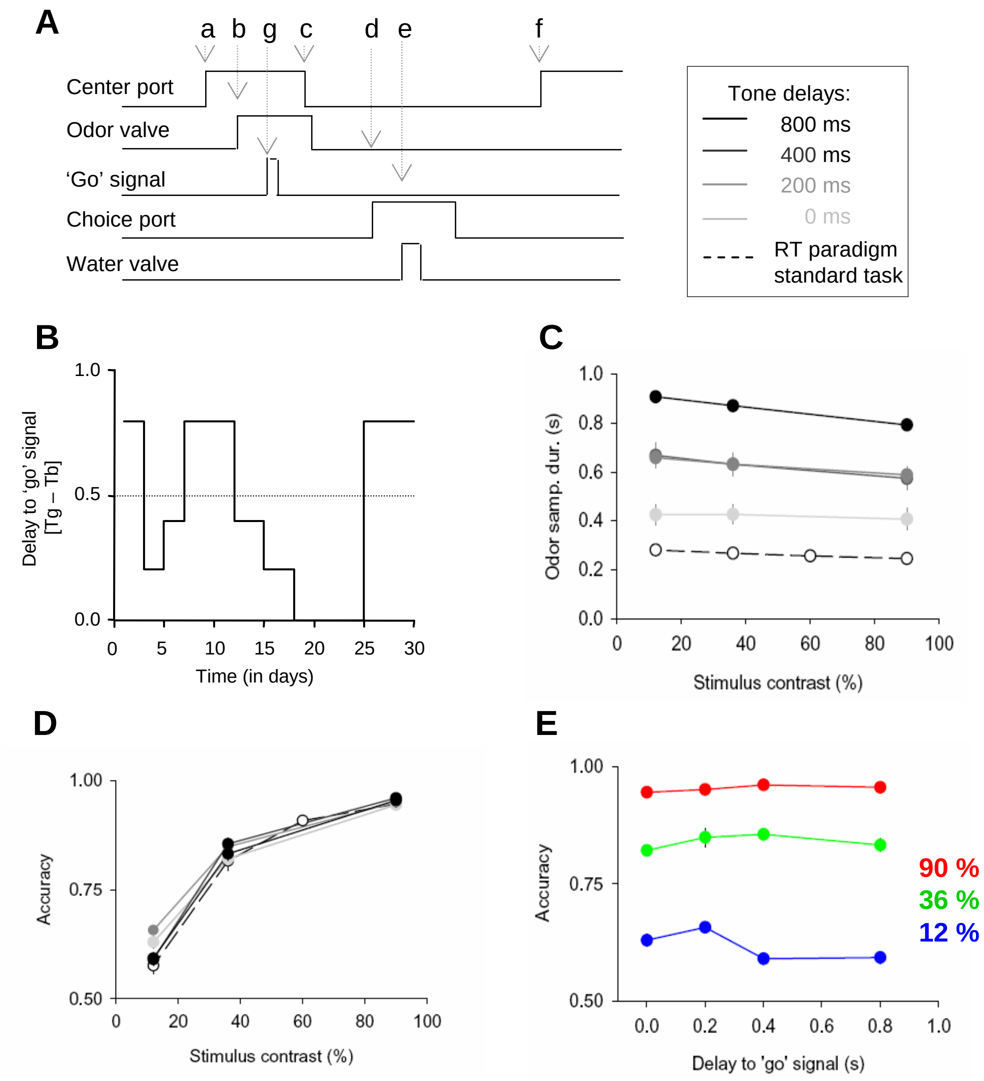


Figure 3 – 7: **Effect of long odor sampling durations instructed with a 'go' signal on the performance accuracy of rats.**

(A) Schematic of a 'go' signal task. 'g' is the time to a 'go' signal presentation. Events a-f are the same as the standard task.

(B) Day-wise change in the delay to the 'go' signal. The delays were kept constant for a few days (3-6) before changing to a new delay.

(C) Mean across rats of the median odor sampling duration on each of the three difficulties. Shades of grey represent different 'go' signal delays (see legend). Also shown are OSD of four rats that performed the standard RT task (open symbols). Error bars are mean +/- SEM (n = 4 rats).

(D) Mean fraction performance accuracy of rats on each of the four difficulties. Symbols and colors same as (C). Error bars are mean +/- SEM (n = 4 rats).

(E) Accuracy on each of the stimulus contrast (difficulty) as a function of delay to the 'go' signal. Error bars are mean +/- SEM (n = 4 rats).

pattern to develop over the course of the session, which could limit the utility of long sampling durations on accuracy. In Figure 3-8A we show that accuracy on each of the stimulus contrast did not vary as a function of trial number within a session.

We noticed (Figure 3-7 C) that the effect of difficulty on odor sampling duration (difference between the median OSD for hard and easy problems) scaled with the delay to ‘go’ signal. In the absence of any effect of long sampling duration on accuracy we cannot reconcile this difference to be due to difficulty. We normalized the difficulty effect to the median OSD in each session for each of the rats and plotted it as a function of this median OSD (Figure 3-8 B). We found that normalized difficulty effect did not vary with median OSD (correlation coefficient $r = -0.011$ $P < 0.912$). This means that the so observed difficulty effect is a function of median OSD obtained for each of the delay to ‘go’ signal and is not actually a function of stimulus difficulty. A difference of OSD for hard and easy problems could be due to motivational differences due to the reward rate differential. We know that subjects respond with less urgency on trials where the reward rates are lower or when actions are not rewarded. More experiments are needed to establish the origin of this difficulty effect seen even in RT paradigms. One testable hypothesis is that motivational effect could scale with the ‘go’ signal delay. Motivational effect on OSD can be studied in a 1-DR experiment (Lauwereyns et al., 2002) where mandatory responses on one side are rewarded while mandatory responses on the other side are not. In a modified1-DR experiment with fixed ‘go’ signal delays we can test how the motivational effect on reaction times scale with the delay to a fixed ‘go’ signal.

Together the results from the ‘go’ signal experiment are further confirmation that the accuracy of odor mixture discrimination does not depend on the OSDs and that in both RT version as well as direct sampling duration manipulation version of the task; the accuracy is dissociated from the speed of performance.

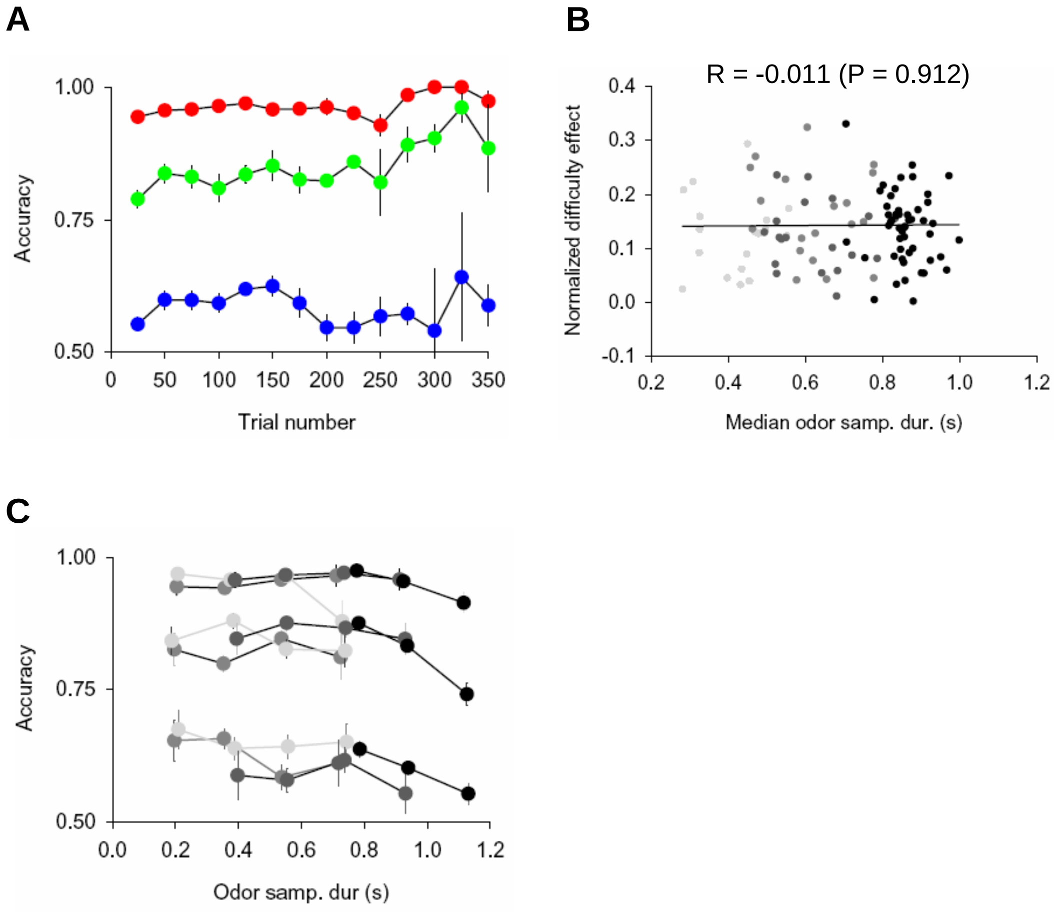


Figure 3 – 8: Stability of performance, difficulty effect and CAFs

(A) Performance accuracy was stable over the course of the session on each of the mixture ratio tested. Each data point is mean +/- SEM (n = 4 rats)

(B) Effect of difficulty on OSD (Mean difference across the ‘easy’ and ‘hard’ problems) scaled as a function of median OSD in each session. Individual data point are from individual sessions of four rats. Color codes (light grey to black) signify delay to ‘go’ signal.

(C) Condition accuracy functions for trials pooled across all rats on each of the ‘go’ signal delays. Color codes signify delay to ‘go’ signal. Error bars are SEM calculated from binomial model.

Chapter 4: Stimulus expectancy and accuracy

In all our experiments so far we presented rats with all difficulties interleaved within one session. This provided us with (a) a rapid method for studying the psychometric performance of rats within a session and (b) to measure the effects on OSDs independent of any change due to the procedural learning over days of training. Despite of the advantages, interleaving the problems limits the ability to motivate higher accuracy on the difficult ones, especially if the benefits associated with solving them are relative insignificant. With a careful control of water intake inside and outside the task, we attempted to surmount that problem and motivate rats to perform better especially on the most difficult stimuli. Though rats lost weight they failed to improve accuracy on difficult problems. Contrarily, mice (Abraham et al., 2004) showed a strikingly high accuracy on all difficulties. Are rats in our study not motivated enough? Or are there differences in the two tasks that could account for the observed results?

Mice performing odor mixture discrimination in Abraham et al (2004) were faced with single difficulty in a session. Taking this difference into account we performed an experiment in which we presented rats with only one difficulty at a time along with performing our standard task and compared their performance accuracy and OSDs. In doing so, we assumed that we simultaneously manipulated two factors, (a) increased the motivation by decreasing the overall reward rate, which is proportional to the difficulty of the problem and (b) provided a context in which they could focus their limited attentional resources on a single problem at a time or in other words increased the expectancy of upcoming stimuli in a session. Both of which could lead to better sensory processing. In case, this manipulation motivated rats to improve accuracy in a speed dependent manner we expected to observe an improvement in accuracy at the cost of speed. On the other hand if it improved expectancy of the upcoming stimuli, we hoped to observe a speed independent effect on accuracy. The case of latter observation would reconcile our results from the motivation study with those of the mice study.

Presenting single difficulty in a session

Six naive Long Evans rats were trained in a reaction time paradigm to perform discrimination of pure odors i.e., enantiomers of 2-octanol. Once they achieved enough training on the basic two alternative discrimination task, other odor mixtures (stimulus contrasts) were introduced and rats were sufficiently trained on all 8 mixtures in a session before the test phase.

Experimental design: We designed a task where rats were tested on the interleaved difficulties before and after testing them on each of the four difficulties alone in a session (non-interleaved). The difficulty in the non-interleaved condition was increased in consecutive stages of testing. Figure 4 – 1 outlines the course of training over days, at the same time it shows the mean discrimination accuracy across rats on each of the four stimulus contrast.

Accuracy improved in the non-interleaved context

The accuracy improvement in the non-interleaved context was (a) transient i.e., specific to the presented context and dropped back to a lower level in the interleaved context (figure 4-1) (b) rapid i.e., the accuracy improved significantly in the non-interleaved context within the first 25 trials that the rats performed (figure 4-2,A). In addition to rapid improvement within 25 trials the

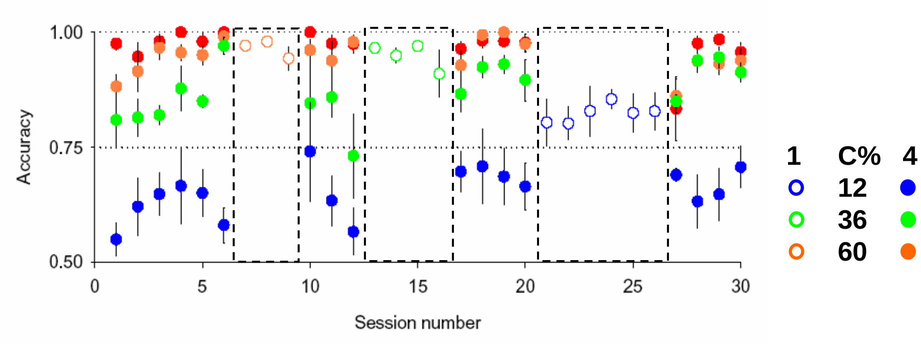


Figure 4 – 1: **Experimental paradigm to test performance of rats in conditions with interleaved and non-interleaved difficulty.**

(A) Mean performance accuracy of rats over the course of 30 days of training. Filled circles - sessions with interleaved difficulty and open circles with dotted box represent sessions with non-interleaved difficulty. Shown here is the mean accuracy on the last 100 trials only. Error bars are mean +/- SEM (n = 6 rats).

performance in the non-interleaved context improved over the course of the session. In the next stage we compared the accuracy and OSD of rats in the two different contexts for the last 100 trials in a session

Accuracy improved at no cost of speed

We compared the best accuracy of rats in the non-interleaved context (last 100 trials) with the best accuracy (last 100 trials) in the interleaved context (figure 4-2B). Accuracy improved remarkably in the context of non-interleaved stimulus contrasts for the most difficult ratio (0.82 +/- 0.03 to 0.63 +/- 0.06, for 12% stimulus contrast, n = 6 rats, Kruskal Wallis test). Interestingly, when we compared the OSDs for the same trials in the two different context (figure 4-2C), we did not see any change in the median OSDs (324ms +/- 21 compared to 302ms +/- 14, for 12% stimulus contrast n = 6 rats). Figure 4-2D shows that while accuracy increased on each of the stimulus contrast, it did not affect the speed of discrimination.

We studied the dependence of accuracy in the two contexts on the OSD by plotting the conditional accuracy curve for the 12% stimulus contrast (figure 4 – 3). We see no effect of OSD beyond 275ms. The T95 values are identical in both contexts. The availability of the complete distribution of OSDs of rats in our tasks (as compared to the stay/no-stay paradigm) allowed us to perform these analyses and show the dependence of accuracy on sampling durations.

In the stay/no-stay paradigm reaction times are assessed only from half the total trials i.e. the no-stay trials. The difference in the reaction times is assessed from the tail of the distribution. In addition to the non-availability of reliable estimates of reaction times, the time taken for rats to pull out of the sampling port (no-stay trials) includes an extra time related with the motivational component, i.e. the extra time subjects take to react on non-rewarded trials

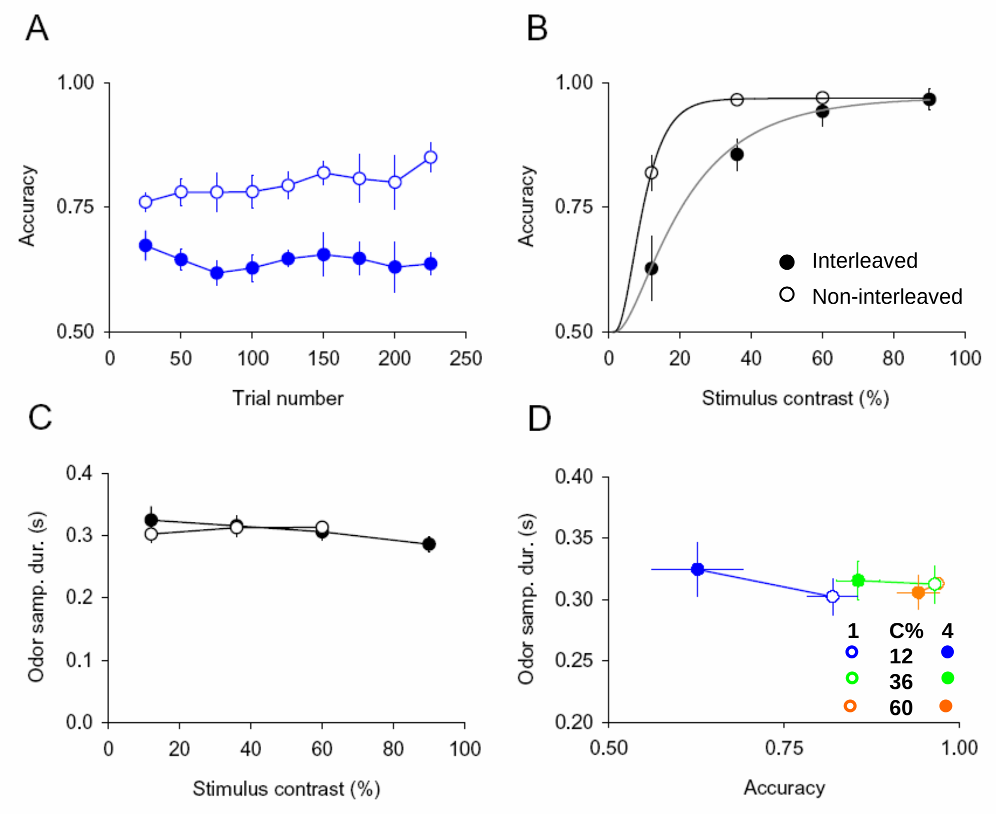


Figure 4 – 2: **Stimulus expectancy affects performance accuracy at no cost of speed**.

(A) Performance improves rapidly and over the course of the session in case of the session in non-interleaved difficulty. Mean performance accuracy on the most difficult mixture ratio (12% stimulus contrast) for a block of 25 trials over the course of session. Filled symbols – session with interleaved difficulties; open symbols – session with non-interleaved difficulty. Error bars are mean +/- SEM (n = 6 rats)

(B) Mean performance accuracy as a function of stimulus contrast (difficulty). Line denotes a Weibull fit. Legends same as in (A). Error bars are mean +/- SEM (n = 6 rats).

(C) Mean odor sampling duration as a function of stimulus contrast (difficulty). Legends same as in (A). Error bars mean +/- SEM (n = 6 rats).

(D) Comparison of the speed and accuracy in the two condition. Each data point is mean performance accuracy across rats. Filled and open symbols as in (A). Each difficulty is represented with a different color. Error bars are mean +/- SEM (n = 6 rats).

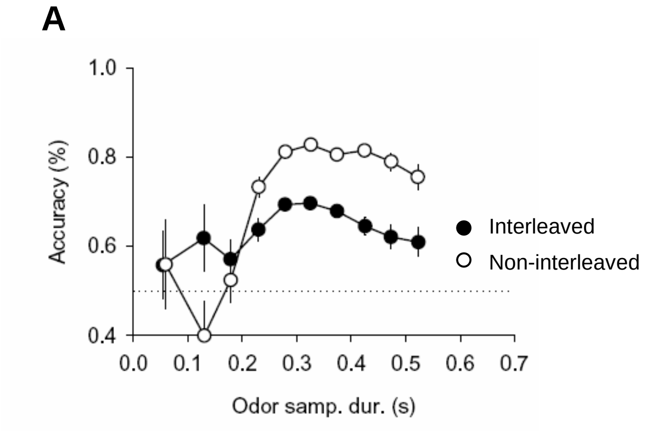


Figure 4 – 3: **Accuracy was affected by stimulus expectancy not odor sampling duration**

(A) Mean performance accuracy on 12% stimulus contrast as a function of odor sampling duration . Filled circles represent sessions with interleaved difficulty and open circles represent sessions with non-interleaved difficulty. Dotted vertical line represents T95 values. T95(interleaved): 277ms and T95(non-interleaved): 278ms. Error bars are calculated from a binomial model.

(Lauwereyns et al., 2002; Matsumoto and Hikosaka, 2007). The motivational consequence confounds the increase in RT observed (Abraham et al., 2004).

Accuracy improvement was independent of training

Perceptual learning of a feature through practice over an extended period could have a lasting effect on the subject's ability to discriminate stimuli based on that feature (Crist et al., 1997; Kuai et al., 2005). Would rats retain their ability to discriminate the most difficult ratio in an interleaved context once trained sufficiently in the non-interleaved context? Four rats were trained on the non-interleaved context for up to 8 sessions on each difficulty and the difficulty was increased in a step-wise manner to up to a 2% stimulus contrast i.e., an odor mixture ratio of 49/51. This data is also part of another experiment detailed in chapter 5 (figure 5-7 & for training history see figure 5-6). Once the rats achieved enough training on non-interleaved condition we tested them for 5 consecutive sessions on interleaved context. Mean accuracy as a function of difficulty is plotted in figure 4-4. The accuracy on the most difficult ratio dropped to 0.73 +/- 0.03 in the interleaved context from 0.9 +/- 0.02 in the non-interleaved context.

This is an important result for our understanding of the basis of decision making process in this task. The speed independent accuracy improvement opens avenues to explore the role of stimulus expectancy like mechanisms in perceptual discrimination. At the same time this effect explains the difference in accuracies in our motivation study interleaved-context and mice study non-interleaved context (Abraham et al 2004). Mice always performed at high accuracy on difficult discriminations consistent with the rapid and transient adaptation seen in non-interleaved context. This result in conjunction with the motivation study indicates that accuracy of odor mixture discrimination is independent of the motivation and the speed of performance.

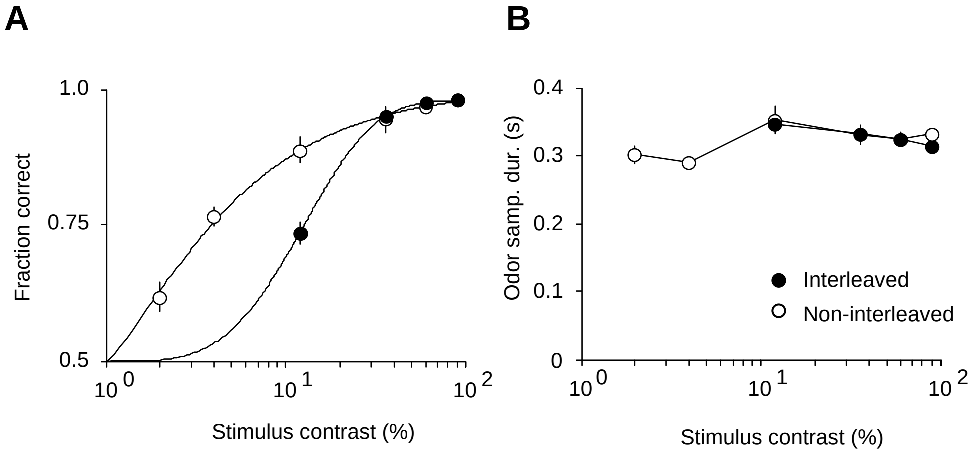


Figure 4 – 4: **Effects of stimulus expectancy are independent of training.**

(A) Mean performance accuracy as a function of stimulus contrast (difficulty). Line denotes a Weibull fit. Filled circles represent sessions with interleaved difficulty and open circles represent sessions with non-interleaved difficulty. Error bars mean +/- SEM (n = 4 rats).

(B) Mean odor sampling duration as a function of stimulus contrast (difficulty). Legends same as in (A). Error bars mean +/- SEM (n = 4 rats).

Chapter 5: Temporal anticipation and accuracy

Stimulus expectancy can affect perceptual accuracy in a speed independent manner. Higher stimulus expectancy can improve the ability recruit the limited attention resources. We have provided a clear demonstration of such an effect in the previous chapter. Stimulus expectancy can also be modulated for a spatial location or to moments in time. There are many studies in psychophysics where attention is recruited (Griffin et al., 2001; Nobre, 2001)) to a specific moment in time by increasing the probability of stimulus presentation. This is akin to a batter striking a ball based on his expectancy of the arrival time of the ball. Can temporal expectancy also affect accuracy in a perceptual task?

In a 'go' signal paradigm rats were forced to sample odors for 2-3 times longer than their voluntary reaction time and still produce no improvement in accuracy (figure 3-6E). This result is in contrast with the mice study (Rinberg et al., 2006) in which mice performing long sampling durations in the 'buzz paradigm' benefited in terms of their accuracy with sampling durations (OSDs) of over 700ms. What accounts for the difference in our results compared to the mice study? In the next set of experiments we demonstrate that the difference in the results is explained by the way the 'go' signals (buzz) were presented within a session; parametrically modulating the temporal expectancy of the 'go' signal.

The random go-signal paradigm (Rinberg et al., 2006) requires the animal to not only weigh sensory evidence, but also time its response in relationship to the external auditory go-signal because the go-signal can occur randomly. The anticipation of the response deadline or 'go' signal in this task is modulated by changing the behaviorally relevant probability of ‘go’ signal occurrence called the hazard rate (Janssen and Shadlen, 2005). A hazard rate is defined as the probability of an event (‘go’ signal) at various time instants (during odor sampling) given that it hasn’t occurred yet (figure 5-1A, B). For a uniformly distributed ‘go’ signal as in Rinberg et al., the hazard rate associated with ‘go’ signal occurrence increases (exponentially) with time. Our hypothesis was that the increasing accuracy in the mice study was a function of the increasing hazard rate (temporal anticipation) and not temporal integration of sensory evidence (speed accuracy trade off).

In order to demonstrate the effect of temporal anticipation on accuracy we used two different ‘go’ signal distributions (uniform and exponential) in a task. These chosen ‘go’ signal distributions had a rising or a flat hazard rate associated with them (figure 5-1 A, B). Our aim was to study the performance accuracy under both conditions and control the temporal anticipation of the ‘go’ signal using a flat hazard condition to isolate the effect of OSD on odor mixture discrimination.

Anticipation of a salient stimulus can help orient attention and improve perception. Anticipation can be modulated dynamically by changing the experienced probability, spatial location or the temporal instant of stimulus occurrence. Temporal anticipation is considered an allocation of attention in time (Nobre, 2001; Ghose and Maunsell, 2002).

Orienting attention to temporally defined moments affects neural activity in the primary sensory cortex (Ghose and Maunsell 2002). Attention affects processing of perceptual stimuli and could improve perceptual sensitivity (McDonald et al., 2000; Correa et al., 2006)) while it could also affect processes beyond sensory processing like motor preparation. Anticipatory sniffing (Zelano et al., 2005) has been shown to affect activity in human primary olfactory cortex even when there odor stimulus was omitted.

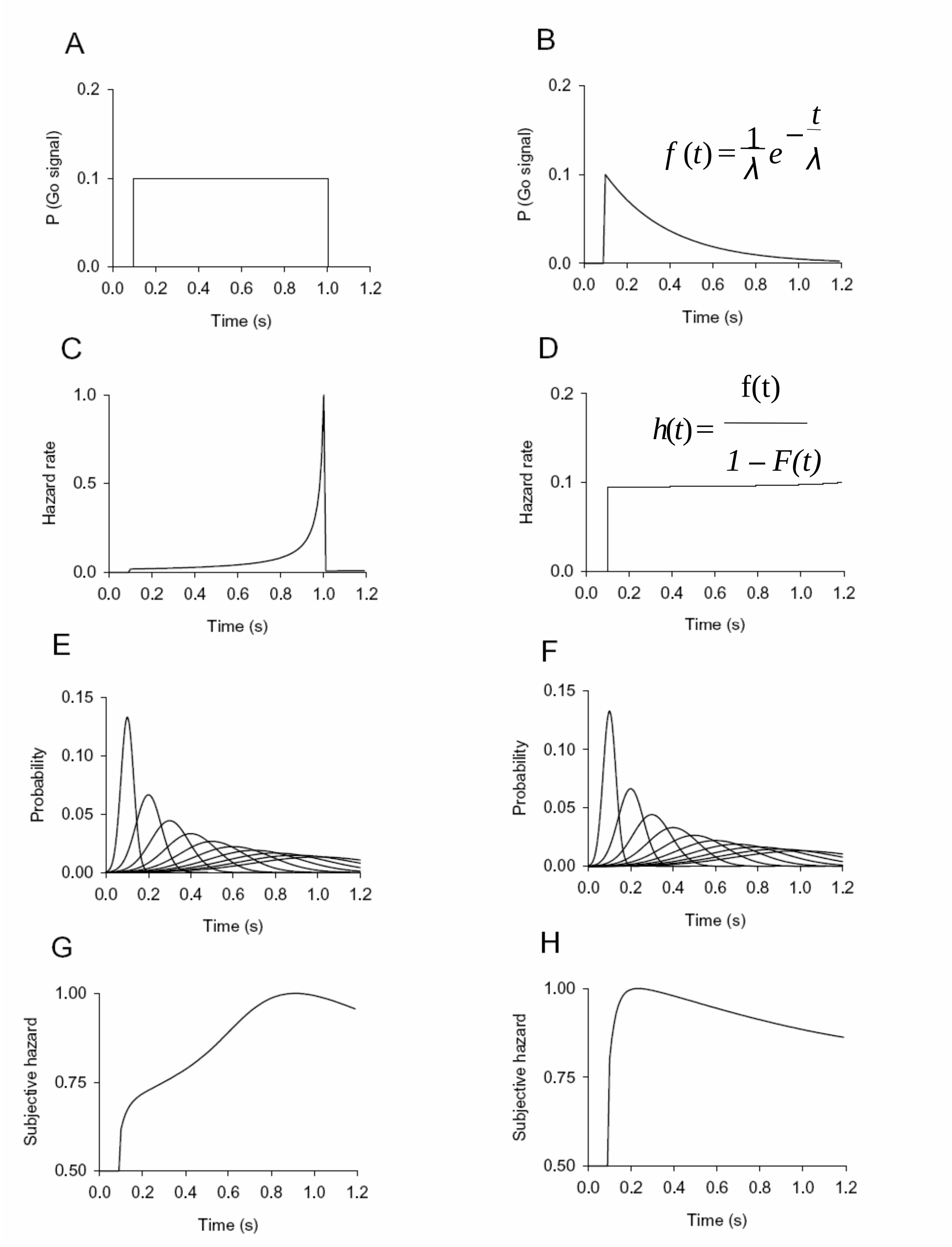


Figure 5 – 1: **Interleaved ‘go’ signal delay affects temporal anticipation.**
(A & B) Theoretical probability distributions uniform & exponential respectively
(C & D) Hazard rates for (A) and (B) respectively. F(t) is cumulative probability of f(t).
(E & F) Subjective time estimation function: a normal distribution whose variance is proportional to the elapsed time. The coefficient of variation is the Weber fraction of time estimation in rats (Φ = 0.3).
(G & H) Anticipation functions (Janssen & Shadlen 2005) obtained by blurring the theoretical probability distributions (in A) with the subjective time estimation functions

Our result is the first demonstration of an effect of temporal attention parametrically varied by changing the hazard rate on sensory discrimination.

Controlling anticipation of the ‘go’ signal occurrence

For a uniformly distributed ‘go’ signal delays, the hazard rate rises exponentially with time. A flat hazard can be achieved with an exponential distribution of the ‘go’ signal delays. We chose an exponential distribution for the ‘go’ signal of mean 0.3s. Human, rats and other experimental animals can only make a subjective estimate of time. This subjective estimate of time is given by the scalar expectancy theory (Gibbon, 1977). It was found that the estimates of time are not only subjective but rescaled with the interval being timed. The rescaling factor was experimentally found to be a scalar (Libby and Church, 1974). The scalar was later found to be the Weber fraction of time estimation established from experiments involving time interval discrimination (Church et al., 1976).To achieve a subjective anticipation function, the chosen probability distribution of the 'go' signals were blurred with the subjective time estimation function for each of the time points before calculating the hazard rate (figure 5-1C). The coefficient of variance is the Weber ratio for time estimation. We used 0.3 as the value of the Weber fraction to obtain our subjective anticipation functions. Previous studies have estimated this value for human, rodents and in pigeons to be in the range of 0.2 – 0.3 (Gibbon et al., 1997; Rakitin et al., 1998; Janssen and Shadlen, 2005).

A randomly delayed ‘go’ signal task

In this task we used a randomly delayed auditory 'go' signal and single stimulus contrast (difficulty) in a session. We trained four naïve rats to respond after randomly delayed ‘go’ signals chosen from a uniform distribution. The delays ranged from 0.1 – 1.0s with increments of 100ms. A typical trial in the test session is shown in figure 5-2 A. Rats initiated a trial with a poke in the center port. A randomly delayed 'go' signal followed odor valve onset. The trials were designed to encourage higher accuracy on the discrimination performance and decrease the frequency of short pokes (odor sampling shorter than the delay to ‘go’ signals). A 8s error inter-trial interval (ITI) followed an error choice with the purpose of delaying the availability of the next trial. A correct choice was followed by a 4s ITI. In an event of a short poke there was a short white noise burst feedback followed by an 8 s error ITI. To encourage quick responding after the ‘go’ signal the water delivery time was made contingent on the time of water poke after a successful odor sampling.

After achieving successful training on the auditory ‘go’ signal (~70% success rate over 7 days of training, data not shown) we introduced the odor mixture discrimination task. The difficulty of the task was gradually increased over the course of training. The mixture ratios used in order of increasing difficulty were 95/05 (5 sessions), 80/20 (8 sessions), 68/32 (8 sessions) and 56/44 (10 sessions).

OSDs and reaction times to the ‘go’ signal, measured as time from onset of ‘go’ tone to the voluntary removal of the rat’s snout from the odor port, are plotted against the delays to the ‘go’ signal (Figure-5-2 B, C). Only trials in which the rats stayed beyond the ‘go’ signal and responded within the response window of 1.0s are used for this analysis. We achieved median OSDs in the range of 0.35 to 1.1s in this task across various ‘go’ signal delays and the OSDs increased monotonically with the ‘go’ signal delays. The OSD are pooled across all rats and across all difficulties. OSDs are adjusted for a 0.1s odor onset delay. The mean reaction time to the ‘go’ signal tone varied from 0.35s to 0.12ms for ‘go’ signal delays of 0.1s to 1.0s (n = 4 rats).

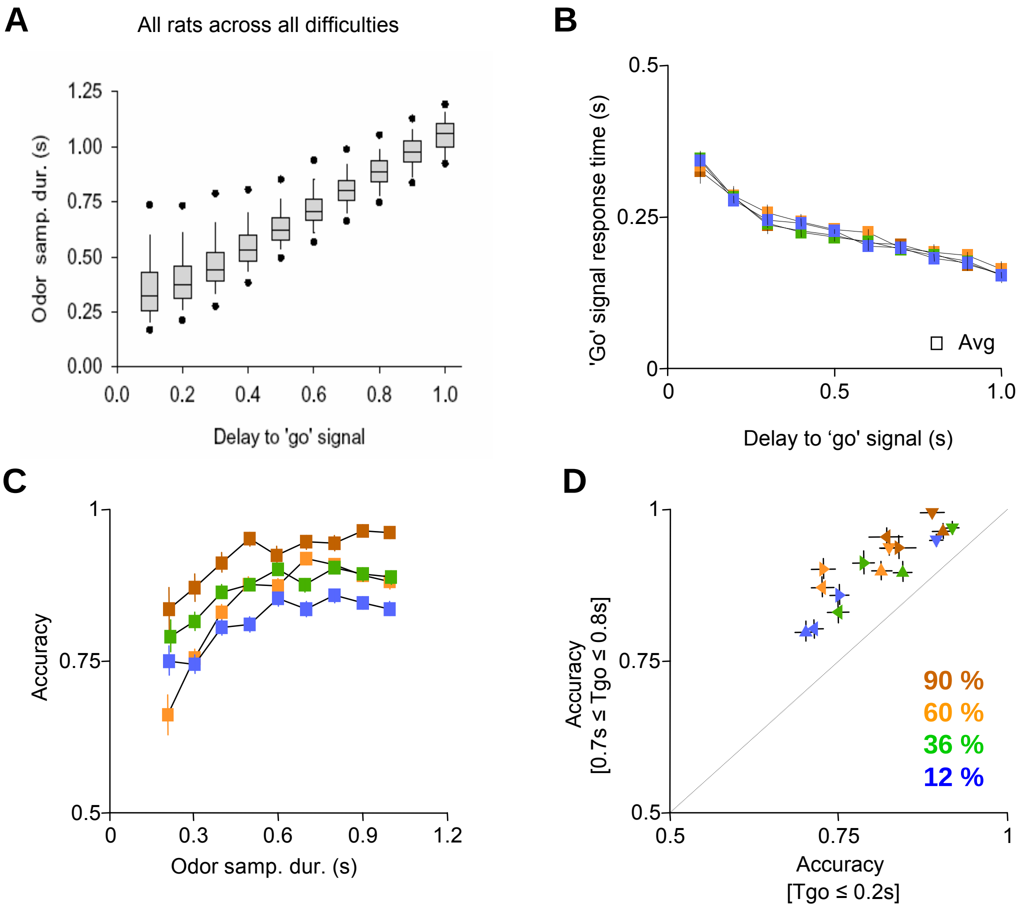


Figure 5 – 2: **Odor sampling duration, response time to 'go' signal and accuracy in randomly delayed 'go' signal paradigm.**

(A) Odor sampling duration distribution is shown as boxplots for each of the 'go' signal delay. The data is pooled across all rats and all sessions in which Tg was uniformly distributed. Box plots show median (horizontal center line), interquartile range (box) and 1.5 times the interquartile range (dots).

(B) Response time to 'go' signal is plotted as a function of delay to 'go' signal for individual stimulus contrast (different colors). Data is pooled across all rats all sessions. Error bars are calculated from a binomial model.

(C) Accuracy as a function of odor sampling duration for individual stimulus contrast (different colors) for all valid trials pooled from all four rats. Error bars are calculated from the binomial model.

(D) Accuracy of individual rats (denoted by different symbols) on individual stimulus contrast (different colors) on the late 'go' signals versus the early 'go' signal.

Performance accuracy for each of the individual mixture ratio tested improved with the OSD. Figure 5-2 C, D shows respectively the mean accuracy across all four rats and summary of accuracy for all four rats on all four difficulties. The mean performance (fraction correct) on late ‘go’ signal was higher than the mean performance for early ‘go’ signal. The improvement is significant ($P < 0.05$, Kruskal Wallis non-parametric statistics for non-matched samples).

Discrimination performance is affected by temporal anticipation.

We predict that temporal anticipation of the ‘go’ signal will have consequences on the performance of rats in our task. Contrarily, if the performance were to depend on temporal integration of sensory evidence it should benefit with longer sampling durations irrespective of the changing anticipation of the ‘go’ signal. We took the four rats and tested them on an exponentially distributed ‘go’ signal (flat hazard, experiment-2) followed by a uniformly distributed ‘go’ signal (rising hazard, experiment-3).

The performance accuracy is affected by the underlying hazard rate (figure 5-3 A & B, example rat and pooled across all rats). Performance accuracy saturated by 300ms of OSD in the flat hazard condition, while in the rising hazard condition it was significantly lower ($P < 0.05$ Kruskal Wallis test, non matched sample across all sessions). We compared the mean T-95 (shortest OSD when the accuracy reaches 95% of the best accuracy) of individual rats in the two conditions and found that it was significantly lower in the case of flat hazard (figure 5-3 C, $P < 0.05$ Kruskal Wallis test).

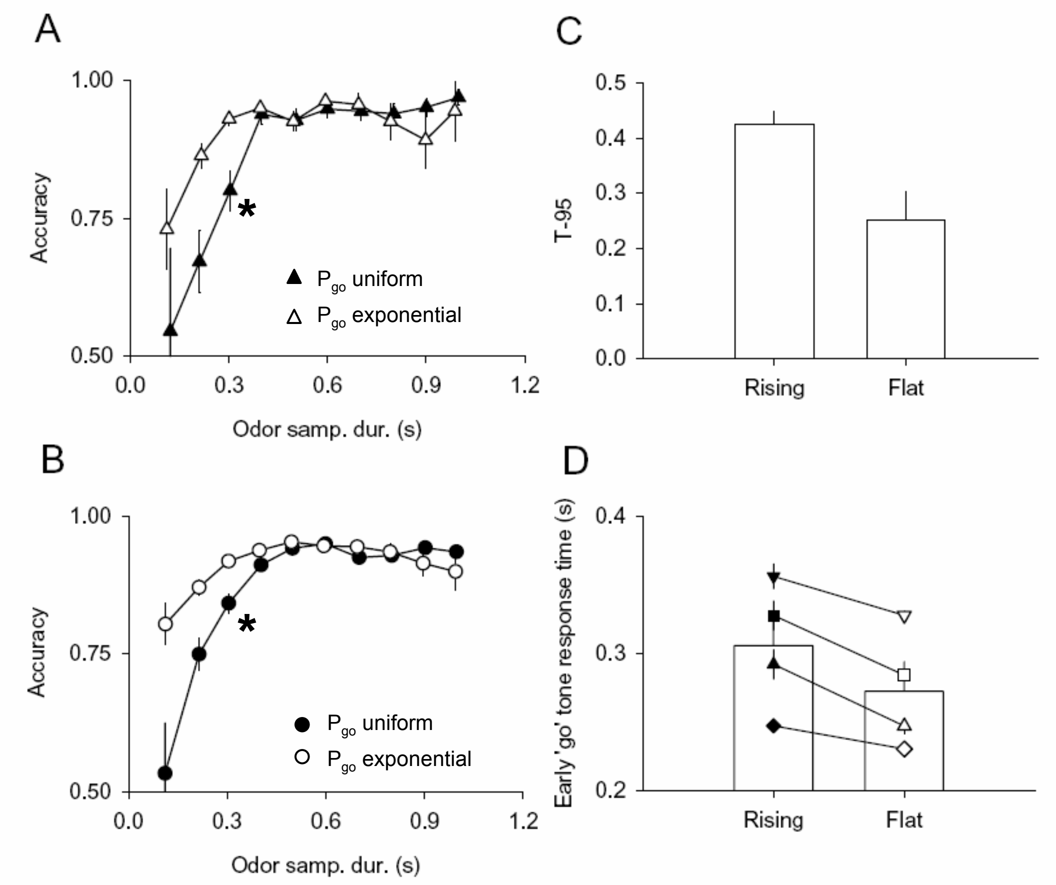


Figure 5 – 3: **Temporal anticipation affects accuracy in a 'go' signal task**.

(A) Mean performance accuracy as a function of odor sampling duration of an example rat performing a go-signal task in two different conditions. Filled symbols – session with uniformly distributed (rising hazard rate) 'go' signal delay; open symbols – exponentially distributed (flat hazard rate) 'go' signal delay. A difficult single mixture pair (12% stimulus contrast) was presented in both conditions. Error bars are calculated using a binomial model. '*' indicate significantly lower accuracy for 300ms OSD compared to accuracy on OSD ≥ 700ms in rising hazard ($P < 0.05$ Kruskal Wallis test).

(B) Same as (A) but data pooled across all four rats. Legends same as in (A). Error bars are calculated using a binomial model.

(C) Accuracy saturated within 300ms under flat hazard condition. Mean T95 (shortest OSD that gave 95% of maximum accuracy) across the four rats tested on two different hazard rates. Error bars mean +/- SEM (n = 4 rats).

(D) As anticipation increases response time to 'go' signal decrease. Each symbol represents individual rat and the mean response times to the early [0:1s to 0.3s] 'go' signals. Filled symbols for rising hazard and open circle for flat hazard. Error bars for each rat is mean +/- SEM (n = 5 sessions).

Orienting attention to temporally estimated moments should improve accuracy and decrease the reaction time. Increasing the attention for the early ‘go’ signal in the flat hazard condition significantly reduced the response time to the early 'go' signals as compared to the early ‘go’ signals in the rising hazard condition (figure 5-3D, $P < 0.05$ Friedman test for matched samples).

Since accuracy of rats is affected by the anticipation function we wanted to see if it follows the subjective anticipation functions (figure 5-1G, H). Since subjective anticipation functions (Au and Ae for uniform and exponentially delayed ‘go’ signal respectively) are dependent on the ‘go’ signal delays and not OSDs we fitted the anticipation function to the accuracy curve plotted as a function of ‘go’ signal time (figure 5-4 A, B, see methods for the fitting procedure). We compared the weights (W(uniform) and W(exponential)) associated with the two anticipation functions (Au and Ae) in the fitted curve for each of individual rats in the two different conditions, in other words, WE and WU for individual rat in uniform and exponentially distributed ‘go’ signal delays (figure 5-4C). the weights for rising subjective anticipation function was larger in the case of uniform ‘go’ signal delay distribution and the weights for flat anticipation function was larger in the case of exponentially distributed ‘go’ signal.

Anticipation functions affect the performance of rats quite significantly but it takes up to three training sessions for rats to learn new anticipation functions. Since anticipation function affects accuracy on early ‘go’ signal we compared two measures that quantify the effect of anticipation on accuracy; (a) the difference in accuracy between late and early ‘go’ signals and (b) accuracy on early ‘go’ signal delays. We looked at the change in these measures over days of training (figure 5-5A, B) in which the rats first experienced uniform distribution (experiment 1) followed by exponential (experiment 2) and once again uniform distribution (experiment 3). It is clear from these plots that a new anticipation function takes up to 2-3 days to show effect accuracy.

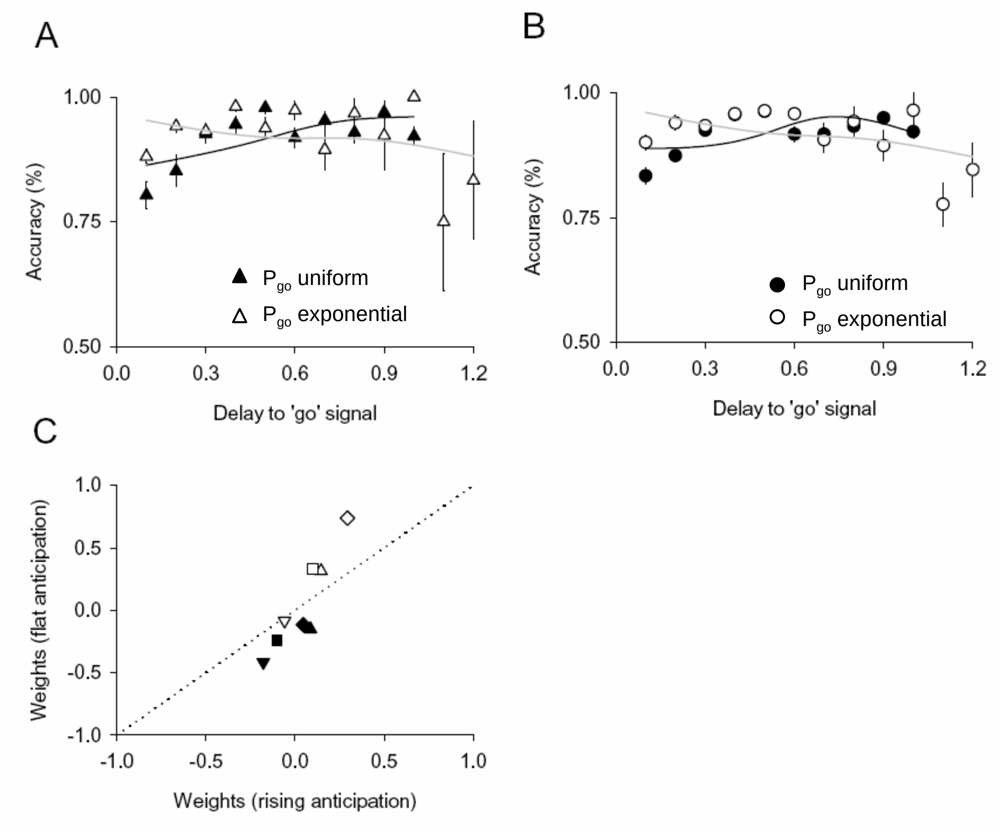


Figure 5 – 4: **Accuracy follows the subjective anticipation function.**

(A) Performance accuracy shown as a function of ‘go’ signal delay for an example rat. closed symbols for rising anticipation and open symbols for flat anticipation. Solid lines are fitted subjective anticipation function in each condition. Error bars are calculated using a binomial model.

(B) Mean performance accuracy for all trials pooled across all four rats as a function of ‘go’ signal delay. Symbols and fitted line same as (A). Error bars are calculated using a binomial model.

(C) W (rising anticipation) and W (flat anticipation) are weights associated with theoretical subjective rising and flat anticipation function respectively; when fitted to the discrimination performance curve of individual rats (different symbols) in two different conditions. Open symbols for weights in flat anticipation condition and filled symbols for weights in rising anticipation. Each rat is represented with the same symbols as in (A) and (B).

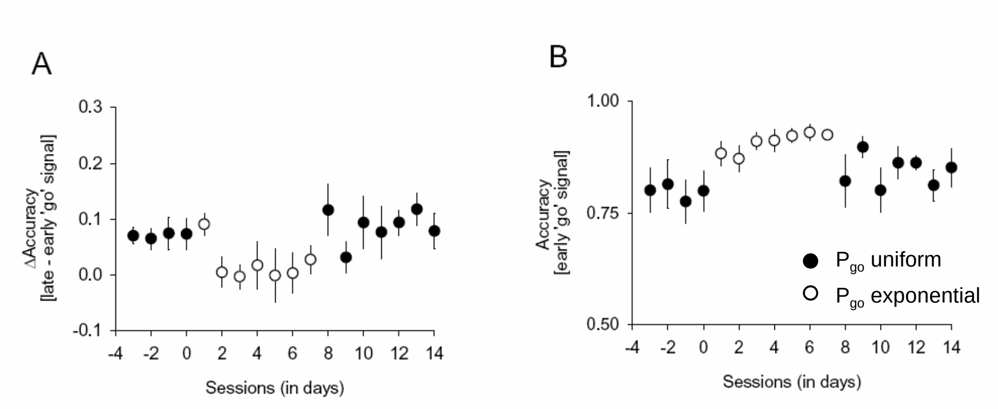


Figure 5 – 5**: A novel anticipation function takes a few (~ 3) sessions to affect discrimination accuracy.**

(A) Mean difference in performance accuracy between late (0.7:1.0s) and early (0.1:0.2) 'go' signal. '0' in the x-axis denote the last point before changing to the flat hazard condition (exponentially distributed 'go' signal delay). Filled symbols for rising anticipation and open symbols for flat anticipation conditions. Error bars are mean +/- SEM (n = 4 rats).

(B) Mean performance accuracy on early 'go' signal (0.1s & 0.2s). Error bars are mean +/-SEM (n = 4 rats).

A 300ms of odor sampling is sufficient for rats to achieve saturating performance in this task. Odor sampling durations in this range is easily achieved by rats in a RT paradigm. We therefore tested these rats in a RT paradigm to compare their accuracy in a task where they voluntarily determine their OSDs and accuracy.

Discrimination performance in a RT paradigm is saturating

Rinberg et al (2006) proposed that mice in a RT paradigm do not perform as well as they do when forced to sample for long times by a 'go' signal. They argue that RT paradigm temporally restricts the processing of odors that require longer processing times.

We took the same rats and tested them in a RT task by removing the 'go' signal but keeping the rest of the task parameters unchanged. We first tested the rats on 44/56 mixture ratio. We compared the accuracy of rats in RT paradigm with that on OSDs greater than 600ms in the rising hazard condition. There was no significant difference in their accuracy ($P < 0.05$ Kruskal Wallis test, not plotted). The accuracy of rats on 44/56 ratios is 90% (+/- 2% n = 4rats) in the RT task. Therefore, we trained the same rats further on two more difficult ratios i.e., 48/52 and 49/51. The accuracy on these ratios was intermediate i.e., 76% and 54% respectively. To test if rats would benefit with longer sampling durations on these two most difficult ratios, we reintroduced a ‘go’ signal paradigm with a fixed 1.0s ‘go’ signal delay. The time line of training of these rats on RT and Fixed 1.0s ‘go’ signal paradigm is shown in figure-5-6.

Fig 5-6A shows the comparison of performance and OSDs of these rats in a RT paradigm and the fixed 1.0s 'go' signal delay paradigm. The performance was not significantly different across these two conditions though the OSDs were very different. This result strongly indicates that accuracy in odor mixture discrimination task is saturating within the reaction times voluntarily chosen by the rats or in other words that their voluntary behavior does not constraint their performance.

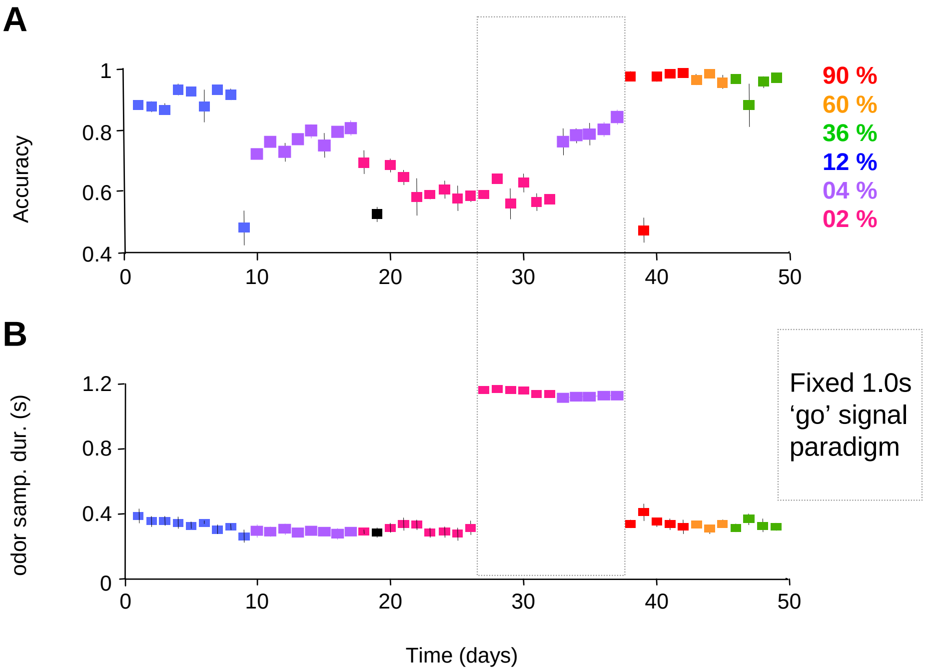


Figure 5 – 6: **Identifying two most difficult ratios in a RT paradigm and test them on a 1.0s fixed 'go' signal (dotted area in (A) and (B))**

(A) Accuracy of rats in a RT paradigm and in fixed 1.0s 'go' signal paradigm.. Difficulty was increased only after obtaining 5 consecutive sessions with stable performance i.e., not more than +/- 5% change in performance level. After testing rats on the most difficult ratio in the RT paradigm, discrimination performance on the two most difficult ratio was tested using longer sampling times implemented by a fixed 1.0s 'go' signal. Finally RT performance was measured on three easy ratio pair for the purpose of obtaining an accuracy curve. Each data point is a mean +/- SEM (n = 4 rats).

(B) Each data point is mean odor sampling duration in seconds. Error bars are +/- SEM (n = 4 rats).

In both (A) and (B) day 9 and 39 are control sessions using the same odor in all odor channels and identical task parameters. Day 19 is a control session with a 50/50 air mixing of two odorized air streams each of a 55/45 and a 45/55 premixed odors (see methods for details on air mixture using premixed odors).

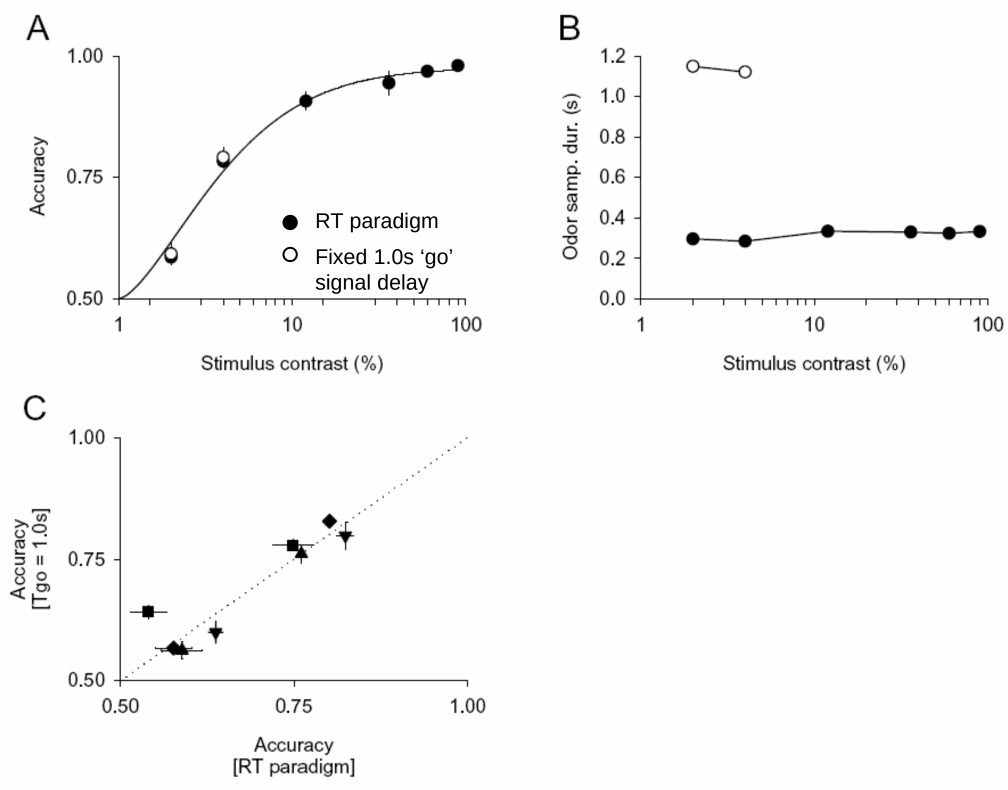


Figure 5 – 7**: Accuracy is saturating in RT paradigm.**

(A) Accuracy as a function of stimulus contrast (%). Open circles, 1.0s ‘go’ signal paradigm; filled circles, RT paradigm. Error bars are mean +/- SEM (n = 4 rats).

(B) Odor sampling duration as a function of stimulus contrast (%). Error bars are mean +/- SEM (n = 4 rats). Note that error bars are smaller than the symbols.

(C) Mean accuracy for individual rats (different symbols) compared in the two paradigms. Error bars are mean +/- SEM (n = 5 sessions).

Chapter 6: Discussion

The speed and accuracy of odor discrimination

Uchida and Mainen (Uchida and Mainen, 2003) reported a limited role of slow temporal code in odor mixture discrimination. Rats in their task performed equally rapidly i.e., within 300ms for all given difficulties. Taking into account the role of speed accuracy trade off in perceptual decision making, we provided strong motivation to rats in the form of punishments and energy depletion to persuade rats to trade their speed for difficult problems. In spite of successful speed trade offs in the range of 50-100ms, rats were unable to improve their accuracy. On the other hand, we saw accuracy to improve only in a speed-independent manner when stimuli were blocked i.e., non-interleaved (single stimulus context) in a session. A similar speed independent accuracy improvement accounts for higher accuracy seen in the mouse study (Abraham et al., 2004) because they employed a similar paradigm. We compared the dependence of accuracy on OSD in both interleaved and non-interleaved contexts using conditional accuracy functions and found that the accuracy saturated equally fast i.e., at 275ms. In case of Abraham et al (2004) it is impossible to achieve accuracy quantized by odor sampling duration (conditional accuracy function) because they do not have a measure of odor sampling time from correct choices. In a stay/no-stay paradigm reaction times are available only from half of the total trials i.e., the no-stay trials. The lack of complete odor sampling duration distribution in the stay/no-stay paradigm and the inability to ascertain the dependence of accuracy on OSDs limits the utility of such paradigms in olfactory psychophysics.

In a single difficulty context and RT paradigm we evaluated the optimality of accuracy voluntarily achieved by rats by first identifying two of the most difficult odor mixtures on which the accuracy was below 75% and then tested rats on long OSDs (>1000ms). We found that accuracy did not improve with additional sampling duration enforced by the ‘go’ signal. The dissociation of speed and accuracy of olfactory discrimination performance indicate a failure of long temporal integration of sensory evidence beyond the 300ms of odor sampling voluntarily achieved by rats in a RT paradigm. While our results strongly support the saturation of accuracy in a RT paradigm we do not rule out temporal processing at fast time scales i.e. within 50ms - 300ms of odor presentation or within a single sniff cycle. Fast temporal processing within single sniffs could be of significance in this task and further experiments are needed with better temporal precision in odor delivery to understand the effect of difficulty on this process. Though surprising these results are consistent with the view of limiting temporal integration to a single sniff cycle (Uchida and Mainen, 2003; Uchida et al., 2006) or within a single saccade (Melcher and Morrone, 2003).

Attention and the effect of reducing stimulus ensemble

Improvement in accuracy as a function of sampling duration is intuitive and well explained by the temporal integration model. Another known cognitive process by which accuracy can improve is broadly classified as attention. Interestingly accuracy improves with oriented attention without any increase in speed. We hypothesize that the interleaved condition represents an increased attentional demand compared to the noninterleaved condition, and that performance improves because there are less stimuli to anticipate in a given session, consistent with the common view of attention as a limited resource for which different stimuli compete (Kahneman, 1973)

Classically attention is thought of as the ability of subjects to select relevant and discard irrelevant stimuli. In a laboratory setting attention is normally studied by instructing subjects to pay attention at particular spatial location or temporal moment. Posner (Posner et al., 1980) found that reaction times of subject decreased for stimuli whose spatial location was better expected (low uncertainty) i.e., when it was at the same location for a block of trials (non-interleaved). But when the stimulus appeared at random locations (50% expectancy) or highly unexpected location (20% expectancy) the reaction time was much slower. Recent studies (McDonald et al., 2000; Correa et al., 2006) implementing near threshold stimuli have shown the effect of orienting attention on perceptual sensitivity measured as discriminability index d'.

Temporal anticipation affects temporal integration

Attention can vary with time and affect perceptual processing depending on the synchrony between expected and actual onset of stimulus (Griffin et al., 2001; Nobre, 2001; Correa et al., 2006). In a fixed delay to 'go' signal experiment rats have a fixed expectancy of the 'go' signal. For a randomly interleaved 'go' signal delay the expectancy is given by the hazard rate as the subjects experience the underlying distribution. Our results show that the hazard rate and temporal attention covary in this task as shown by the fits of the subjective anticipation function to performance accuracy.

Subjects show sensitivity to the hazard rate by virtue of their capability of time estimation. Rats use this capability to learn temporal contingencies in a task and make associations between temporally contiguous events like stimulus and reward. Why do rats care for the hazard rate of 'go' signal and how does the sensitivity affect performance accuracy? We believe that the sensitivity to the hazard rate allow rats to make time estimates of response deadlines. These temporal estimates seem extremely relevant in this task for rats in order to receive rewards. But then rats make their decision within 300ms of odor sampling. Why should the delay beyond 300ms have any bearing on accuracy? It is plausible that an unanticipated early ‘go’ signal disrupts sensory processing, however a rapid and optimal performance is restored once the rats learn to anticipate early ‘go’ signals i.e., with exponentially distributed ‘go’ signal delays.

Temporal anticipation in general affects perceptual processing and has been shown to affect processing of stimuli where temporal integration over longer time scales is extremely beneficent to the subjects. Newsome (Shadlen and Newsome, 2001) showed that rate of temporal integration process in LIP is affected by the anticipated time of response i.e., either 500ms to 2.0s. The slope of the ramping activity of LIP neurons varied inversely with the delay to the anticipated response deadline. LIP neurons ramp up faster for early i.e. 0.5s response deadline as compared to a late, 2.0s response deadline. These same LIP neurons are also modulated by the hazard rate or temporal anticipation of ‘go’ signal occurrence in the absence of a perceptual task (Janssen and Shadlen, 2005). Such a slowing down of decision-making process, i.e., temporal integration could also explain the effects of unanticipated early ‘go’ signals on performance accuracy of rats in our ‘go’ signal task. Based on temporal integration to the threshold model, the early ‘go’ signals enforce an early termination of the integration processes leading to suboptimal performance. In fact slowing down monkeys with delayed response deadline did not improve accuracy through temporal integration; it only changed the slope of the integration process. This could be an explanation for difference in integration time observed for rats in a RT paradigm and ‘go’ signal task with rising hazard.

Voluntary orienting attention to ‘go’ signal tone affects performance cross modally on odor discrimination

In the ‘go’ signal task the auditory ‘go’ signal is as salient as the odor discrimination and therefore rats orient their attention to the ‘go’ signal. McDonald et al (McDonald et al., 2000) found that human subjects showed improved sensitivity (d’) on a visual detection task when their attention was involuntarily oriented to the moment of visual stimulus presentation by a random auditory signal. In the same task when the visual stimulus presentation and the auditory signal occurred incoherently the performance was significantly lower on the visual detection task. Our results depart from this observation in two ways. First, the attention in our task is oriented to temporal moments in time by a voluntary process of time estimation and sensitivity to increasing hazard rate. Secondly, our task is stimulus discrimination task. The ability to study an intersensory integration process in neurophysiologically amicable rodents have obvious advantageous.

Odor encoding and sensory motor integration

It has been argued (Uchida et al., 2006) and shown (Melcher and Morrone, 2003) that temporal integration in many visual discrimination tasks are limited to within a saccade. Similar results have been obtained for rats performing odor mixture discrimination (Uchida and Mainen, 2003) and stereo odor detection (Rajan et al., 2006). Integration across saccades is only possible when the spatial location of the stimulus on the retina is conserved (Melcher and Morrone, 2003). This strategy is useful for creating visual scenes using saccadic eye movements. It has been proposed that a single sniff in a similar manner gives a snapshot of the olfactory environment (Uchida and Mainen, 2003; Rajan et al., 2006; Uchida et al., 2006).

More recently olfactory coding strategies based on active sniffing have been explored (Sobel et al., 1998; Kepecs et al., 2007). Human fMRI studies have reported attention modulation in the piriform cortex based on anticipatory sniffing (Zelano & Sobel 2005) even when the odors were absent. In the odor mixture discrimination task, the duration (or frequency) of sniffing correlates with the accuracy on difficult problems (Kepecs et al., 2007). How does an active sensation process affect the encoding of sensory stimuli? It would be of interest to explore the modulation of sniffing rate with temporal attention and accuracy of performance. Such a study would shed light on attentional effects on sniffing rate dependent olfactory code.

METHODS

Subjects: Male Long-Evans hooded rats (300 – 400 grams) were water restricted for motivation. Rats were handled following the standard procedures and guidance provided by the National Institute of Health and approved by the Cold Spring Harbor laboratory institutional animal care and use committee. Motivated rats performed 250 to 400 successful trials per session.

Odor delivery: The test odors were a1:10 dilution of S-(+) and R-(-) stereo isomers of 2-octanol in all our studies. Stereo isomers were chosen because they have similar vapor pressures and intensities and can be premixed in the liquid phase. Odors were diluted in mineral oil. Odor mixtures were generated by mixing into a carrier air stream two odorized air streams (odorized with pure odors) whose flow rates were controlled using a pair of mass flow controllers (Aalborg GFM). The mixture ratios of 05/95, 20/80, 32/68 and 44/56 were reliably generated by this method given the precision of mass flow controllers. The ratios 48/52 and 49/51 were also generated by air flow mixing but from two odorized air streams each consisting of premixed (in liquid phase) ratio of 55/45 and 45/55 of the two pure test odors.

Odor travel delay: The minimum odor travel delay measured for our odor delivery apparatus using EOG probe in an anesthetized animal was found to be 0.1s and was subtracted from all measures of the OSDs (Feierstein 2006).

Behavior

Rats where trained in a two alternative choice paradigm with a custom built behavioral set up and olfactometer (Uchida 2003). All data acquisition was done in Matlab® 6.5 data acquisition tool box. The analysis was performed in Matlab® 6.5 Release 13.

The reaction time paradigm

Training: Rats were first trained on an operant task where a poke in to one of the choice ports led to a drop of water as a reward. Rats were then trained to sample odors in the center port before making a poke in the choice port (odor sampling training). The required sampling durations was gradually increased from 0 to 300ms and rats were sufficiently trained on this task. A third odor other than the test odor pair was used during this stage of training. After training rats for 2-4 days on odor sampling two test odors were introduced and rats were trained to perform the discrimination task. Odor and choice ports were balanced across all the rats in a group i.e., half the rats were rewarded at the left choice port for S-(+)-2-octanol and the other half at the right choice port for the same odor. Rats were intermittently tested on a negative control using a single odor out of the pair to odorize air streams keeping all other task parameters like reward contingencies constant. The odor mixture difficulty was gradually increased over 4-8 sessions before data collection. The task difficulty was increased gradually from 05/95 Vs 95/05 mixture ratio to 20/80 & 20/80, 32/68 & 68/32 and 44/56 & 56/44 mixture ratios in the same session.

Standard task: Four LE rats were trained and tested on a binary odor mixture discrimination task using a set of task parameters identical to Uchida 2003.

Low urgency to respond: Four naive LE rats were trained on a 2.0s fixed water delay from the time of odor onset during the odor sampling phase of early training. The inter-trial interval in this task was fixed at 6.0s from the time of odor onset. In order to control for motor stereotypy and temporal anticipation of odor onset, an odor stimulus fore period of 0.1 – 2.0s was chosen randomly from an exponential distribution with a mean of 0.5s.

Air puff punishment: Four naive LE rats were trained with an air puff punishment for error choices right from the onset of discrimination task training. The air puff was given using an outlet right above the water delivery tube using a three way solenoid valve that diverted a continuous 2.0 liter per minute air flow into the nose cone for punishment. On successful trial the water delivery was randomly delayed by 0.1 - 0.6s (uniformly distributed) from the time of water port entry. On error trials the air puff was given immediately after water port entry.

Protocol for water control in water regulation task: Eight naïve rats were individually housed in a cage and trained on the standard task. The task parameters, type and number of stimuli and a typical trial are as outlined in figure 3-4. After achieving sufficient training on the basic task, these rats were put on ad libidum food and water. Their weights and water intake was recorded for each day (fig 3-4). After achieving asymptotic weights on ad libidum food and water, water deprivation was re-introduced. Each of the rats was given 50% of its ad libidum water intake. Weights were monitored for a few days of deprivation. Training ensued on the full task. The 50% of the delivered water was divided between inside the task (In-task) and outside the task (free water). Rats received 34% of their ad libidum water in-task and 16% of the ad libidum water as free water in the cage. The reward amount per correct trial was customized for individual rats and estimated for 90% success rate in a task consisting of 256 performed (correct & error) trials. The maximum session length was 50 minutes. All the rats finished their allocated total trials before the maximum session length. After achieving sufficient baseline performance (8 sessions) we took away the free in-cage water from the test group and tested them on another 6 sessions.

The ‘go’ signal paradigm

Training: Rats were trained to respond after the onset of an auditory ‘go’ signal tone (2 kHz frequency and 0.1s duration) by pulling out of the odor port. During this phase the delay to the ‘go’ signal was chosen to be 0.8s for the fixed ‘go’ signal task and was drawn randomly from a uniform distribution in the random ‘go’ signal delay task. We enforced the rats to respond to the ‘go’ signal within a 1.0s window after its onset. A short 0.12s or a long 3.0s white noise burst signaled all short OSDs (response before the ‘go’ signal) and very long OSDs (response after the 1.0s ‘go’ signal window) respectively. A 4.0s minimum inter-trial interval was enforced from the time of switching off the water valve. Rats were punished with an additional 4.0s for short and long pokes. Rats made very few choice pokes (~5%) after the white noise feedback and therefore learned the feedback signals. A 1:100 dilution of ethyl butyrate was presented during the OSDs. This odor was different from the test odor pair in the experimental phase. Water was available at the choice port for up to 4.0s after a ‘go’ signal response. Water delivery was delayed randomly for 0.1s – 0.6s (uniformly distributed) from the time of the choice port entry. In the training phase rats were free to respond at either choice ports and were rewarded with a drop of water (34 µl) for a successful ‘go’ signal response. We began the experiments after achieving a stable successful trial rate of approximately 70%

Fixed ‘go’ signal paradigm: Four new rats were used for this task. Three odor mixture ratios (5/95, 32/68 and 44/56) were randomly interleaved in this task. The ‘go’ signal delay was fixed for the entire session and few consecutive days. A new delay was introduced after a few test sessions. The sequence of change in ‘go’ signal delays over days is shown in figure 3-B.

Random ‘go’ signal paradigm: Four new rats were used for this task. The single odor mixture ratio pair was presented per session and the ‘go’ signal delays were randomly interleaved within a task.

Experiment I: The ‘go’ signals were drawn from uniform distribution ranging from 0.1s to 1.0s in 0.1s steps. The mixture ratio difficulty was gradually increased after sufficient training on each ratio (8-10 sessions, data not shown). The difficulty was increased once the performance did not change +/- 5% over consecutive 5 sessions. The mixture ratios used were 5/95, 20/80, 32/68 and 44/56 (data not shown).

Experiment 2: ‘Go’ signals were drawn from an exponential distribution with a mean (μ) of 0.3s. Rats were then tested on this distribution of ‘go’ signal delays for 7 sessions.

$$f(x, \mu) = - \mu e^{-\mu x}, \qquad x >= 0$$

Experiment 3: same four rats performed another 8 sessions once again on uniformly distributed ‘go’ signal delays to test if the anticipation dependent effect disappeared with learning.

Experiment 4: After completing all ‘go’ signal experiments rats where then tested on the RT paradigm keeping other task parameters constant. The difficulty was increased over days. The analysis included last five sessions when the performance did not change for more than +/- 5%.

Experiment 5: All four rats were tested on 1.0s fixed ‘go’ signal delay on two mixture ratios 48/52 and 49/51.

Data analysis

All the analysis was performed in Matlab® 6.5 Release 13. We used mean +/- SEM in all analysis and figures except where mentioned.

Binomial model: Standard errors for binomially distributed trials (correct or incorrect) were calculated using a standard model for binomial statistics. We used these error bars for individual rat data after pooling all trials for individual 'go' signal delays. The standard error is given as

$$SE = \sqrt{p(1-p)/n}$$

Where p is probability of correct choices, n is total number of trials performed.

Psychometric fit: The logistic regression fits to the psychometric performance of rats were performed using the generalized linear model in Matlab® (glmfit) using a binomial distribution of choices. The fitted parameters were the slope and the bias of the fit. The plotted values were obtained from the fitted parameters using glmval function in Matlab®.

Trial selection for 'go' signal task: The analysis of discrimination performance, OSDs and the response times to 'go' signal includes only those trials where the rats OSDs was longer than the delay to 'go' signal and shorter than the 'go' signal response window.

Trial selection for reaction time paradigm: All the trials where the sampling durations were longer than the odor onset delay where included in the analysis.

Hazard rate: The hazard rate of upcoming 'go' signal was calculated using the following formula:

$$h(t) = f(t)/(1-F(t))$$

Where h(t) is the hazard rate, f(t) the distribution of 'go' signal delays (uniform or exponential) and F(t) the cumulative distribution.

Subjective anticipation function: The subjective anticipation function for the 'go' signal was calculated by blurring the probability distribution (uniform & exponential) with a subjective time estimation function (described below). The hazard rate of this blurred probability distribution gave the subjective anticipation functions which we used for fitting the performance curves.

Subjective time estimation function: The estimation of time by rats is subjective. The uncertainty in the estimate of time scales with elapsed time, therefore, a subjective estimate functions are normal distributions whose standard deviation (σ) increase with elapsed time (figure 5-1 E, F). In other words an elapsed time 't' is estimated as 't+/-σ'. The coefficient of variation is given by the Weber fraction for time estimation (Φ). For our analysis we used a value of Weber fraction (Φ) = 0.3.

Fitting subjective anticipation function to performance accuracy of rats: The performance of rats was plotted as a function of delay to the 'go' signal because the subjective anticipation is a function of 'go' signal times and not OSDs. The subjective anticipation functions (figure 5-1 G, H) were scaled between 0.5 and 1.0 which is the minimum and maximum performances accuracy for rats in this task. The two scaled subjective anticipation functions (for uniform and exponential distributions) were fitted to the performance curve using the following equation:

$$C(t) = Wn + Wu * Au(t) + We * Ae(t)$$

Where C(t) is instantaneous performance of rats, Wn is a constant term, Wu is the weight associated with Au, We is the weight associated with Ae. Au & Ae are the subjective anticipation function for uniformly and exponentially distributed 'go' signal delays. The minimization of mean squared errors was performed using the 'fminsearch' program in Matlab®.

List of References

Abraham NM, Spors H, Carleton A, Margrie TW, Kuner T, Schaefer AT (2004) Maintaining accuracy at the expense of speed: stimulus similarity defines odor discrimination time in mice. Neuron 44:865-876.

Blackwell HR, Schlosberg H (1943) Octave generalization, pitch discrimination and loudness thresholds in the white rats. Journal of experimental psychology:407-419.

Bodyak N, Slotnick B (1999) Performance of mice in an automated olfactometer: odor detection, discrimination and odor memory. Chem Senses 24:637-645.

Britten KH, Shadlen MN, Newsome WT, Movshon JA (1992) The analysis of visual motion: a comparison of neuronal and psychophysical performance. J Neurosci 12:4745-4765.

Buck L, Axel R (1991) A novel multigene family may encode odorant receptors: a molecular basis for odor recognition. Cell 65:175-187.

Carr WJ, Solberg B, Pfaffmann C (1962) The olfactory threshold for estrous female urine in normal and castrated male rats. J Comp Physiol Psychol 55:415-417.

Chittka L, Dyer AG, Bock F, Dornhaus A (2003) Psychophysics: bees trade off foraging speed for accuracy. Nature 424:388.

Church RM, Getty DJ, Lerner ND (1976) Duration discrimination by rats. Journal of the Experimental Psychology: Animal Behavior Processes:303 - 312.

Correa A, Lupianez J, Madrid E, Tudela P (2006) Temporal attention enhances early visual processing: a review and new evidence from event-related potentials. Brain Res 1076:116-128.

Crist RE, Kapadia MK, Westheimer G, Gilbert CD (1997) Perceptual learning of spatial localization: specificity for orientation, position, and context. J Neurophysiol 78:2889-2894.

Donders F (1869) On the speed of mental processes. Acta Psychol (Amst)

30:412-431.

Feierstein CE, Quirk MC, Uchida N, Sosulski DL, Mainen ZF (2006) Representation of spatial goals in rat orbitofrontal cortex. Neuron 51:495-507.

Finger FW (1941) Quantitative studies of "conflict". I. Variations in latency and strength of the rat's response in a discrimination-jumping situation. Journal of comp psychology 31:97-127.

Friedrich RW, Laurent G (2001) Dynamic optimization of odor representations by slow temporal patterning of mitral cell activity. Science 291:889-894.

Ghose GM, Maunsell JH (2002) Attentional modulation in visual cortex depends on task timing. Nature 419:616-620.

Gibbon J (1977) Scalar Expectancy Theory and Weber law of animal timing. Psychological Review 84:279-325.

Gibbon J, Malapani C, Dale CL, Gallistel C (1997) Toward a neurobiology of temporal cognition: advances and challenges. Curr Opin Neurobiol 7:170-184.

Goldberg SJ, Moulton DG (1987) Olfactory bulb responses telemetered during an odor discrimination task in rats. Exp Neurol 96:430-442.

Green DM, Swets JA (1988) Signal detection theory and psychophysics.

Griffin IC, Miniussi C, Nobre AC (2001) Orienting attention in time. Front Biosci 6:D660-671.

Janssen P, Shadlen MN (2005) A representation of the hazard rate of elapsed time in macaque area LIP. Nat Neurosci 8:234-241.

Jennings JR, Wood CC (1976) Speed-accuracy trade off function in choice reaction time: Experimental designs and computational procedures. Perception and Psychophysics 9:93-102.

Kahneman D (1973) Attention and Effort. New York: Prentice-Hall.

Kay LM, Beshel J, Martin C (2006) When good enough is best. Neuron 51:277-278.

Kepecs A, Uchida N, Mainen ZF (2007) Rapid and Precise Control of Sniffing during Olfactory Discrimination in Rats. J Neurophysiol.

Khan RM, Sobel N (2004) Neural processing at the speed of smell. Neuron 44:744-747.

Kuai SG, Zhang JY, Klein SA, Levi DM, Yu C (2005) The essential role of stimulus temporal patterning in enabling perceptual learning. Nat Neurosci 8:1497-1499.

Laing DG (1983) Natural sniffing gives optimum odour perception for humans. Perception 12:99-117.

Laming D (1968) Information theory of choice reaction time. Academic Press.

Laurent G (2002) Olfactory network dynamics and the coding of multidimensional signals. Nat Rev Neurosci 3:884-895.

Lauwereyns J, Watanabe K, Coe B, Hikosaka O (2002) A neural correlate of response bias in monkey caudate nucleus. Nature 418:413-417.

Libby ME, Church RM (1974) Timing of avoidance response by rats. Journal of the Experimental Analysis of Behavior 513-517.

Link SW, Heath RA (1975) A sequential theory of psychological discrimination. Psychometrika:77-105.

Luce R (1960) Response time.

Luce R (1986) Response times: Their role in inferring elementary mental organization. Oxford: Oxford University Press.

Matsumoto M, Hikosaka O (2007) Lateral habenula as a source of negative reward signals in dopamine neurons. Nature 447:1111-1115.

Mazurek ME, Roitman JD, Ditterich J, Shadlen MN (2003) A role for neural integrators in perceptual decision making. Cereb Cortex 13:1257-1269.

McDonald JJ, Teder-Salejarvi WA, Hillyard SA (2000) Involuntary orienting to sound improves visual perception. Nature 407:906-908.

Medin DL, Borkhius ML, Davis RT (1970) Response latency of brightness judgements by monkeys. Journal of experimental psychology 83:480-485.

Melcher D, Morrone MC (2003) Spatiotopic temporal integration of visual motion across saccadic eye movements. Nat Neurosci 6:877-881.

Moody DB (1970) Reaction time as an index of sensory function. In: Stebbins W (Ed) Animal Psychophysics: The Design and Conduct of Sensory Experiments Appleton-Century-Crofts, New York, pp 277-302.

Muenzinger KF (1934) Motivation in learning. I. Electric shock for correct response in the visual discrimination habit. Journal of comp psychology 17.

Niemi P, Naatanen R (1981) Foreperiod and simple reaction time. Psychological

Bulletin:133-162.

Nigrosh BJ, Slotnick BM, Nevin JA (1975) Olfactory discrimination, reversal learning, and stimulus control in rats. J Comp Physiol Psychol 89:285-294.

Nobre AC (2001) Orienting attention to instants in time. Neuropsychologia 39:1317-1328.

Ollman RT (1977) Choice reaction time and the problem of distinguishing task effects from strategy effects. In S. Dornic (ed.), Attention and Performance. VI:99-113.

Palmer J, Huk AC, Shadlen MN (2005) The effect of stimulus strength on the speed and accuracy of a perceptual decision. J Vis 5:376-404.

Paton JJ, Belova MA, Morrison SE, Salzman CD (2006) The primate amygdala represents the positive and negative value of visual stimuli during learning. Nature 439:865-870.

Pfaff DW, Pfaffmann C (1969) Olfactory and hormonal influences on the basal forebrain of the male rat. Brain Res 15:137-156.

Pfaffmann C (1958) An olfactometer for the rat. Science 128:1007-1008.

Posner MI, Snyder CR, Davidson BJ (1980) Attention and the detection of signals. J Exp Psychol 109:160-174.

Rajan R, Clement JP, Bhalla US (2006) Rats smell in stereo. Science 311:666-670.

Rakitin BC, Gibbon J, Penney TB, Malapani C, Hinton SC, Meck WH (1998) Scalar expectancy theory and peak-interval timing in humans. J Exp Psychol Anim Behav Process 24:15-33.

Rinberg D, Koulakov A, Gelperin A (2006) Speed-accuracy tradeoff in olfaction. Neuron 51:351-358.

Roitman JD, Shadlen MN (2002) Response of neurons in the lateral intraparietal area during a combined visual discrimination reaction time task. J Neurosci 22:9475-9489.

Schlosberg H, Solomon R (1943) Latency of response in a choice discrimination. Journal of experimental psychology 33:22-39.

Schultz EF, Tapp JT (1973) Olfactory control of behavior in rodent. Psychological Bulletin 79:21-44.

Shadlen MN, Newsome WT (2001) Neural basis of a perceptual decision in the parietal cortex (area LIP) of the rhesus monkey. J Neurophysiol 86:1916-1936.

Slotnick BM, Nigrosh BJ (1974) Olfactory stimulus control evaluated in a small animal olfactometer. Percept Mot Skills 39:583-597.

Smith PL, Ratcliff R (2004) Psychology and neurobiology of simple decisions. Trends Neurosci 27:161-168.

Sobel N, Prabhakaran V, Desmond JE, Glover GH, Goode RL, Sullivan EV, Gabrieli JD (1998) Sniffing and smelling: separate subsystems in the human olfactory cortex. Nature 392:282-286.

Talwar SK, Gerstein GL (1998) Auditory frequency discrimination in the white rat. Hear Res 126:135-150.

Uchida N, Mainen ZF (2003) Speed and accuracy of olfactory discrimination in the rat. Nat Neurosci 6:1224-1229.

Uchida N, Kepecs A, Mainen ZF (2006) Seeing at a glance, smelling in a whiff: rapid forms of perceptual decision making. Nat Rev Neurosci 7:485-491.

Warden CJ, Aylesworth M (1927) The relative value of reward and punishment in the formation of a visual discrimination habit in the white rat. Journal of comp psychology 7:117-127.

Wehr M, Laurent G (1996) Odour encoding by temporal sequences of firing in oscillating neural assemblies. Nature 384:162-166.

Youngentob SL, Hornung DE, Mozell MM (1991) Determination of carbon dioxide detection thresholds in trained rats. Physiol Behav 49:21-26.

Youngentob SL, Mozell MM, Sheehe PR, Hornung DE (1987) A quantitative analysis of sniffing strategies in rats performing odor detection tasks. Physiol Behav 41:59-69.

Zelano C, Bensafi M, Porter J, Mainland J, Johnson B, Bremner E, Telles C, Khan R, Sobel N (2005) Attentional modulation in human primary olfactory cortex. Nat Neurosci 8:114-120.